

Cerebral blood flow and cerebrovascular reactivity at rest and during sub-maximal exercise: Effect of age and 12-week exercise training

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Abstract Chronic reductions in cerebral blood flow (CBF) and cerebrovascular reactivity to CO₂ are risk factors for cerebrovascular disease. Higher aerobic fitness is associated with higher CBF at any age; however, whether CBF or reactivity can be elevated following an exercise training intervention in healthy individuals is unknown. The aim of this study was to assess the effect of exercise training on CBF and cerebrovascular reactivity at rest and during exercise in young and older individuals. Ten young (23±5 years; body mass index (BMI), 26±3 kg m⁻²; \dot{V}_{O_2} max, 35±5 ml kg⁻¹ min⁻¹) and 10 older (63±5 years; BMI, 25±3.0 kg m⁻²; \dot{V}_{O_2} max, 26±4 ml kg⁻¹ min⁻¹) previously sedentary

individuals breathed 5 % CO₂ for 3 min at rest and during steady-state cycling exercise (30 and 70 % heart rate range (HRR)) prior to and following a 12-week aerobic exercise intervention. Effects of training on middle cerebral artery blood velocity (MCAv) at rest were unclear in both age groups. The absolute MCAv response to exercise was greater in the young (9 and 9 cm s⁻¹ (30 and 70 % HRR, respectively) vs. 5 and 4 cm s⁻¹ (older), $P<0.05$) and was similar following training. Cerebrovascular reactivity was elevated following the 12-week training at rest (2.87±0.76 vs. 2.54±1.12 cm s⁻¹ mm Hg⁻¹, $P=0.01$) and during exercise, irrespective of age. The finding of a training-induced elevation in cerebrovascular reactivity provides further support for exercise as a preventative tool in cerebrovascular and neurological disease with ageing.

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Introduction

The maintenance of adequate cerebral blood flow (CBF) is critical to maintain a constant supply of oxygen and nutrients to the metabolically active brain. The control of CBF is multi-factorial, influenced largely by the partial pressure of arterial carbon dioxide (PaCO₂) and to a lesser extent by cerebral metabolism, mean arterial pressure (MAP), cardiac

output (\dot{Q}) distribution and neurogenic activity (Ainslie and Duffin 2009). Chronic reductions in CBF and impairments in its control are risk factors for cerebrovascular disease. A reduced absolute cerebral blood flow may result in stroke (Markus 2004) and is a major risk factor for Alzheimer's disease and dementia (de la Torre 2010). In addition, an impaired ability of the cerebral vasculature to respond to changes in PaCO₂ (termed cerebrovascular reactivity) is a risk factor for—and a consequence of—cerebrovascular disease (Gur et al. 1996; Markus and Cullinane 2001; Silvestrini et al. 2000). Assessment of the cerebrovascular response to alterations in PaCO₂ (by measuring changes in middle cerebral artery blood flow velocity (MCAv)) is a well-established means to estimate the physiological 'reserve' of cerebral perfusion (Ainslie and Duffin 2009).

Global CBF declines gradually with ageing, by 25–30 % between 20 and 80 years of age (Ainslie et al. 2008; Buijs et al. 1998; Demirkaya et al. 2008; Krejza et al. 1999; Liu et al. 2011) or ~5 % per decade (Grolimund and Seiler 1988). The effect of healthy ageing on cerebrovascular CO₂ reactivity is less clear, however. Hypercapnic reactivity has been found to be both lower (Reich and Rusinek 1989; Yamamoto et al. 1980) and unchanged (Davis et al. 1983; Ito et al. 2002; Kastrup et al. 1998; Schwertfeger et al. 2006; Galvin et al. 2010) with advancing age, while hypocapnic reactivity has been found to be elevated (Galvin et al. 2010), unchanged (Ito et al. 2002) and reduced (Yamaguchi et al. 1979; Tsuda and Hartmann 1989). Differences in measurement technique, stimulus magnitude (i.e. PaCO₂ change) and subject age make inter-study comparison difficult.

In addition, MCAv has been shown to be increased ~10–25 % with higher aerobic fitness across the adult lifespan (Ainslie et al. 2008), but the reasons remain unclear. One cross-sectional study indicated that higher fitness is related to larger frontal and temporal volumes of grey matter (Colcombe et al. 2006). Other potential mechanisms may involve more endothelium-dependent vasodilatation as a result of more NO bioavailability (Green et al. 2004) and/or cerebral angiogenesis (Rhyu et al. 2010; Swain et al. 2003; Ding et al. 2006; Black et al. 1990). In support of this, age-induced cerebral atrophy appears to be less marked in those with higher aerobic fitness (Colcombe et al. 2003). Furthermore, 6 months of aerobic exercise training can even increase brain volume in previously sedentary older individuals (Colcombe et al. 2006), so

a corresponding increase in CBF would seem plausible. Yet Ivey et al. (2011) found no increase in CBF following 6 months of aerobic-based exercise training in chronic hemiparetic stroke patients. In summary, cross-sectional data indicate that fitness training may increase CBF, consistent with the demonstrated improvements in neuronal mass and metabolism, but it remains to be shown whether CBF is actually increased with training or whether this occurs in both young and older healthy individuals.

Another important consideration is whether ageing impacts on CBF responses to common daily activity (e.g. exercise) and if so, why? Only two studies have assessed the effect of age on the CBF response to sub-maximal exercise, and both observed no differential effect of age on the exercise-induced increase in CBF (Fisher et al. 2008; Heckmann et al. 2003). In addition to the CBF response, cerebrovascular CO₂ reactivity is also increased during exercise (Ogoh et al. 2008; Rasmussen et al. 2006). Although the effects of exercise on CBF (Ogoh and Ainslie 2009; Querido and Sheel 2007) and cerebrovascular reactivity (Ogoh et al. 2008; Rasmussen et al. 2006) have been relatively well documented in young individuals, any influencing effect of older age on these parameters has not been established. Due to the conflicting evidence on the effect of age on cerebrovascular reactivity at rest, it is unclear what effect (if any) age has on cerebrovascular reactivity during exercise.

Although cerebrovascular diseases such as stroke have been associated with an impaired cerebrovascular reactivity, and regular physical activity is known to reduce the risk of stroke, only one study has assessed the effect of fitness on cerebrovascular reactivity to CO₂. Ivey et al. (2011) demonstrated an elevation in cerebrovascular reactivity to CO₂ following 6 months of exercise training in stroke survivors. Whether similar improvements can be observed in healthy young and older individuals following a lesser amount of exercise training is unknown. This fitness-induced reduction in the risk of stroke is due to upregulation of endothelial NO synthase (Endres et al. 2003; Gertz et al. 2006; Lee and Paffenbarger 1998). Furthermore, it is known that hypercapnia-induced cerebrovascular vasodilatation is, at least in part, NO dependent (Peebles et al. 2007). Since systemic NO production/bioavailability is increased with exercise training (Kingwell et al. 1997), it seems possible that increases in aerobic fitness will be associated with increases in cerebrovascular reactivity.

The main purposes of this study were twofold: (1) to assess the effect of age on CBF and its reactivity at rest and during sub-maximal exercise and (2) assess improvements in the aforementioned parameters across 12 weeks of aerobic-based exercise training. We hypothesised that (1) cerebrovascular reactivity would be maintained in healthy older age and age would have no effect on the elevation in CBF or cerebrovascular reactivity during sub-maximal exercise and (2) 12 weeks of aerobic-based exercise training would increase CBF and its reactivity at rest and during exercise regardless of age.

Methods

Participants

Twenty-six participants (13 young and 13 older) volunteered for this study which was approved by the University of Otago's Human Ethics Committee and complied with the Declaration of Helsinki. All were inactive, participating in less than 30 min of exercise three times per week for ≥ 2 years. Six participants withdrew from the study during the training intervention for various reasons (too busy with work/university, loss of interest, poor health of family members). Consequently, 20 participants (10 young and 10 older, Table 1) completed the study. Participants were informed of the experimental procedures and potential risks involved in the study before their written consent was obtained. Participants were not taking any cardiovascular medications, all were non-smokers and none had any history of cardiovascular, cerebrovascular or respiratory disease. Participants all had a body mass index (BMI) ≤ 30 kg m⁻², and none had any musculoskeletal problems which prevented them from exercising. All older females were post-menopausal.

Experimental design

Participants reported to the laboratory on four occasions prior to training and twice after training. Before inclusion into the study, all participants were screened by means of a thorough medical history, physical activity history and 12-lead ECG. If any significant ECG abnormality or arrhythmia was detected, participants underwent a clinical transthoracic echocardiogram to exclude overt pathology. Following successful

screening, participants underwent a maximal aerobic power ($\dot{V}_{O_2 \text{ max}}$) test. Participants then underwent the main testing protocol on two separate occasions, the first as a familiarisation and the second for data collection (pre-training). Prior to each session, participants were asked to abstain from alcohol and caffeine in the 12 h prior to testing and avoid a large meal in the 2 h prior to testing. Participants were also asked to arrive well-hydrated by consuming 1 L of water the evening prior to and 0.5 L the morning of testing. On arrival at the laboratory for the main session, participants voided their bladder prior to body composition assessment using bioelectrical impedance (Inbody 230, Biospace, Seoul, Korea). Following procurement of a venous blood sample and instrumentation, participants assumed a seated position for assessment of cerebrovascular reactivity. Following this assessment, participants were transferred to a cycle ergometer (Excalibur Sport, Lode B.V., The Netherlands) where they exercised at 30 % and then 70 % of heart rate range (HRR) whilst breathing room air for 5 min and 5 % CO₂ (in air) for a further 3 min.

Participants then began the 12-week aerobic-based exercise intervention, as detailed below. Following the 12 weeks of training, participants underwent a second $\dot{V}_{O_2 \text{ max}}$ test and then a repeat of the main (pre-training) testing protocol. Relative exercise intensities for the post-training protocol were recalculated from the post-training $\dot{V}_{O_2 \text{ max}}$ test. To control for menstrual cycle phase in the young female participants, pre- and post-training testing was completed in the early follicular phase, when oestrogen and progesterone concentrations are at their lowest. In addition, each participant's post-training testing session was completed at the same time of the day (± 2 h) as their pre-training session.

Exercise training protocol

The 12-week mostly supervised, aerobic-based exercise intervention took place in Dunedin, New Zealand in spring 2008 (1st September–23rd November). At the beginning of the intervention, participants were issued with a recordable heart rate monitor to use during each exercise session (Polar S610i monitors, Polar, Finland) and a physical activity diary. Participants were asked to record information about each exercise session in the

Table 1 Pre- and post-training characteristics of the young and older participants

	Young					Older					
	Pre-training (n=13)	Pre-training (n=10)	Post-training (n=10)	Change (absolute (relative))	Pre-training (n=13)	Pre-training (n=10)	Post-training (n=10)	Change (absolute (relative))	Pre-training (n=13)	Post-training (n=10)	Change (absolute (relative))
Male to female ratio	7:6	5:5	5:5	–	6:7	5:5	5:5	–	6:7	5:5	–
Age (years)	24±5	23±5	23±5	–	64±5	63±5	63±5	–	64±5	63±5	–
Height (cm)	175±9	175±9	175±9	–	164±8*	165±8*	165±8*	–	164±8*	165±8*	–
Body mass (kg)	78.9±13.1	80.1±13.9	79.9±14.3	-0.1±2.5 (-0±4 %)	68.1±8.8*	68.4±9.4*	67.6±9.6*	-0.8±2.4 (-1±4 %)	68.1±8.8*	67.6±9.6*	-0.8±2.4 (-1±4 %)
BMI (kg m ⁻²)	25.7±3.1	26.0±3.0	26.0±3.1	-0.0±0.9 (-0±4 %)	25.2±2.9	25.1±2.9	24.8±3.0	-0.3±0.9 (-1±4 %)	25.2±2.9	24.8±3.0	-0.3±0.9 (-1±4 %)
Muscle mass (kg)	32.9±6.8	33.8±7.2	34.5±8.1	0.7±0.9** (2±3 %)	26.8±4.4*	27.2±4.8*	27.2±4.6*	0.1±0.6** (2±4 %)	26.8±4.4*	27.2±4.6*	0.1±0.6** (2±4 %)
Body fat (%)	26±9	25.4±7.2	24.1±7.1	-1.2±2.4** (-4±10 %)	29±8	28.1±8.4	27.0±8.9	-1.1±2.5** (-5±12 %)	29±8	27.0±8.9	-1.1±2.5** (-5±12 %)
\dot{V}_{O_2} max (L min ⁻¹)	2.62±0.66	2.63±0.71	2.85±0.69	0.22±0.22** (8±8 %)	1.62±0.28*	1.69±0.23*	1.76±0.26*	0.07±0.14** (4±7 %)	1.62±0.28*	1.76±0.26*	0.07±0.14** (4±7 %)
\dot{V}_{O_2} max (mL kg ⁻¹ min ⁻¹)	32.5±6.5	31.9±5.3	35.0±4.8	3.0±3.3** (9±10 %)	23.4±4.7*	24.4±4.1*	25.7±4.1*	1.4±2.0** (5±7 %)	23.4±4.7*	25.7±4.1*	1.4±2.0** (5±7 %)

Values are means ± SD based on 10 or 13 young and 10 or 13 older participants

BMI body mass index, \dot{V}_{O_2} max maximal oxygen consumption

P*<0.05 (different from young); *P*<0.05 (different from pre-training)

diary (mode, duration and intensity). Fourteen supervised exercise sessions were scheduled each week (five circuit gym-based (treadmill, stationary bike, rowing machine, stepper, arm crank, skipping etc.), nine walking/jogging group-based), and participants could attend whichever sessions they wished. Participants were encouraged to attend the supervised scheduled sessions as much as possible; however, some preferred to exercise alone and/or at times that best suited their schedules. All participants followed the same progression in frequency (from three sessions per week (weeks 1–4) to four sessions per week (weeks 9–12)), intensity (from 50–65 % HRR (weeks 1–4) to 65–80 % HRR (weeks 9–12)) and duration (from 20–30 min per session (weeks 1–4) to 40–50 min (weeks 9–12)) of exercise sessions during the intervention. Exercise intensity (in percent of HRR) was determined for each participant from their maximal heart rate (HR) obtained during the initial \dot{V}_{O_2} max test.

Protocols and measurements

Assessment of maximal aerobic power (\dot{V}_{O_2} max)

Participants performed the incremental maximal exercise test on a cycle ergometer (Velotron Elite, RacerMate Inc., Seattle, WA, USA), with starting workloads and increments dependent upon age and sex. Expired O_2 and CO_2 fractions were measured using an online gas analyser (Quark CPET, Cosmed, Rome, Italy), and \dot{V}_{O_2} max was determined from the highest 20-s value.

CBF velocity arterial BP and heart rate Blood flow velocity in the right middle cerebral artery (MCAv) was measured using a 2-MHz pulsed Doppler ultrasound system (DWL Doppler, Sterling VA, USA). Beat-to-beat BP was measured using finger photoplethysmography (Finometer, TPD Biomedical Instruments, The Netherlands). From the blood pressure waveform, HR, stroke volume (SV) and \dot{Q} were calculated using the Modelflow method which incorporates sex, age, height and mass data (BeatScope 1.0 software; TNO-TPD Biomedical Instruments). All data were sampled continuously at 200 Hz using an analogue digital converter (Powerlab/16SP ML795; ADInstruments, Colorado Springs, CO, USA) interfaced with a computer. Data were later analysed using a

commercially available software (Chart version 5.4.2, ADInstruments). Total peripheral resistance (TPR) was estimated as MAP/\dot{Q} . Cerebrovascular resistance was estimated as $MAP/MCAv$.

Respiratory variables During each session, participants breathed through a leak-free respiratory mask (Hans-Rudolph 8980, Kansas City, MO), attached to a one-way valve (Hans-Rudolph 2700) and fitted with a spirometer (Hans-Rudolph bi-directional and linear pneumotachometer, Series 3830, Hans-Rudolph). Minute ventilation (\dot{V}_E) was calculated from the spirometer, and the partial pressures of end-tidal CO_2 and O_2 ($P_{ET}CO_2$ and $P_{ET}O_2$) were sampled from the leak-free mask and measured using a fast responding gas analyser (model CD-3A (CO_2) and model S-3A/I (O_2), AEI Technologies, Pittsburgh, PA). Changes in $P_{ET}CO_2$ adequately reflect those of $PaCO_2$, especially in the hypocapnic range (Thomas et al. 2009), and there is no evidence to indicate that healthy ageing affects this linear relationship between $PaCO_2$ and $P_{ET}CO_2$ (Marsden et al. 2011).

Biochemical variables Prior to and following training, a 10-mL fasted venous blood sample was procured for analysis of lipid profile and glucose concentration. Lipid and glucose samples were analysed in duplicate using photometric analysis (Cobas c111, Roche Diagnostics Ltd., Basel, Switzerland).

Assessment of cerebrovascular CO_2 reactivity

Cerebrovascular reactivity to hypercapnia and hypocapnia was assessed whilst seated at rest. Following baseline recordings (of MCAv and $P_{ET}CO_2$), participants breathed a 5 % CO_2 (in room air) gas mixture for 3 min (hypercapnia), and the change in $P_{ET}CO_2$ from baseline was recorded. Following a recovery period to restore $P_{ET}CO_2$, participants were then hyperventilated for 3 min to reach an equivalent reduction in $P_{ET}CO_2$ (hypocapnia) to match the increase observed with prior hypercapnia. In addition, hypercapnic cerebrovascular reactivity was assessed during steady-state sub-maximal cycling exercise at 30 and 70 % of HRR. Cerebrovascular reactivity was calculated from the data obtained in the final minute of the 3-min intervention and assessed as $\Delta MCAv/\Delta P_{ET}CO_2$. The use of only one level of hypercapnia (5 %) may over- or under-estimate MCAv reactivity to CO_2 because there

is an exponential relationship between the two variables. However, 5 % CO₂ was chosen as the concentration for the CO₂ stimulus because this is the maximum limit tolerated by most people during exercise whilst also wishing to limit the total period of exercise and hypercapnia required of (initially) untrained participants.

Statistical and data analysis

Steady-state resting data were obtained whilst seated following instrumentation and averaged over a 2-min period. A second 2-min baseline period (immediately prior to the assessment of cerebrovascular reactivity) was used for all resting reactivity analysis. Steady-state resting reactivity data were obtained from the average final 60 s of the 3-min hypercapnia or hypocapnia intervention. Reactivity was calculated as both absolute (change in MCAv per millimetre Hg change in P_{ET}CO₂) and relative (change in MCAv (as a percentage of resting MCAv) divided by the change in P_{ET}CO₂). Steady-state baseline data during cycling exercise were averaged over a 60-s period >4 min after the onset of exercise (or the increase in workload). Similar to rest, hypercapnia data were obtained in the final 60 s of the 3-min intervention.

To evaluate the effect of age on pre-exercise resting variables (i.e. purpose 1), one-way ANOVA were used. Effects of age and training on resting variables were assessed by two-factor repeated measures ANOVA. Furthermore, three-factor repeated measures ANOVA were conducted to assess the effect of training (purpose 2) on cerebrovascular responses and cerebrovascular reactivity during exercise (between-subjects factor: age; within-subjects factors: training and exercise). Any significant interactions were isolated using Bonferroni-controlled post hoc *t* tests. All data were analysed using SPSS statistical software (SPSS version 17.0, SPSS Inc., Chicago, IL). Statistical significance was established at an α level of 0.05, and data are expressed as means (\pm SD). In addition, reliability of the MCAv signal was compared from seated baseline measures during the familiarisation and main testing sessions prior to training.

Post-training MCAv at rest was corrected (to account for the lowered post-training P_{ET}CO₂), by multiplying the difference in P_{ET}CO₂ by each individual's post-training hypocapnic cerebrovascular reactivity

and adding this to the MCAv. Coefficient of variation was calculated as the total unbiased error, divided by the grand mean and multiplied by 100 %. Intra-class correlation was also used to determine reliability (Hopkins 2000). Age effects on pre-training variables were analysed using the 26 individuals ($n=13$ in each group) who began the study. Effects of training were analysed using the 20 individuals who completed the training (i.e. $n=10$ in each group). There were no apparent sex-related statistical differences in our main outcome variables; thus, data for males and females were pooled for analysis. Finally, due to many poor Finometer traces during exercise, the blood pressure data are not reported.

Results

Effect of age on resting variables (pre-training data)

Participant characteristics and cardiorespiratory and cerebrovascular variables Despite greater height, body mass and muscle mass in the young ($P<0.05$, Table 1), both age groups had a similar BMI and body fat percentage. As expected, \dot{V}_{O_2} max was lower in the older individuals ($P<0.01$). Apart from an elevated systolic BP (~ 15 %, $P<0.05$; Table 2) in the older participants, all other cardiovascular variables (i.e. HR, MAP, diastolic BP, SV, \dot{Q} and TPR) were not different with age. Mean MCAv, systolic MCAv and diastolic MCAv were lower (~ 27 , ~ 22 and ~ 32 %, respectively; $P<0.01$), and CVR was higher (~ 53 %, $P<0.01$) in the older participants. All respiratory variables were not different with age.

Cerebrovascular reactivity Resting cerebrovascular reactivity (i.e. the change in MCAv (in centimetres per second) per millimetre Hg change in P_{ET}CO₂) to both hypocapnia (1.4 (young) vs. 1.8 cm s⁻¹ mm Hg⁻¹ (older)) and hypercapnia (1.5 (young) vs. 1.2 cm s⁻¹ mm Hg⁻¹ (older), Table 3) was similar between young and older participants (both $P>0.05$, Fig. 2). However, when cerebrovascular reactivity is considered in relative terms (i.e. percent change in MCAv per millimetre Hg change in P_{ET}CO₂), the hypocapnic reactivity was elevated in the older participants (3.9 (older) vs. 2.3 %mm Hg⁻¹ (young), $P<0.01$), whilst hypercapnic reactivity was not (2.5 (older) vs. 2.4 %mm Hg⁻¹ (young), $P=0.55$).

Table 2 Steady-state cardiovascular, cerebrovascular, respiratory and biochemical measures during seated rest in young and older participants prior to and following the 12-week aerobic exercise intervention

	Young				Older			
	Pre-training (n=13)	Pre-training (n=10)	Post-training (n=10)	Change (absolute (relative))	Pre-training (n=13)	Pre-training (n=10)	Post-training (n=10)	Change (absolute (relative))
Cardiovascular								
HR (beats min ⁻¹)	73±8	73±9	66±11	-7±5* (-10±8)	65±15	62±13	59±9	-2±5** (-3±8)
MAP (mm Hg)	83±7	83±8	85±10	1±12 (2±14)	89±13	91±14	93±13	-2±11 (-1±12)
SBP (mm Hg)	120±10	121±10	124±13	3±15 (3±13)	138±24**	141±26	143±22	-8±22 (-3±14)
DBP (mm Hg)	65±7	65±8	65±9	1±10 (2±16)	65±8	66±9	69±9	0±7 (2±11)
SV (mL)	99±22	105±21	105±22	0±11 (1±11)	113±20	112±21	102±16	-10±16 (-8±12)
\dot{Q} (L min ⁻¹)	7.1±1.2	7.4±1.0	6.6±0.8	-0.8±0.8* (-10±10)	7.4±2.3	6.9±1.6	6.1±1.5	-0.8±0.7* (-11±8)
TPR (mm Hg L ⁻¹ min)	12.4±3.1	11.5±2.6	13.4±3.2	1.8±3.0* (18±28)	13.3±3.3	14.0±2.6	15.5±3.2	1.6±1.8* (10±12)
Cerebrovascular								
MCAv (cm s ⁻¹)	67±12	68±8	69±7	1±4 (0±5)	49±11**	50±10	52±13	2±7 (4±14)
SMCAv (cm s ⁻¹)	111±22	116±18	115±14	0±8 (0±6)	87±18**	88±17	91±20	3±12 (3±14)
DMCAv (cm s ⁻¹)	44±8	46±8	45±5	-1±3 (-1±7)	30±7**	31±7	32±9	1±5 (4±18)
CVR (mm Hg cm ⁻¹ s ⁻¹)	1.30±0.27	1.23±0.17	1.25±0.21	0.03±0.19 (3±15)	1.99±0.53**	1.93±0.51	1.89±0.38	-0.11±0.39 (-3±18)
CVc (cm s ⁻¹ mm Hg ⁻¹)	0.81±0.16	0.84±0.14	0.82±0.14	-0.01±0.13 (-1±16)	0.54±0.14	0.56±0.15	0.55±0.12	0.01±0.09 (5±19)
Total oxygenation index (%)	65.9±4.0	66.1±4.2	64.5±4.7	-1.8±3.5 (-3±5)	66.0±5.6	65.3±6.2	67.3±9.7	2.0±8.2 (3±12)
Respiratory								
P _{ET} O ₂ (mm Hg)	111±5	109±4	107±3	-3±5 (-3±5)	111±5	110±6	106±7	-4±10 (-3±9)
P _{ET} CO ₂ (mm Hg)	39±3	39±3	37±4	-2±2* (-4±5)	37±3	38±3	36±3	-1±2* (-3±5)
$\dot{V}_{\dot{E}}$ (L min ⁻¹)	8.2±2.4	8.2±2.2	9.6±2.1	1.4±2.1 (23±36)	6.9±2.2	6.2±2.0	5.9±2.8	-0.5±1.7 (-7±27)
Biochemical								
Total cholesterol (mmol L ⁻¹)	4.1±0.5	4.2±0.5	3.8±0.6	-0.4±0.8 (-8±19)	5.1±0.9	4.9±0.7	4.8±0.6	-0.1±0.5 (-1±7)
HDL cholesterol (mmol L ⁻¹)	1.36±0.24	1.40±0.23	1.34±0.20	-0.07±0.10 (-4±7)	1.41±0.40	1.43±0.44	1.49±0.54	0.07±0.14** (3±9)
LDL cholesterol (mmol L ⁻¹)	2.7±0.5	2.7±0.6	2.4±0.5	-0.3±0.8 (-7±27)	3.7±0.7	3.4±0.4	3.3±0.5	-0.1±0.4 (-4±12)
Triglycerides (mmol L ⁻¹)	1.09±0.41	1.11±0.45	0.93±0.49	-0.18±0.46 (-11±38)	1.32±0.46	1.18±0.23	1.04±0.32	-0.14±0.30 (-11±24)
Glucose (mmol L ⁻¹)	4.9±0.3	4.9±0.3	4.6±0.3*	-0.2±0.4 (-4±9)	5.1±0.5	5.2±0.5	4.9±0.5*	-0.2±0.3 (-4±6)

Values are means ± SD from 13 or 10 young and 13 or 10 older participants

HR heart rate, MAP mean arterial blood pressure, SBP systolic blood pressure, DBP diastolic blood pressure, SV stroke volume, \dot{Q} cardiac output, TPR total peripheral resistance, MCAv middle cerebral artery blood flow velocity, SMCAv systolic MCAv, DMCAv diastolic MCAv, CVR cerebrovascular resistance, CVc cerebrovascular conductance, P_{ET}O₂ partial pressure of end-tidal oxygen, P_{ET}CO₂ partial pressure of end-tidal carbon dioxide, $\dot{V}_{\dot{E}}$ minute ventilation, HDL high-density lipoprotein, LDL low-density lipoprotein

*P<0.05 (different from pre-training); **P<0.05 (different from young)

Table 3 Pre-training cerebrovascular reactivities and ventilatory sensitivities

	Young	Older
Hypocapnic reactivity (rest) ($\text{cm s}^{-1} \text{ mm Hg}^{-1}$)	1.43±0.53	1.79±0.74
Hypercapnic reactivity ($\text{cm s}^{-1} \text{ mm Hg}^{-1}$)		
- Rest	1.46±0.63	1.20±0.71
- 30 % HRR	2.73±0.98	2.09±0.67
- 70 % HRR	2.63±0.60	2.46±0.99
Full reactivity (rest) ($\text{cm s}^{-1} \text{ mm Hg}^{-1}$)	1.52±0.35	1.51±0.41
Ventilatory sensitivity ($\text{L min}^{-1} \text{ mm Hg}^{-1}$)	0.91±0.51	0.63±0.52

Values are means ± SD from 13 young and 13 older participants

HRR heart rate range

Ventilatory sensitivity to hypercapnia tended to be higher in the young participants at rest ($P=0.07$); however, the variability in this group was twice that of the older individuals.

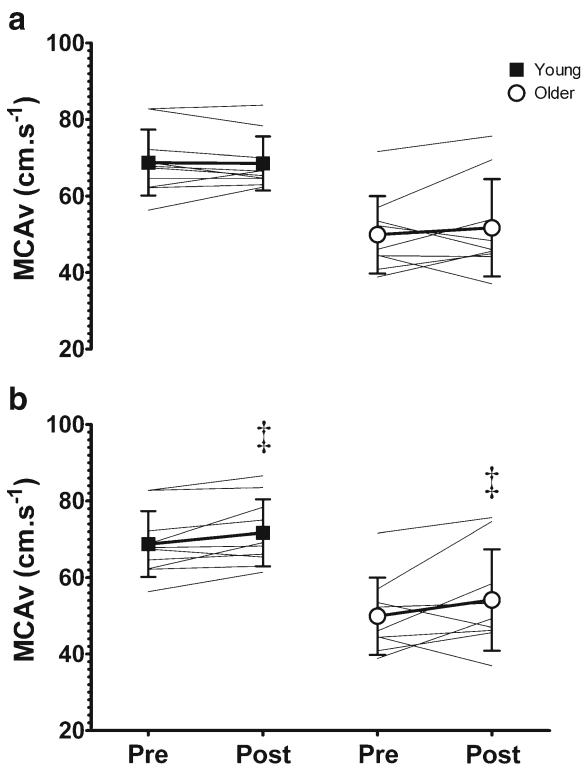


Fig. 1 Absolute (a) and corrected (for $P_{\text{ET}}\text{CO}_2$, ± SD) (b) middle cerebral artery blood flow velocity (MCAV) at rest, prior to (Pre) and following 12 weeks of aerobic-based exercise training (Post) in up to 10 young and 10 older individuals. ‡ $P<0.05$, different from pre-training

Effect of age on responses to acute exercise (pre-training data)

Cardiorespiratory and cerebrovascular variables MCAV was elevated above resting values during sub-maximal cycling exercise at 30 and 70 % of HRR in both young and older participants ($P<0.01$). The younger individuals exhibited a greater absolute increase in MCAV during exercise (7 and 9 cm s^{-1} (30 and 70 % (uncorrected for exercise-induced reductions in $P_{\text{ET}}\text{CO}_2$) HRR, respectively) vs. 2 and 2 cm s^{-1} , respectively (older); $P=0.02$); however, when expressed as a relative change, there was no reliable difference between age groups (11 and 14 % (30 and 70 % HRR, respectively; young) vs. 5 and 4 %, respectively (older); $P=0.21$). Mean $P_{\text{ET}}\text{CO}_2$ was also elevated with exercise (~2–3 mm Hg, $P<0.01$), in both young and older participants. As expected, greater absolute increases in HR were observed in the young ($P<0.01$), and the absolute \dot{V}_E response to exercise tended to be higher in the young ($P=0.07$). Cerebral TOI was unchanged during exercise ($P>0.05$).

Cerebrovascular reactivity Hypercapnic cerebrovascular reactivity was increased during exercise at both 30 and 70 % of HRR ($P<0.01$), independent of age. Both groups exhibited approximately twofold increases in the MCAV response to elevations in $P_{\text{ET}}\text{CO}_2$ at 70 % HRR (1.5 (rest)–2.6 $\text{cm s}^{-1} \text{ mm Hg}^{-1}$ (70 %, young) and 1.2–2.5 $\text{cm s}^{-1} \text{ mm Hg}^{-1}$ (70 %, older), Table 3). Ventilatory sensitivity to hypercapnia was also elevated above resting levels during exercise ($P<0.01$).

Effect of training on resting variables

Training Of the 26 participants who started the training programme, 20 (77 %; 10 young, 10 older) completed the entire 12-week training intervention. Although body mass and BMI were unchanged ($P>0.05$, Table 1), body composition was altered favourably, with an increase in muscle mass (~2 %) and reduction in body fat (~5 %, both $P<0.05$). In addition, $\dot{V}_{O_2 \text{ max}}$, a measure of cardiorespiratory fitness, was increased by ~6 % ($P<0.05$). All training-induced anthropometric changes were comparable across age. Fasted plasma glucose was lowered across training ($P<0.05$, Table 2), but lipid variables were unchanged ($P>0.05$).

Cardiorespiratory and cerebrovascular variables On initial assessment, resting MCAv was unchanged with training ($P>0.05$, Table 2); however, independent of changes in alveolar ventilation, measured $P_{ET}CO_2$ was lowered post-training ($P<0.05$). When this effect of resting hypocapnia on MCAv was corrected for (see ‘Methods’), MCAv was elevated following training ($P=0.02$, Fig. 1). This elevation was apparent in 17/20 participants and averaged $4\pm 6 \text{ cm s}^{-1}$ (range, -8 to $+18 \text{ cm s}^{-1}$). Resting HR was reduced following training; however, this reduction was greater in the young ($P<0.05$).

Effect of training on cerebrovascular reactivity at rest Resting hypercapnic cerebrovascular reactivity was elevated following training in both young and older individuals (combined, 1.9 ± 0.7 vs. $1.4\pm 0.6 \text{ cm s}^{-1} \text{ mm Hg}^{-1}$ (pre-training); $P=0.01$; Fig. 2), whereas hypocapnic cerebrovascular reactivity was unchanged ($P>0.05$). Furthermore, ventilatory sensitivity to hypercapnia was unchanged ($P>0.05$).

Effect of training on responses to acute exercise

Absolute workrates were increased by 28 % and 31 % for younger and older participants respectively, following training (i.e., to attain the same proportion of HRR).

Cardiorespiratory and cerebrovascular variables Similar to pre-training, the young individuals exhibited a greater absolute increase in MCAv in response to exercise (9 and 9 cm s^{-1} (30 and 70 % HRR,

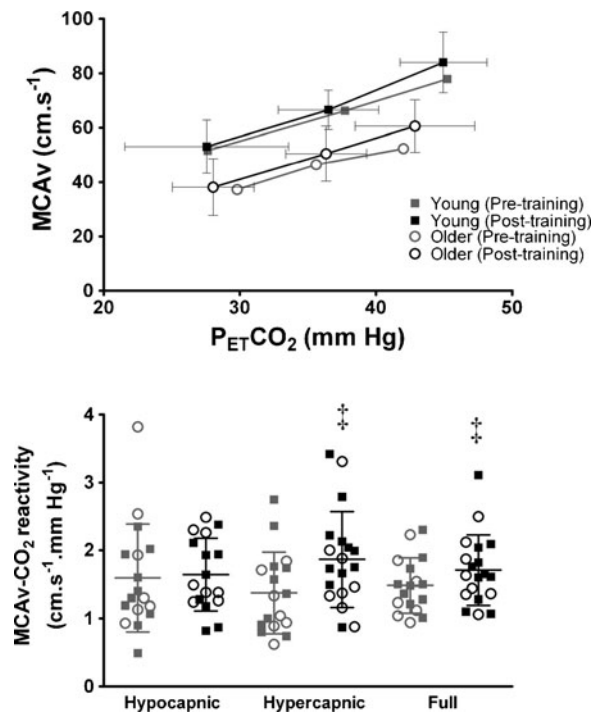


Fig. 2 Absolute (\pm SD) changes in middle cerebral artery blood flow velocity (MCAv, *top*) during normocapnia, hypercapnia (5 % CO₂) and hyperventilation-induced hypocapnia (P_{ET}CO₂) at rest, prior to (*grey*) and following (*black*) 12 weeks of aerobic-based exercise training in up to 10 young and 10 older individuals and the corresponding calculated cerebrovascular CO₂ reactivity (MCAv-CO₂ reactivity, *bottom*). ‡ $P<0.05$, different from pre-training

respectively) vs. 5 and 4 cm s^{-1} , respectively (older); age \times exercise interaction, $P<0.05$; Fig. 3). Training itself had no effect on the MCAv response to exercise in either age group ($P>0.05$). The \dot{V}_E and P_{ET}CO₂ responses to exercise were different following training (training \times exercise interactions, $P<0.05$), with an increased \dot{V}_E at 70 % HRR ($+14 \text{ L min}^{-1}$ vs. pre-training, $P<0.05$) and reduced P_{ET}CO₂ at 30 and 70 % HRR (-4 mm Hg vs. pre-training (at 70 % HRR), $P<0.05$). When MCAv was corrected for the exercise-induced hypocapnia at 70 % HRR, the measured MCAv was significantly lower than the corrected value ($P<0.05$); however, this did not differ with age. Like pre-training, the HR response to exercise was lower in the older individuals ($P<0.05$). In addition, training resulted in an elevated HR response at 70 % HRR ($+5 \text{ beats min}^{-1}$ vs. pre-training, $P<0.05$).

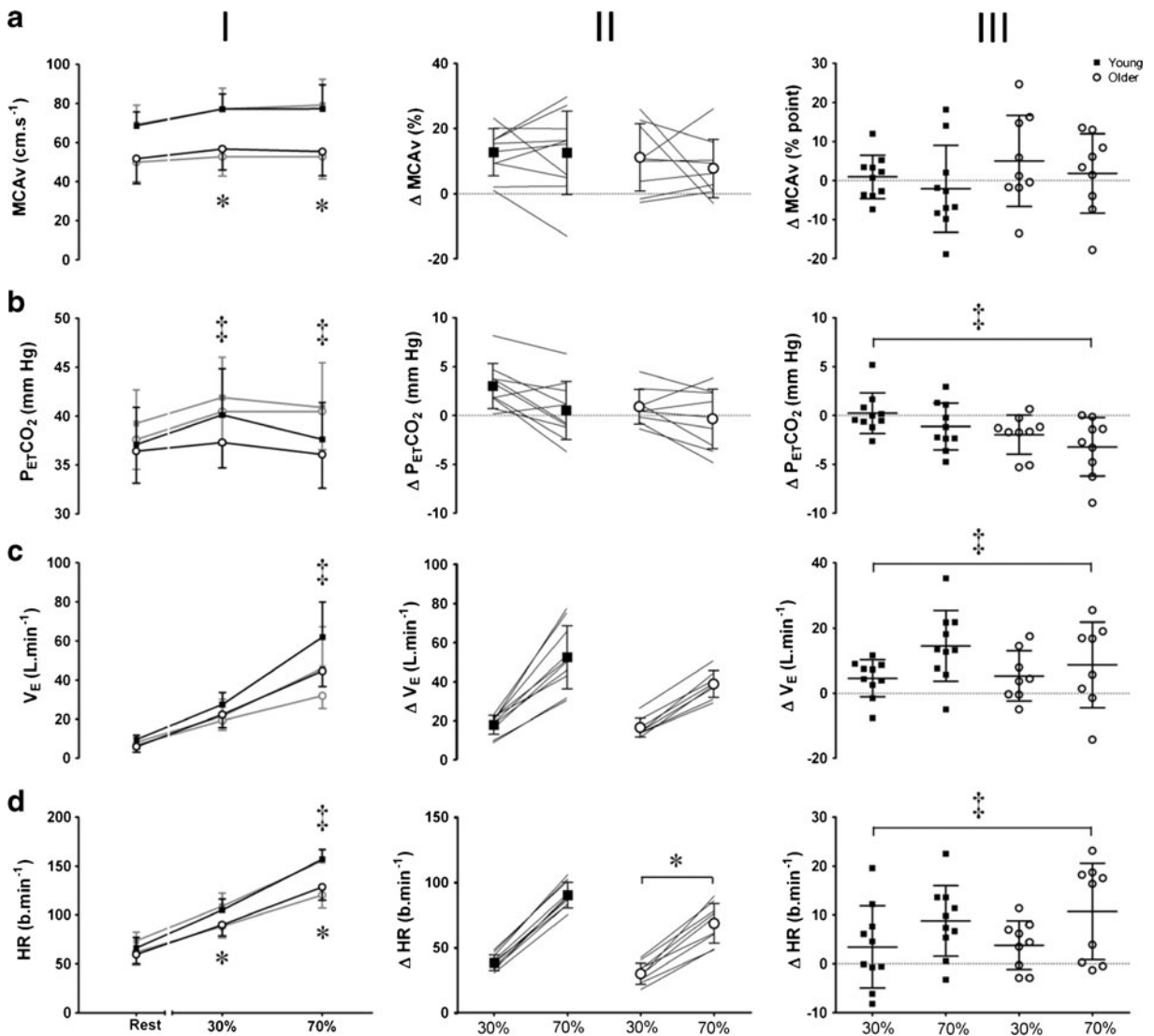


Fig. 3 Middle cerebral artery blood flow velocity (MCAv) (a), partial pressure of end-tidal CO₂ (P_{ET}CO₂) (b), minute ventilation (V_E) (c) and heart rate (HR) (d) response (I). Absolute or relative change from rest (II) to sub-maximal cycling exercise at 30 and 70 % of HRR in young and older individuals prior to and following a 12-week exercise training intervention. In (I), pre-

training values are depicted in grey and post-training in black. Furthermore, change score differences (i.e. absolute value or percentage point change) in response to exercise between pre- and post-training are displayed in the right column (III). **P*< 0.05 different from young; ‡*P*<0.05, different from pre-training

Cerebrovascular reactivity Similar to pre-training values, cerebrovascular reactivity to hypercapnia was increased during sub-maximal exercise (*P*<0.05), with a similar response across age. Similar to resting hypercapnic reactivity, the 12 weeks of training increased reactivity responses during the sub-maximal exercise in both groups (by 0.3–0.6 cm s⁻¹ mm Hg⁻¹, *P*=0.03 vs. pre-training values; Fig. 4). Although

ventilatory sensitivity was elevated during exercise, the response did not differ to pre-training (*P*>0.05).

Discussion

This is the first known study to assess the effect of age and training on MCAv and cerebrovascular CO₂

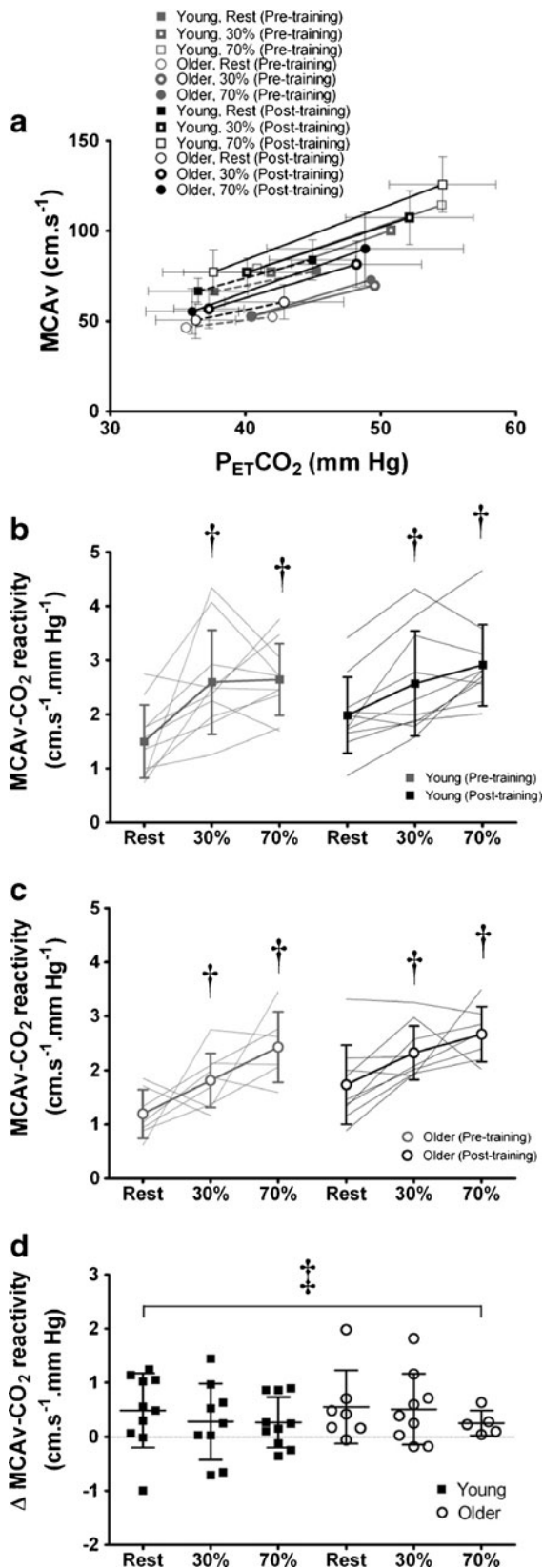


Fig. 4 Absolute changes in middle cerebral artery blood flow velocity (MCAv) (a) during normocapnia, hypercapnia (5 % CO₂) and hyperventilation-induced hypocapnia (P_{ET}CO₂) at rest and during cycling exercise at 30 and 70 % HRR, prior to (grey) and following (black) 12 weeks of aerobic based exercise training in up to 10 young and 10 older individuals. Corresponding calculated cerebrovascular CO₂ reactivity (MCAv-CO₂ reactivities in young (b) and older (c) individuals) and individual change scores between pre- and post-training (d). †P<0.05, different from rest; ‡P<0.05, different from pre-training

reactivity both at rest and during exercise. The major finding from this study was that following a 12-week aerobic-based exercise training intervention, we observed an elevation in the cerebrovascular reactivity to hypercapnia, both at rest and during acute exercise. Of note, this fitness-induced elevation in cerebrovascular reactivity occurred independently of age and was possibly associated with increased MCAv at rest. These findings support the use of exercise as a therapeutic tool in the prevention of cerebrovascular disease.

Influence of age on cerebral blood flow and cerebrovascular reactivity at rest and during exercise

Older individuals have been reported to have a maintained (Davis et al. 1983; Ito et al. 2002; Kastrup et al. 1998; Schwertfeger et al. 2006; Galvin et al. 2010) and reduced (Reich and Rusinek 1989; Yamamoto et al. 1980) hypercapnic cerebrovascular reactivity compared to younger individuals. The reasons for the discrepancy are likely to be multi-factorial and may include differences in CBF measurement technique (i.e. transcranial Doppler (TCD), Xe inhalation and positron emission tomography), the extent of the CO₂ stimulus and whether incorporated into a room air or hyperoxic solution, the age ranges used to describe ‘older’, and posture (Galvin et al. 2010). Data from this study are in support of a maintained cerebrovascular reactivity to hypercapnia in older age. Indeed, Kastrup et al. (1998), who used TCD to assess changes in MCAv to 5 % CO₂ (a similar protocol to this study), observed no effect of age on hypercapnic reactivity in men. Interestingly, they did observe an elevated reactivity to CO₂ in pre-menopausal women, but not in post-menopausal women. That finding indicates a potential effect of oestrogens on cerebrovascular reactivity to CO₂ (Kastrup et al. 1998). In contrast, we observed no effect of sex on cerebrovascular reactivity; however, all older females were post-menopausal

and all young females were tested in the low oestrogen (early follicular) phase of the menstrual cycle or during the ‘sugar-pill’ week if using oral contraceptives (when oestrogen was low).

The effect of age on hypocapnic cerebrovascular reactivity is also controversial, with reports of an unchanged (Ito et al. 2002), reduced (Yamaguchi et al. 1979; Tsuda and Hartmann 1989) and even increased (Galvin et al. 2010) reactivity in older age. In the current study, absolute cerebrovascular reactivity to hypocapnia was comparable whereas relative cerebrovascular hypocapnic reactivity was actually *higher* with older age. However, because of the lower absolute MCAv in the older participants, any change in MCAv to hypocapnia will manifest in a disproportionately larger percent change in the older compared to the young. Thus, it seems that there is a little effect of age on global cerebrovascular reactivity.

MCAv was elevated ~5–15 % in response to sub-maximal exercise in both young and older individuals. This magnitude of increase is similar to that observed by other authors using transcranial Doppler to assess blood flow velocity in the MCA during exercise (Hellstrom et al. 1996; Jorgensen et al. 1992; Linkis et al. 1995; Moraine et al. 1993; Ogoh et al. 2005). However, MCAv at 70 % HRR was similar or slightly reduced compared with that observed at 30 % HRR. This is in accordance with previous reports that the increase in MCAv is intensity dependent up until ~60 % of \dot{V}_{O_2} max, after which it declines back towards resting levels due to hyperventilation-induced hypocapnia (Hellstrom et al. 1996; Moraine et al. 1993). Below ventilatory threshold, the increase in CBF with exercise is likely driven by increases in cerebral metabolism and PaCO₂, as a result of increased functional activation with motor activity (Hellstrom et al. 1996; Moraine et al. 1993); however, above this, the large disproportional increase in ventilation reduces PaCO₂ and thus CBF despite progressive elevations in cerebral metabolism (Hellstrom et al. 1996; Moraine et al. 1993).

When expressed in absolute terms, the younger participants exhibited a greater increase (9 vs. 2 cm s⁻¹ (older)) in MCAv during sub-maximal exercise. When expressed as a percent change from rest (14 % (young) vs. 4 % (older), at 70 % HRR), the age effect was not statistically significant ($P=0.21$); however, this likely represents a lack of statistical power. Fisher et al. (2008) and Heckmann et al. (2003) have

both assessed the effect of age on the MCAv response to sub-maximal exercise, and both found no differential effect of age. Surprisingly however, in both of these studies, there was no difference in resting MCAv with age, making comparison with this study difficult. Fisher et al. (2008) compared the MCAv response to 30 and 50 % HRR. Interestingly, although not significant, the absolute change in MCAv during exercise at 50 % was lower in their older individuals (~7 % increase from rest in the young vs. ~2 % in the older individuals), similar to our observations at 70 % HRR. In opposition to these findings by Fisher et al. and Heckmann et al., a recent study (Marsden et al. 2011) demonstrated similar findings to ours of a lesser increase in MCAv in older individuals in comparison to the young (15 vs. 28 %) during sub-maximal intensities. It seems that younger individuals with an already higher CBF have a greater absolute capacity to increase CBF. Whether the greater exercise-induced increase in MCAv in the young individuals is merely a function of a higher starting point or is due to the higher absolute workload required in the young to elicit the same percentage heart rates (thereby resulting in an increased neural activation and consequently metabolism) is unknown. It is not attributable to a larger exercise-induced rise in the perfusion pressure (MAP). Overall, the effect of age on the cerebrovascular response to exercise (both sub-maximal and maximal) requires further investigation.

An increase in hypercapnic cerebrovascular reactivity during exercise, as observed in this study, is similar to previous reports (Ogoh et al. 2008; Rasmussen et al. 2006; Ogoh et al. 2009). The exact mechanisms responsible for the increased reactivity are unknown; however, increases in cerebral activation, core temperature (Rasmussen et al. 2006) and CO₂-induced increases in MAP (Ainslie et al. 2005) have been suggested. Similar to the findings from this study, Rasmussen et al. (2006) observed an approximately twofold increase in hypercapnic reactivity during exercise at 67 % \dot{V}_{O_2} max. The exercise-induced elevations in reactivity observed by Ogoh et al. (2008) were substantially less, at only 15–20 %, despite similar CBF measurement technique and CO₂ stimulus to that in the present study. However, in that study, participants were only exercising at ~32 % \dot{V}_{O_2} max, potentially indicating a relationship with exercise intensity. Although data from our study support that of Rasmussen et al. (2006) with regards to the extent of

the MCAv response to changes in CO₂, we did not observe further elevations in hypercapnic reactivity with increasing exercise intensity. Overall, in this study we observed that the increase in hypercapnic reactivity occurred independent of age, indicating a maintained control of CBF during exercise with healthy ageing.

Effect of training on MCAv

Measured resting MCAv was unchanged following the 12 weeks of training. However, training was associated with a lowered (measured) resting P_{ET}CO₂. The reason for the lower P_{ET}CO₂ following training is unknown; however, one group has found that the act of imagining dynamic exercise increased ventilation in athletes compared to non-athletes (Wuyam et al. 1995), potentially accounting for the 2-mm Hg reduction in P_{ET}CO₂ following the training intervention, as participants were aware that they were about to perform exercise. When MCAv was corrected for the post-training alterations in P_{ET}CO₂ (using the post-training hypocapnic reactivity), resting MCAv was significantly elevated following 12 weeks of aerobic-based exercise training. Fitness-induced elevations in MCAv have been observed in a cross-sectional cohort (Ainslie et al. 2008); however, this has not yet been demonstrated in a longitudinal study, and the present data provide some support for such an effect, but not clear support. It may be that the small sample size in this study was insufficient to accurately assess the effect of exercise training on MCAv.

Effect of training on resting variables

The 12 weeks of exercise training was associated with the expected reductions in body fat percentage and increases in muscle mass and maximal oxygen consumption. Although training had no effect on MAP, resting HR was reduced in both groups. A lowered resting HR is a common feature of exercise training (Thompson et al. 2001; Boutcher and Stein 1995) and may indicate an increased vagal outflow.

Cerebrovascular reactivity at rest

Cerebrovascular reactivity to hypercapnia was elevated, by 10–15 %, following the 12-week training intervention and without the confounding effects that were evident for the MCAv response. This elevation also

occurred independently of age, indicating that healthy ageing does not affect the ability of the cerebrovascular bed to adapt to exercise training. This is only the second known study to report on the effect of fitness on cerebrovascular reactivity in humans. Ivey et al. (2011) demonstrated a ~30 % elevation in cerebrovascular reactivity to 6 % CO₂ in stroke survivors following 6 months of exercise training. Interestingly, the elevation in fitness across the training intervention was positively correlated with the change in cerebrovascular reactivity, indicating that the increased aerobic capacity is at least, in part, responsible for the improved cerebrovascular reactivity. Swain et al. (2003) used MRI in rats to assess the cerebral responses to 10 % CO₂ prior to and following at least 30 days of voluntary exercise or sedentary living. They found a 22 % increase in signal intensity during CO₂ inhalation (when compared to pre-training) in the exercise group, indicating a more pronounced CBF response to hypercapnia, which supports the findings from this study and is independent of inferring an increased perfusion based on velocity measurements. Whether similar differences in cerebrovascular reactivity are observed in a cross-sectional cohort requires further investigation. An impaired cerebrovascular reactivity has been identified as a risk factor for stroke [4–6]. In addition, an elevated cardiorespiratory fitness has been associated with a reduced risk of stroke (Endres et al. 2003; Gertz et al. 2006; Lee and Paffenbarger 1998; Hooker et al. 2008). This fitness-induced elevation in cerebrovascular reactivity may provide an important physiological link in the relationship between fitness and cerebrovascular disease.

The mechanisms responsible for this increase in hypercapnic cerebrovascular reactivity following chronic exercise training are not well understood but likely involve NO. Cerebrovascular vasodilatation in response to hypercapnia is, in part, dependent upon NO bioavailability (Peebles et al. 2007). Nitric oxide has been reported to increase with exercise training (Green et al. 2004; Kingwell et al. 1997). Exercise training has also been shown to increase systemic endothelium-dependent vasodilatation (a NO-mediated response) (Green et al. 2004; Clarkson et al. 1999; Tinken et al. 2008). Furthermore, a link between the systemic endothelial function and cerebrovascular reactivity has been established (Ainslie et al. 2007; Lavi et al. 2006), indicating that any improvements in systemic vascular function might also be

reflected in the cerebral circulation (as now demonstrated in this study). In addition, the reduced stroke risk and improved long-term outcome as a result of physical activity have been postulated to be due to upregulation of endothelial NO synthase (Endres et al. 2003; Gertz et al. 2006). Additional mechanisms potentially contributing to this increased cerebrovascular reactivity include brain-derived neurotrophic factor (Seifert et al. 2010) and insulin-like growth factor-1 (Llorens-Martin et al. 2010). Furthermore, hypocapnic cerebrovascular reactivity—a response which is *not* NO mediated (Peebles et al. 2007)—was unchanged following training, further supporting the suggestion that the training-induced elevation in hypercapnic reactivity is mediated by NO. Although the role of NO is a possibility based on this information, we acknowledge that this suggestion is purely speculative because we did not measure systemic or cerebrovascular NO.

Cerebral blood flow and its reactivity during exercise

Despite some indication of an elevated resting MCAv following exercise training, the MCAv response to exercise was unchanged, showing a 10–15 % increase. $P_{ET}CO_2$ was elevated during exercise but was lower at both 30 and 70 % HRR (post-training) when compared with pre-training; however, this was also accompanied by a reduced resting $P_{ET}CO_2$. Nevertheless, the overall observation is a relatively unchanged cerebrovascular response to sub-maximal exercise following training; thus, it seems that the control of CBF during exercise is maintained or even elevated with both increasing age and fitness.

Similar to pre-training, cerebrovascular reactivity was increased during sub-maximal exercise in both young and older individuals, indicating that the improved cerebrovascular control at rest is also apparent during exercise. This finding seems likely since the mechanisms responsible for the increased cerebrovascular reactivity at rest (i.e. potentially NO) following training should still be apparent during exercise. Although the mechanisms are unknown, it may reflect training/exercise-induced alterations in cerebral metabolism, which apart from $PaCO_2$ is the most potent regulator of CBF. Whilst our findings are encouraging, we acknowledge that the extent of adaptation in reactivity across this 12-week intervention is small and may have a limited physiological or clinical relevance. Nevertheless, the findings were evident despite the

improvements in aerobic fitness also being modest (6 % mean increase in \dot{V}_{O_2} max) in this training study. Whether longer term or more intensive exercise might help improve CBF by the extent seen cross-sectionally for aerobically fit young (~12 %, ~10 cm s⁻¹) and older (~20 %, ~10 cm s⁻¹) adults remains unknown. Future studies might also quantify the ‘time course’ of such changes in MCAv and reactivity, as well as concomitant changes in autoregulation during a prolonged training intervention.

Technical considerations

Although resting MCAv was unchanged following training, when it was corrected for the post-training hypocapnia, a training-induced elevation in MCAv was observed. This was only slightly greater (7 %) than our calculated day-to-day reproducibility of the MCAv technique (5.4 % older, 6.1 % young); therefore, the observed 7 % higher MCAv post-training may be due, in part, to day-to-day variation. We attempted to reduce this error as much as possible by ensuring that the MCAv signal was obtained by the same experimenter throughout the study. Although the operator was aware of the depth and gain of the Doppler settings from the baseline (pre-training) testing session, they were not aware of the pre-training MCAv value during the post-training testing session. The reasons for this reduction in $P_{ET}CO_2$ following training are unclear. Alveolar ventilation (and its components, including chemoreflex control) was not measurably altered following training. Methodological issues might explain this reduction, yet this seems unlikely as the same gas analysers were used throughout the training study and were always calibrated with a 5 % (± 0.2 %) CO_2 gas mixture. It therefore seems most likely that this reduction in $P_{ET}CO_2$ represented a normal day-to-day variation. Overall, further assessment of the effect of longitudinal exercise training on MCAv is warranted in a larger study population.

Another potential limitation of this study is the absence of a control group. The inclusion of a control group could have been helpful in determining the reason for the post-training reduction in $P_{ET}CO_2$, whether due to a training effect or some other reason applicable to both groups (e.g. measurement error or seasonal effects). Nevertheless, it was felt that by ensuring participants were fully familiarised to the testing protocol before the main data collection period,

we would be able to confidently assume that any differences following 12 weeks of training would be primarily a result of the training. In addition, all measures were deemed to be relatively insensitive to any placebo/expectation effects of having knowingly participated in a training study. All measures were reflective of actual physiological, rather than perceptual stress. Finally, calibration errors were considered improbable because the researchers were experienced in these procedures and the reactivity testing and calibrations used multiple gas cylinders both before and after the training intervention.

In summary, in addition to showing that age had no effect on cerebrovascular reactivity at rest or during exercise, we have revealed that cerebrovascular reactivity to hypercapnia (and possibly also resting MCAv) was increased following 12 weeks of aerobic-based exercise training in both young and older individuals. This finding is important as it (1) indicates that healthy older age does not compromise cerebrovascular function at rest or during sub-maximal exercise and (2) provides insight into the relationship between fitness and cerebrovascular disease. This is the first study to demonstrate this significant finding and provides important supporting evidence for the role of aerobic exercise in the improved control of CBF and in the prevention of cerebrovascular disease.

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