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Muscle metaboreflex and cerebral blood flow regulation in humans: implications for exercise with blood flow restriction

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ABSTRACT

We investigated the effect of activating metabolically sensitive skeletal muscle afferents (muscle metaboreflex) on cerebral blood flow and the potentially confounding influence of concomitant changes in the partial pressure of arterial carbon dioxide. Eleven healthy males (25±4 years) performed submaximal leg cycling exercise on a semi-recumbent cycle ergometer (heart rate ~120 b·min^{-1}), and assessments made of the partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$), internal carotid artery blood flow (ICA_Q) and conductance (ICA_{CVC}), middle cerebral artery mean blood velocity (MCA_{Vm}) and conductance index (MCA_{CVCi}).The muscle metaboreflex was activated during cycling with leg blood flow restriction (BFR) or isolated with post exercise ischemia (PEI). In separate trials, $P_{ET}CO_2$ was either permitted to fluctuate spontaneously (control trial) or was clamped at 1 mmHg above resting levels ($P_{ET}CO_2$ clamp trial). In the control trial, leg cycling with BFR decreased $P_{ET}CO_2$ (Δ-4.8±0.9 mmHg vs. leg cycling exercise) secondary to hyperventilation, while ICA_Q, ICA_{CVC}, and MCA_{Vm} were unchanged, and MCA_{CVCi} decreased. However, in the $P_{ET}CO_2$ clamp trial, leg cycling with BFR increased both MCA_{Vm} (Δ5.9±1.4 cm·s^{-1}) and ICA_Q (Δ20.0±7.8 ml·min^{-1}), and attenuated the decrease in MCA_{CVCi}, while ICA_{CVC} was unchanged. In the control trial, PEI decreased $P_{ET}CO_2$ (Δ-7.0±1.3 mmHg vs. rest), MCA_{Vm} and MCA_{CVCi}, whereas ICA_Q and ICA_{CVC} were unchanged. In contrast, in the $P_{ET}CO_2$ clamp trial both ICA_Q (Δ18.5±11.9 ml·min^{-1}) and MCA_{Vm} (Δ8.8±2.0 cm·s^{-1}) were elevated, while ICA_{CVC} and MCA_{CVCi} were unchanged. In conclusion, when hyperventilation-related decreases in $P_{ET}CO_2$ are prevented the activation of metabolically sensitive skeletal muscle afferent fibres increases cerebral blood flow.

New & Noteworthy: Muscle metaboreflex activation increases cerebral blood flow, but only when hyperventilation mediated reductions in the partial pressure of end-tidal carbon dioxide are prevented. These findings may have implications for individuals practicing exercise training with blood flow restriction and patient populations in whom exaggerated muscle metaboreflex sensitivity has been identified.

Key words: cerebrovascular circulation; exercise; metabolic activation

INTRODUCTION

Increases in cerebral blood flow during exercise are associated with upsurges in brain activation and metabolism within regions such as the motor-sensory cortex and supplementary motor area (23). Several other interacting mechanisms also contribute to the cerebral circulatory response accompanying exercise, including chemical, hemodynamic, autoregulatory and neural factors (42). The stimulation of group III and IV skeletal muscle afferents has also been implicated in the cerebral blood flow responses to exercise, but this remains incompletely understood (19, 20, 27). Group III and IV skeletal muscle afferents are responsive to metabolic (muscle metaboreflex) and mechanical (muscle mechanoreflex) perturbation. Alongside central command (feedforward signals from higher brain centres) and the arterial and cardiopulmonary baroreceptors, group III and IV skeletal muscle afferents play a key role in mediating the cardiovascular adjustments to exercise (18). Early studies established two approaches for the assessment of the muscle metaboreflex (3, 4, 52). The first approach involved blood flow restriction (BFR) to the exercising muscles using proximally placed inflatable occlusion cuffs, in order to create a mismatch between oxygen delivery and demand. This in turn evoked an accelerated accumulation of exercise-induced metabolites and enhanced activation of the metabolically sensitive skeletal muscle afferents. The second approach involved the complete circulatory arrest of the exercising skeletal muscle continuing into the recovery period while the muscle is quiescent (i.e., post-exercise ischemia; PEI). In this manner, metabolically sensitive skeletal muscle afferents may be activated in isolation by the trapping of exercise-induced metabolites within the muscle.

During the isolated activation of the muscle metaboreflex with PEI following handgrip, exercise-induced increases in middle cerebral artery (MCA) mean blood velocity ($_{Vm}$) are not sustained and MCA_{Vm} returns to baseline (28, 46). This is in conflict with reports that the use of local anaesthesia to block sensory feedback from group III and IV skeletal muscle afferent fibres abolished the normal increase of MCA_{Vm} during static and dynamic handgrip (19, 20, 27). We recently observed that such contradictory reports may be attributable to muscle metaboreflex mediated increases in ventilation during PEI, which lead to a confounding reduction in the partial pressure of arterial carbon dioxide (P_aCO_2; indexed by the partial pressure of end-tidal carbon dioxide; $P_{ET}CO_2$) and a cerebral

vasoconstriction that prevents a muscle metaboreflex mediated increase in MCA_{Vm}. Indeed, the clamping of $P_{ET}CO_2$ at baseline values during PEI following fatiguing static handgrip resulted in an elevation in MCA_{Vm} (13).

The aforementioned studies possess limitations with regards to the assessment of cerebral blood flow and mode of muscle metaboreflex activation that we seek to address in the present investigation. First, for transcranial Doppler ultrasound measures of MCA_{Vm} to be representative of cerebral blood flow it must be assumed that MCA diameter remains constant. To circumvent this issue, direct measures of internal carotid artery (ICA) diameter ($_d$), velocity ($_{Vm}$) and thus ICA blood flow (ICA_Q) can be employed, but to date the contribution of skeletal muscle afferents to the ICA_Q responses to exercise remain unknown. Second, while the cerebral circulatory responses to muscle metaboreflex activation following exercise with PEI have been investigated, the responses to the enhanced muscle metaboreflex activation during exercise with BFR have not been considered. During PEI the muscle metaboreflex is activated in isolation from central command and skeletal muscle mechanoreflex (11, 16, 17). However, under clinical conditions such as peripheral vascular disease and chronic heart failure, where there is a hypoperfusion of the skeletal muscles, the muscle metaboreflex is not activated in isolation (7, 9, 24). Increasing metaboreflex signalling during exercise with BFR at the same time that central command and mechanoreflex are also activated would potentially provide a more realistic simulation of this paradigm. Furthermore, exercise with BFR is becoming an increasingly popular athletic training practice due to the potential for gains in muscle strength and endurance to occur without high-intensity training (54, 60). Spranger et al. (57) recently raised a 'call for concern' regarding the practice of exercise with BFR on the basis of the exaggerated increases in blood pressure and the associated risk of cardiovascular and cerebrovascular insult. At present the effects of BFR exercise on cerebral blood flow are unknown.

Given this background, we sought to determine; 1) the influence of the muscle metaboreflex activation on cerebral blood flow, 2) if changes in $P_{ET}CO_2$ are a key determinant of the cerebral blood flow response to muscle metaboreflex activation, and 3) whether the mode of muscle metaboreflex activation (i.e., during vs. following exercise) influences the corresponding cerebral blood flow response. To achieve this MCA_{Vm} and ICA_Q were measured during exercise with BFR to enhance

muscle metaboreflex activation and during isolated activation of the muscle metaboreflex with PEI. Trials were conducted where $P_{ET}CO_2$ was permitted to fluctuate spontaneously and where $P_{ET}CO_2$ was clamped at baseline values. We hypothesized that muscle metaboreflex activation would evoke an increase in MCA_{Vm} and ICA_Q only when $P_{ET}CO_2$ was clamped at baseline.

111 METHODS

112 The study was approved by the Health, Safety and Ethics Committee of the School of Sport,
113 Exercise and Rehabilitation Science at the University of Birmingham and was undertaken according to
114 Declaration of Helsinki. Eleven male participants were recruited (age 25±4 years; height 180±1 cm;
115 weight 71±7 kg; mean±SD). After receiving a detailed verbal and written explanation of the
116 experimental protocol, all participants signed the consent form. All participants were free of any
117 cardiovascular, respiratory, neurological, renal or metabolic diseases and were not using any
118 prescription or over-the-counter medication. Abstinence of caffeinate beverages, alcohol or exercise was
119 requested 24 hours prior to experimental sessions. The room temperature was kept constant at 20-22ºC,
120 and external stimuli were kept to a minimum.

121

122 Measurements

123 Heart rate (HR) was monitored using lead II electrocardiogram and blood pressure was measured
124 beat-to-beat from the middle finger of the right hand (Finometer Pro, Finapres Medical Systems,
125 Arnhem, The Netherlands). Mean arterial pressure (MAP) was calculated offline by the integration of
126 the arterial blood pressure waveform over a cardiac cycle. Resting blood pressure was verified by
127 brachial artery blood pressure measurement made from the left arm using an automated
128 sphygmomanometer (Tango+, SunTech Medical, USA). ICA_{Vm} and ICA_d were measured from the left
129 side of the neck with duplex Doppler ultrasound (Logiq, GE Medical Systems, Milwaukee, USA) using
130 a 10-MHz multifrequency linear-array transducer with a constant insonation angle of 60º relative to the
131 skin. ICA measurements were performed 1 to 1.5 cm distal to the carotid bifurcation while the subject's
132 chin was slightly elevated. To measure the ICA_d the brightness mode was used in a longitudinal section,
133 the systolic and diastolic diameters were measured over 10 cardiac cycles, the mean diameter was
134 calculated as: Mean diameter (cm) = [(systolic diameter ×1/3)] + [(diastolic diameter ×2/3)]. The
135 Doppler velocity spectrum was analyzed using the pulsed wave mode and the time-averaged mean flow
136 velocity obtained over 10 cardiac cycles. The internal carotid blood flow (ICA_Q; $ml \cdot min^{-1}$) was

calculated as [$ICA_{Vm} \times \pi \times (diameter/2)^2$] x 60. ICA conductance (ICA_{CVC}; $ml \cdot mim^{-1} \cdot mmHg^{-1}$) was calculated as ICA_Q/ MAP.

MCA_{Vm} was measured with a 2 MHz pulse wave transcranial Doppler ultrasound system (Doppler Box X; Compumedics Germany GmbH, Singen, Germany). The MCA was insonated via the temporal window above the zygomatic arch on the left side of the head. After finding a satisfactory signal, the probe was fixed in place with a headband and ultrasonic gel. The MCA vascular conductance index (MCA_{CVCi}) was calculated as MCA_{Vm} /MAP.

Participants wore a mouthpiece and nose-clip to permit breath-by-breath determination of minute ventilation (V_E) via a turbine volume transducer (VMM400; Interface Associates, Aliso Viejo, CA, USA). The end-tidal partial pressures of O_2 and CO_2 were determined using rapid response gas analyzers (Moxus Modular; AEI Technologies Inc, Pittsburg, USA). Analog data were digitally converted at 1 kHz and stored on a PC for offline analysis (Powerlab and LabChart Pro; ADInstruments, Dunedin, New Zealand).

Experimental Protocol

An initial familiarization session was first conducted where the participants experienced all the experimental methods and protocols. The experimental protocol was subsequently conducted over two laboratory visits separated by 3-7 days with the order of protocol 1 and 2 (day 1 or 2) decided according to a coin toss. For each protocol, two trials were performed. In one trial, $P_{ET}CO_2$ fluctuated normally while subjects breathed medical grade air, in another trial $P_{ET}CO_2$ was clamped at ~1 mmHg above the resting partial pressure. Trials were counterbalanced and separated by a minimum of 20 min. $P_{ET}CO_2$ clamping was undertaken using a dynamic end-tidal forcing system which uses a prediction – correction system, whereby $P_{ET}CO_2$ is controlled at the desired level by altering the composition of the inspired gas on a breath by breath basis (50).

Protocol I: Leg cycling with BFR

After instrumentation participants sat quietly in a semi-recumbent cycle ergometer and respiration was monitored for 10 min to determine the normal $P_{ET}CO_2$. Participants were then instructed to commence cycling exercise at 60 rpm. During the first ~3 to 5 min the workload was adjusted to reach a target HR of 120 beats·min^{-1} after which ~10 min of steady-state cycling exercise was performed (Ex1). Following this period, bilateral thigh cuffs were inflated to 130 mmHg (Rapid Cuff Inflation System E20 AG101, Hokanson, Bellevue, USA) in order to partially restrict blood flow to exercising muscles and engage the muscle metaboreflex (BFR). The thigh cuffs were deflated after 3 min and a further 3 min of steady-state cycling exercise was performed under free-flow conditions (Ex2). ICA assessments were not carried out during Ex2. Ratings of perceived exertion (RPE) were obtained using the 1-10 Borg scale (12) at the end of the Ex1, BFR, and Ex2 periods. Mean HR, BP, respiratory and MCA_{Vm} data were obtained on a beat-to-beat basis and averages calculated at rest (3 min), Ex1 (last 1 min), BFR (last 1 min) and Ex2 (last 1 min). Ultrasound images for calculation of ICA_Q were obtained during the last 1 min of rest, last 1 min of Ex1 and last 1 min of leg cycling with BFR. Measurements were then pooled to provide a mean value for each experimental phase.

Protocol II: Leg cycling with PEI

As described above, following a 10 min rest period during which the normal $P_{ET}CO_2$ was determined, participants undertook cycling exercise on a semi-recumbent cycle ergometer (60 rpm). After a ~3-5 min period during which the workload was adjusted in order to reach the reach the target HR of 120 beats·min^{-1} participants undertook steady-state cycling exercise for 10 min (Ex). Fifteen seconds before the end of the exercise, bilateral thigh cuffs were inflated to 300 mmHg in order to occlude the blood flow to the exercising muscles and remained inflated for 3 min in order to isolate the activation of the muscle metaboreflex (PEI). RPE was obtained after 5 min of steady-state cycling exercise. Mean HR, BP, respiratory and MCA_{Vm} data were obtained on a beat-to-beat basis and averages calculated at rest (3 min), Ex (last 1 min) and PEI (last 1 min). Ultrasound images for calculation of

190 ICA_Q were obtained during last 1 min of rest, last 1 min of Ex and last 1 min of PEI. Measurements
191 were then pooled to provide a mean value for each experimental phase.
192
193 Data and statistical analysis
194 Values are reported as means ±SEM. Main effects of experimental phase (Rest, Ex, PEI or Rest,
195 Ex1, BFR, Ex2), trial (control, $P_{ET}CO_2$ clamp) and interaction (phase x trial) were made using two-way
196 repeated measures ANOVA followed by Student-Newman-Keuls post hoc test. Between trial
197 comparisons of exercise workload were made using Student t-tests. Statistical significance was set to
198 $p<0.05$. Analyses were conducted using SigmaPlot 12.5 (Systat Software Inc, London, UK).

199 RESULTS

200 Leg cycling with BFR

201 The exercise workload was not different between trials (control 90±9 W and $P_{ET}CO_2$ clamp trial
202 85±9 W; P>0.05). In the control trial, $P_{ET}CO_2$ was slightly increased from rest during leg cycling (Ex1
203 Δ2.2±0.3 mmHg, P<0.05), decreased with BFR (Δ-4.8±0.9 mmHg, P<0.05), and returned to resting
204 values upon the cessation of BFR (Ex2 Δ0.8±0.5 mmHg, P>0.05 vs. Rest; Figure 1). By design, in the
205 clamp trial $P_{ET}CO_2$ remained unchanged from the rest throughout all experimental phases. Leg cycling
206 evoked similar increases in MCA_{Vm} during the control and $P_{ET}CO_2$ clamp trials (P<0.05 Rest vs. Ex1;
207 Figure 1). In the control trial, no change in MCA_{Vm} was observed during exercise with BFR, whereas in
208 the $P_{ET}CO_2$ clamp trial MCA_{Vm} was increased (P<0.05 vs. Rest, Ex1, and between conditions). In both
209 trials, MCA_{Vm} was not different during leg cycling before and after BFR (P>0.05 Ex1 vs. Ex2).
210 MCA_{CVCi} was unchanged from rest during leg cycling (P>0.05, Ex1 vs. Rest, Ex2 vs. Rest), but was
211 decreased with BFR (P<0.05 vs. Rest and Ex1; Table 1). The magnitude of this decrease was greater in
212 the control trial than in the $P_{ET}CO_2$ clamp trial. ICA_Q was unchanged from rest to leg cycling (Ex1) in
213 both trials, but was increased with BFR in the $P_{ET}CO_2$ clamp trial (P<0.05 vs. Rest and between trials;
214 Figure 1), secondary to an increase in ICA_{Vm}. ICA_d was unchanged throughout all experimental phases
215 in both trials (Table 1). ICA_{CVC} was not different between trials and was similarly decreased from rest
216 during leg cycling (P<0.05 Rest vs. Ex1) then further decreased during leg cycling with BFR (P<0.05
217 Ex1 vs. Rest) in both trials.

218 HR was not different between the control and $P_{ET}CO_2$ clamp trials at any experimental phase
219 (Table 1). MAP was slightly, but higher in the $P_{ET}CO_2$ clamp trial (Figure 1). Leg cycling evoked
220 increases in MAP, HR in both trials (P<0.05 Ex1 vs. Rest), which were further increased during BFR
221 (P<0.05 vs. Ex1). During leg cycling following BFR, MAP and HR returned to values observed during
222 leg cycling prior to BFR (Ex1 vs. Ex2, P>0.05). RPE was not different between the control trial [Ex1
223 median, 4 (interquartile range, 4-5), BFR, 8 (7-8) and Ex2 5 (4-6); P<0.05] and the $P_{ET}CO_2$ clamp trial
224 [Ex1 4 (3-6), BFR 8, (7-8), Ex2 5 (4-7); P<0.05] (Wilcoxon signed-rank test).

225

Leg cycling with PEI

Exercise workload was not different between trials (control 89± 9 W and $P_{ET}CO_2$ clamp trial 89±9 W; $P>0.05$). In the control trial, $P_{ET}CO_2$ was unchanged from rest during leg cycling and decreased with PEI (Δ-7±1 mmHg, $P<0.05$; Figure 2), but by design, in the $P_{ET}CO_2$ clamp trial it remained not different from rest throughout all experimental phases. In both the control and $P_{ET}CO_2$ clamp trials, MCA_{Vm} increased from rest during leg cycling ($P\leq0.05$; Figure 2). During PEI, in the control trial MCA_{Vm} was not different from rest ($P>0.05$), whereas in the $P_{ET}CO_2$ clamp trial MCA_{Vm} remained elevated ($P<0.05$ vs. rest and between trials). In the control trial, MCA_{CVCi} was not different from rest during leg cycling and was decreased from rest during PEI ($P<0.05$ vs. Rest and between trials; Table 2). MCA_{CVCi} was not different from rest throughout $P_{ET}CO_2$ clamp trial ($P>0.05$; Table 2). ICA_Q was not different from rest during leg cycling in both control and $P_{ET}CO_2$ clamp trials ($P>0.05$; Figure 2). However, during PEI, ICA_Q was higher in the $P_{ET}CO_2$ clamp trial than in the control trial ($P<0.05$; Figure 2). ICA_d was not different throughout all experimental phases in both trials (Table 2). ICA_{CVC} was greater in the $P_{ET}CO_2$ clamp trial, but decreased similarly from rest during leg cycling ($P<0.05$ vs. Rest) in both trials. During PEI, ICA_{CVC} remained at levels sustained during exercise ($P<0.05$ vs. Rest, $P>0.05$ vs. Ex) in both trials. MAP and HR (Table 2; Figure 2) were not different between the control and $P_{ET}CO_2$ clamp trials at any experimental phase. Leg cycling evoked increases in MAP and HR in both trials ($P<0.05$ vs. rest). In both trials, MAP was further increased with PEI from the level observed during leg cycling ($P<0.05$ vs. Rest and Ex), whereas HR fell, but remained above resting levels ($P<0.05$ vs. Rest and Ex).

RPE were not different during leg cycling in the control [median, 5 (interquartile range, 4-7) and the $P_{ET}CO_2$ clamp trials [5 (4-6)] (Wilcoxon signed-rank test).

248 DISCUSSION

249 The major novel finding of this study is that the muscle metaboreflex failed to elevate either
250 MCA_{Vm} or ICA_{Q}, when engaged by leg cycling with BFR or isolated during PEI following leg cycling
251 under control conditions. However, a significant reduction in $P_{ET}CO_2$, secondary to an increase in V_E,
252 was induced by muscle metaboreflex activation with either BFR or PEI. Accordingly, when $P_{ET}CO_2$ was
253 clamped at resting levels muscle metaboreflex-mediated increases in MCA_{Vm} and ICA_{Q} were revealed
254 during both BFR and PEI. Thus, in accordance with our original hypothesis, these findings demonstrate
255 that when hyperventilation-related decreases in $P_{ET}CO_2$ are prevented the muscle metaboreflex increases
256 cerebral blood flow, and this occurs irrespective of the mode of muscle metaboreflex activation.

257 A potential explanation why exercise with BFR, or indeed PEI, does not increase cerebral
258 perfusion may be that the activation of metabolically sensitive skeletal muscle afferents evokes an
259 increase in ventilation (5), which leads to a confounding reduction in P_aCO_2 (indexed by $P_{ET}CO_2$). The
260 contribution of group III and IV skeletal muscle afferents to the control of breathing remains
261 controversial, nevertheless in agreement with several previous reports we observed an increase in V_E
262 during muscle metaboreflex activation (1, 13, 15, 44) and a reduction in $P_{ET}CO_2$. CO_2 is a powerful
263 dilator of the cerebral vasculature (31) and decreases in P_aCO_2 lead to cerebral vasoconstriction (2). In
264 the present study, the clamping of $P_{ET}CO_2$ at resting levels unmasked a muscle metaboreflex-mediated
265 increase in MCA_{Vm} and ICA_{Q} during leg cycling with BFR and during PEI following leg cycling.
266 Furthermore, the degree of cerebral vasoconstriction (i.e., the magnitude of the reduction in MCA_{CVCi})
267 during PEI and exercise with BFR was attenuated in the $P_{ET}CO_2$ clamp trial, although a similar effect
268 was not observed for ICA_{CVC}. These observations are in concordance with our earlier report that PEI
269 following fatiguing ischemic handgrip exercise only increases MCA_{Vm} when $P_{ET}CO_2$ is clamped at
270 resting levels (13). Such observations may help to explain why others have not shown MCA_{Vm} to be
271 elevated during PEI. Indeed, Jorgensen et al., (28) also reported that P_aCO_2 was decreased below resting
272 levels during PEI following cycling exercise. In agreement with Friedman et al., (19, 20) and Jorgensen
273 et al., (29), who demonstrated that pharmacological blockade of sensory feedback from skeletal muscle
274 afferents diminished the increase in cerebral perfusion during exercise, the results of the present study

support a role for the muscle metaboreflex in the regulation of cerebral blood flow during exercise. This may be attributable to the pairing of local neuronal activation and perfusion (i.e., neural-vascular coupling), that is to say cerebral flow increases in order to increase O_2 delivery in accordance with increased metabolic. Studies employing advanced imaging techniques have shown that isolated metaboreflex stimulation (PEI) evokes increased activity in discrete brain regions (e.g., medial and lateral dorsal medulla, contralateral insula, primary and secondary somatosensory cortex) (53). It is acknowledged that part of the cerebrovascular responses to PEI arises on account of the discomfort associated with this manoeuvre (35). In the present study we are not able to quantify the contribution of local discomfort to the cerebrovascular responses to PEI and BFR, but note that without the clamping of $P_{ET}CO_2$ at resting levels no changes in cerebral perfusion were observed. As such, the combination of $P_{ET}CO_2$ clamping and brain imaging modalities may provide additional insights into the effects of muscle metaboreflex activation on regional brain activation. The influence of exercise-induced increases of MAP on cerebral blood flow is somewhat controversial. The observation that during PEI, MCA_{Vm} remains at resting levels while MAP is elevated is part of the reason why the direct influence of blood pressure on the exercise-induced increase of cerebral perfusion has on occasion been discounted (26, 47, 55). However, when considering the effects of MAP on cerebral perfusion during exercise the potentially confounding effects of changes in $P_{ET}CO_2$ should be considered. With P_aCO_2 controlled, MCA_{Vm} changes by ~0.8% per mmHg change in MAP within the so called "autoregulatory range" (34). We observed that, BFR evoked a 44 mmHg increase from rest in MAP and a 26% increase in $MCA_{Vm,}$ while during PEI, MAP was elevated by 29 mmHg and MCA_{Vm} elevated by 17%. As such, increases in MAP may reasonably be expected to contribute, at least in part, for the observed increase in cerebral blood flow. The ventilatory response to leg cycling with BFR was enhanced when $P_{ET}CO_2$ was clamped. As neither the exercise workload nor the thigh cuff pressure were different between conditions, it is unlikely that this is attributable to a difference in the degree of muscle afferent activation within the active skeletal muscles. However, in the absence of a direct assessment of local metabolites this possibility cannot be excluded. Alternatively, the clamping of $P_{ET}CO_2$ may have eliminated part of the chemoreflex-mediated inhibition of the ventilatory response. It is possible that the greater ventilatory

response to exercise observed when $P_{ET}CO_2$ was clamped, could evoke a respiratory muscle metaboreflex and augment the concomitant blood pressure response (51). However, an exaggerated increase in blood pressure did not accompany the greater ventilatory response to exercise with BFR in the clamp trial.

The present study extends earlier reports examining the contribution of skeletal muscle afferents to cerebral blood flow regulation in two important ways. Rather than relying on transcranial Doppler ultrasound measures of MCA_{Vm} to estimate cerebral perfusion, we have used duplex Doppler ultrasound measures of cerebral blood flow (ICA_Q). In order that transcranial Doppler ultrasound measures of MCA_{Vm} are representative of cerebral blood flow, it must be assumed that MCA_d remains constant. As such, this is the first study to determine the influence of the muscle metaboreflex activation on cerebral blood flow in humans. In addition to use of PEI to assess the effect of isolated skeletal muscle metaboreflex activation on cerebral blood flow, exercise with BFR was undertaken. As indicated above, this has applied relevance to those undertaking such practices to induce athletic enhancements, but also patient populations in whom muscle metaboreflex activation may be heightened during exercise as a consequence of skeletal muscle under-perfusion (e.g., chronic heart failure, peripheral vascular disease). Despite exercise with BFR and PEI evoking similar reductions from rest in $P_{ET}CO_2$ (Δ-2.6 and Δ-5.0 mmHg for BFR and PEI, respectively), MCA_{Vm} was significantly elevated from rest during exercise with BFR such that it was similar to that observed during free-flow exercise, whereas MCA_{Vm} was not different from rest during PEI. Such findings may be explained by a greater elevations in cardiac output and the concomitant activation of central command during exercise with BFR compared to PEI (36). Nevertheless, independent of the mode of muscle metaboreflex activation, when muscle metaboreflex mediated reductions in $P_{ET}CO_2$ were prevented increases in cerebral blood flow were observed.

Regular exercise with BFR (e.g., Kaatsu training) has been reported to enhance cardiorespiratory fitness (60) and skeletal muscle mass in healthy young and older individuals (32, 33, 63, 64), and patients (56). It is believed that exercise training with BFR exaggerates the normal accumulation of metabolites within the active skeletal muscle, thus promoting muscle growth and force generating capacity without the need for high-intensity training (21, 22, 43, 62). In a recent article, Spranger et al.,

329 (57) raised a 'call for concern' about this practice on the basis that it engages the muscle metaboreflex,
330 which is known to powerfully increase sympathetic nerve activity to the heart and peripheral vasculature
331 and can inhibit cardiac parasympathetic activity (18). As a consequence of these autonomic alterations,
332 exercise with BFR evokes pronounced increases in peripheral vascular resistance, cardiac output and
333 blood pressure (59). This could be of particular concern to patients in whom exaggerated skeletal
334 muscle afferent sensitivity has been identified (e.g., hypertension, chronic heart failure, chronic
335 obstructive pulmonary disease, type 2 diabetes) (7, 9, 24, 25, 45) and could raise the risk of
336 cardiovascular and cerebrovascular events (57). Indeed, in the present study of healthy individuals,
337 exercise with BFR raised MAP by ~27 mmHg and heart rate by ~22 beats·min^{-1} from levels established
338 during a preceding period of leg cycling under free-flow conditions. However, despite such
339 hemodynamic alterations cerebral perfusion was unchanged during exercise with BFR, thus calling into
340 question the contention that exercise with BFR may increase the risk of cerebrovascular injury as a
341 consequence of a large local hyperaemic response. In fact we observed that exercise with BFR evoked a
342 lower than expected cerebral blood flow, on account of the coincident hyperventilation and hypocapnia
343 linked cerebral vasoconstriction. During high intensity dynamic exercise, particularly when combined
344 with hypoxia, a reduced cerebral oxygenation has been postulated as a fatigue mechanism (8, 40, 55).
345 As such, in patients who exhibit exaggerated skeletal muscle afferent feedback and an excessive
346 hyperventilatory response to exercise (e.g., chronic heart failure, congestive obstructive pulmonary
347 disease) (7, 9, 58), the associated reduction in P_aCO_2 and cerebral vasoconstriction may precipitate
348 exercise intolerance via a central mechanism (41, 48). Future studies utilizing arterial-internal jugular
349 venous blood sampling are required, particularly in chronic disease populations, to better understand
350 how cerebral metabolism (e.g., O_2 delivery, cerebral metabolic rate for O_2, fractional O_2 extraction) is
351 affected by the activation of skeletal muscle afferents while $P_{ET}CO_2$ is clamped at baseline levels.

352 There are several limitations on the present study, $P_{ET}CO_2$ was used as a surrogate for P_aCO_2,
353 although during exercise $P_{ET}CO_2$ could have overestimated P_aCO_2 (49) there is a strong correlation
354 between $P_{ET}CO_2$ and $PaCO_2$ across all levels of physiologic dead space (37). We used external thigh
355 cuffs to decrease/occlude blood flow to the legs, although no direct measures of the leg blood flow or

local metabolite concentrations were possible, we observed marked increases in MAP during this manoeuvre indicative of muscle metaboreflex activation, which strongly suggest that reductions in blood flow were successfully induced. BFR may have increased in central command, which could have contributed to the increase of cerebral blood flow (6). Indeed, increases in RPE, historically related to central command (38, 39), were noted during leg cycling with BFR. Human studies are needed in which the cardiovascular and cerebrovascular responses to the application of BFR during leg cycling are evaluated before and after pharmacological inhibition of feedback from group III and IV skeletal muscle afferents (e.g., intrathecal fentanyl) in order to test the contribution of central command to this manoeuvre. Automated edge-tracking software was not used in the present study and this may be a limitation (61), although intraclass correlations between repeated rest and exercise measures made during a study visit were high (i.e., >0.8). Finally, we have only evaluated the effect of skeletal muscle afferent feedback with BFR during moderate cycling exercise, and care should be taken when directly extrapolating our findings to other exercise intensities and modalities. This is important in light of the concept that athletes may utilise BFR training in order to try to obtain a desirable training effects but at a lower exercise intensity (21, 22, 43, 62).

In conclusion, the findings of the present study indicate that only when hyperventilation-related decreases in $P_{ET}CO_2$ are prevented does the activation of metabolically sensitive skeletal muscle afferent fibres evoke an increase in cerebral blood flow, irrespective of the mode of activation (i.e. during or following Ex).

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Conflict of interest

None to declare.

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FIGURE LEGENDS

Figure 1. Cardiorespiratory and cerebrovascular responses to leg cycling under free-flow conditions (Ex1, Ex2) and with blood flow restriction (BFR). Partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$); middle cerebral artery blood velocity (MCA_{Vm}); internal carotid artery blood flow (ICA_Q); mean arterial pressure (MAP). Ex, exercise; BFR, blood flow restriction. Values are mean±SEM. * P<0.05 vs. rest, †P<0.05 vs. Ex1, ‡P<0.05 vs. control.

Figure 2. Cardiorespiratory and cerebrovascular responses to leg cycling and post-exercise ischemia (PEI). Partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$); middle cerebral artery blood velocity (MCA_{Vm}); internal carotid artery blood flow (ICA_Q); mean arterial pressure (MAP). Ex, exercise; PEI, post exercise ischemia. Values are mean±SEM. * P<0.05 vs. rest, †P<0.05 vs. Ex, ‡P<0.05 vs. control.

Table 1. Cardiorespiratory and cerebrovascular responses to leg cycling under free-flow conditions (Ex1, Ex2) and with blood flow restriction (BFR)

	Experimental Phase				P value		
	Rest	Ex1	BFR	Ex2	Phase	Condition	Interaction
V_E ($l \cdot min^{-1}$)							
Control	9 ± 1	35 ± 2*	45 ± 3*†	39 ± 2*	<0.001	0.002	0.002
$P_{ET}CO_2$ Clamp	11 ± 2	37 ± 3*	56 ± 4*†‡	46 ± 3*†‡			
MCA_{CVCi} ($cm \cdot s^{-1} \cdot mmHg^{-1}$)							
Control	0.66 ± 0.06	0.65 ± 0.05	0.49 ± 0.03*†	0.66 ± 0.06	<0.001	0.927	0.026
$P_{ET}CO_2$ Clamp	0.67 ± 0.06	0.63 ± 0.04	0.54 ± 0.04*†‡	0.63 ± 0.06			
ICA_{Vm} ($cm \cdot s^{-1}$)							
Control	28 ± 1	28 ± 1	26 ± 1		0.573	0.007	0.003
$P_{ET}CO_2$ Clamp	28 ± 1	29 ± 2*	31 ± 1*†‡				
ICA_d (cm)							
Control	0.46 ± 0.01	0.45 ± 0.01	0.45 ± 0.01		0.467	0.486	0.213
$P_{ET}CO_2$ Clamp	0.45 ± 0.01	0.45 ± 0.01	0.46 ± 0.01				
ICA_{CVC} ($ml \cdot min^{-1} \cdot mmHg^{-1}$)							

Control	3.3 ± 0.2	2.9 ± 0.2	2.0 ± 0.1		<0.001	0.321	0.108
$P_{ET}CO_2$ Clamp	3.2 ± 0.2	2.8 ± 0.2	2.4 ± 0.1				
SBP (mmHg)							
Control	117 ± 2	153 ± 7	191 ± 8	146 ± 7	<0.001	0.611	0.402
$P_{ET}CO_2$ Clamp	115 ± 3	153 ± 5	195 ± 6	146 ± 6			
DBP (mmHg)							
Control	67 ± 3	72 ± 3	96 ± 3	75 ± 4	<0.001	<0.001	0.522
$P_{ET}CO_2$ Clamp	70 ± 2	79 ± 3	101 ± 5	80 ± 3			
HR (beats·min^{-1})							
Control	64 ± 3	122 ± 1	144 ± 3	124 ± 2	<0.001	0.450	0.760
$P_{ET}CO_2$ Clamp	65 ± 3	121 ± 1	145 ± 2	125 ± 2			

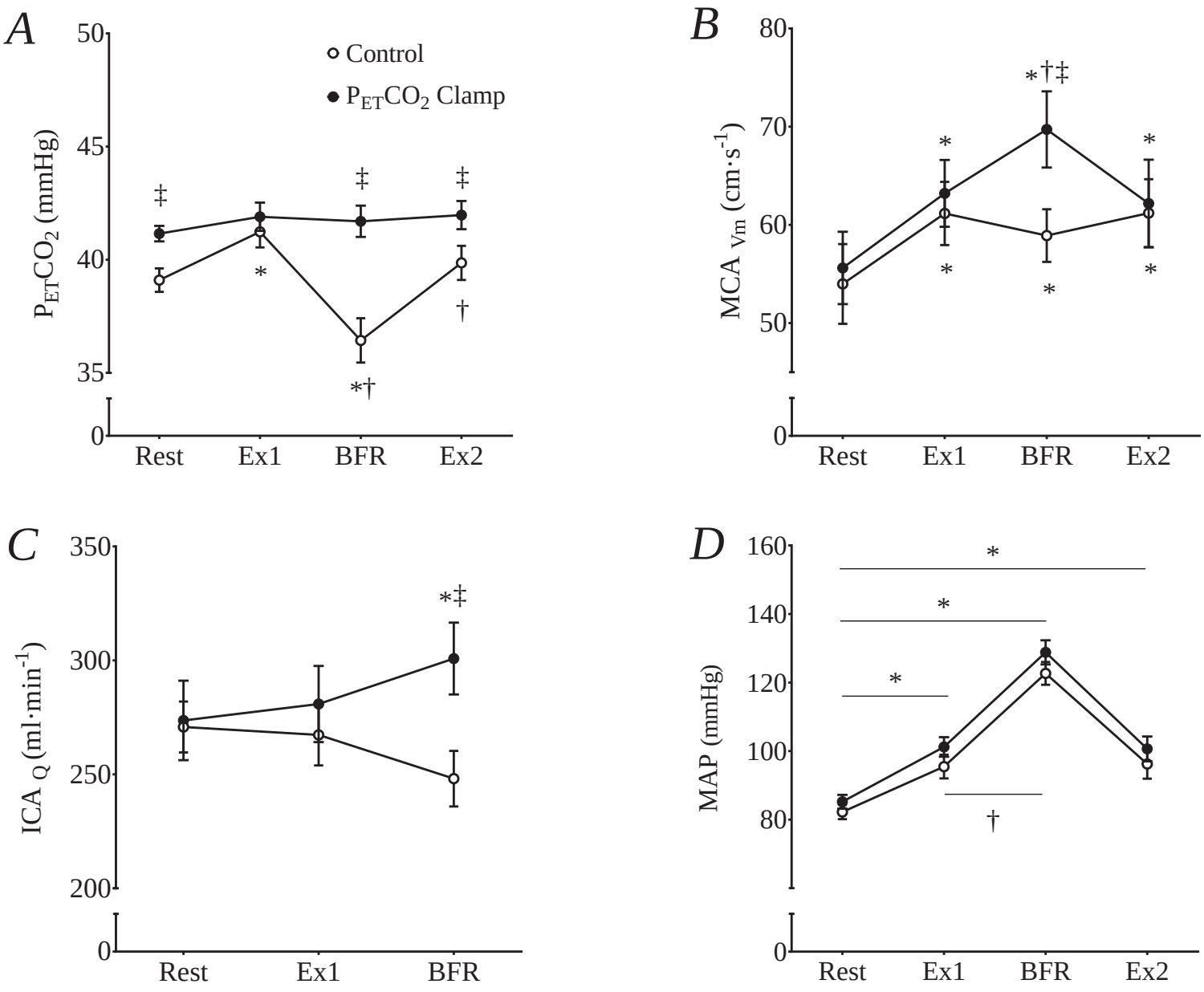
Values are mean±SEM. V_E, ventilation; MCA_{CVCi}, middle cerebral artery conductance; ICA_{Vm}, internal carotid artery mean velocity; ICA_d, internal carotid artery diameter; ICA_{CVC}, internal carotid artery conductance; SBP, systolic blood pressure; MAP, mean arterial pressure; HR, heart rate; Ex, exercise; BFR, blood flow restriction. Values are mean ± SEM. * P<0.05 vs. Rest, †P<0.05 vs. Ex1, ‡P<0.05 vs. Control.

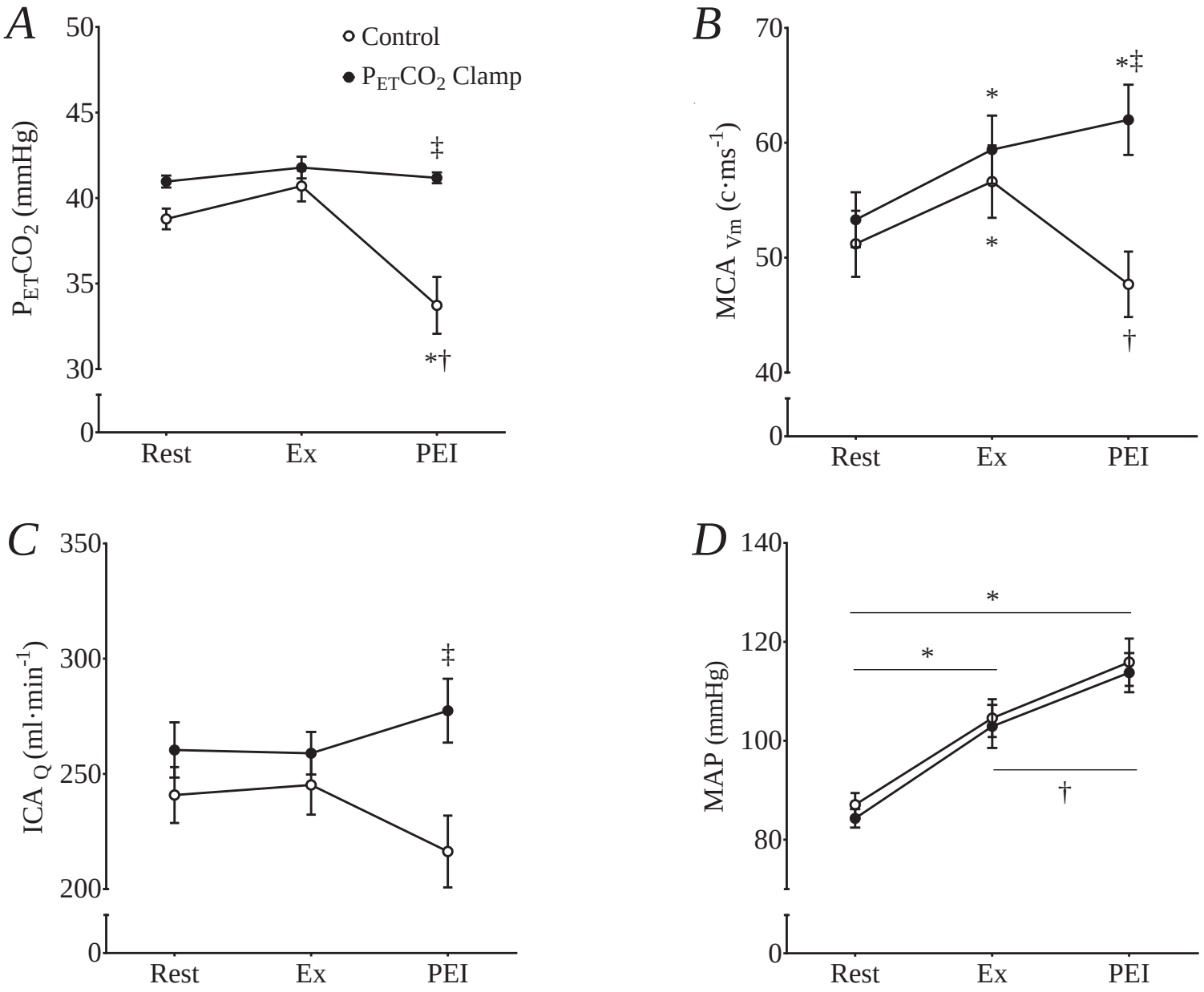
Table 2. Cardiorespiratory and cerebrovascular responses to leg cycling and post-exercise ischemia (PEI)

	Experimental Phase			P value		
	Rest	Ex	PEI	Phase	Condition	Interaction
V_E ($l \cdot min^{-1}$)						
Control	9 ± 1	40 ± 2*	16 ± 3†	<0.001	0.011	0.001
$P_{ET}CO_2$ Clamp	13 ± 1	40 ± 3*	25 ± 3*†‡			
MCA_{CVCi} ($cm \cdot s^{-1} \cdot mmHg^{-1}$)						
Control	0.60 ± 0.05	0.55 ± 0.03	0.43 ± 0.03*†	0.001	0.004	0.031
$P_{ET}CO_2$ Clamp	0.64 ± 0.04	0.59 ± 0.04	0.56 ± 0.04‡			
ICA_{Vm} ($cm \cdot s^{-1}$)						
Control	25 ± 1	26 ± 1	23 ± 2	0.735	<0.001	0.019
$P_{ET}CO_2$ Clamp	27 ± 1	28 ± 1	29 ± 2‡			
ICA_d (cm)						
Control	0.45 ± 0.01	0.45 ± 0.01	0.45 ± 0.01	0.306	0.572	0.784
$P_{ET}CO_2$ Clamp	0.46 ± 0.01	0.45 ± 0.01	0.45 ± 0.01			
ICA_{CVC} ($ml \cdot min^{-1} \cdot mmHg^{-1}$)						
Control	2.8 ± 0.2	2.4 ± 0.2	1.9 ± 0.2	<0.001	<0.001	0.122

$P_{ET}CO_2$ Clamp	3.1 ± 0.2	2.6 ± 0.1	2.5 ± 0.2			
SBP (mmHg)						
Control	118 ± 4	155 ± 7	145 ± 7	<0.001	0.729	0.469
$P_{ET}CO_2$ Clamp	116 ± 4	159 ± 9	148 ± 8			
DBP (mmHg)						
Control	72 ± 3	83 ± 4	98 ± 5	<0.001	0.157	0.932
$P_{ET}CO_2$ Clamp	69 ± 2	80 ± 3	94 ± 3			
HR (beats·min^{-1})						
Control	61 ± 3	121 ± 2	95 ± 5	<0.001	0.764	0.416
$P_{ET}CO_2$ Clamp	63 ± 3	123 ± 1	93 ± 3			

Values are mean±SEM. V_E, ventilation; MCA_{CVC}, middle cerebral artery conductance; ICA_{Vm}, internal carotid artery mean velocity; ICA_d, internal carotid artery diameter; ICA_{CVCi}, internal carotid artery conductance; SBP, systolic blood pressure; MAP, mean arterial pressure; HR, heart rate; Ex, exercise; PEI, post exercise ischemia. Values are mean ± SEM. * P<0.05 vs. Rest, †P<0.05 vs. Ex, ‡P<0.05 vs. Control.


A
Control
$P_{ET}CO_2$ Clamp
$P_{ET}CO_2$ (mmHg)
50
45
40
35
0
Rest
Ex1
BFR
Ex2
B
MCA $_{Vm}$ (cm·s^{-1})
80
70
60
50
0
C
ICA $_Q$ (ml·min^{-1})
350
300
250
200
0
D
MAP (mmHg)
160
140
120
100
80


A
Control
$P_{ET}CO_2$ Clamp
$P_{ET}CO_2$ (mmHg)
50
45
40
35
30
0
‡
*†
Rest
Ex
PEI
B
MCA_{Vm} ($c \cdot ms^{-1}$)
70
60
50
40
0
*
*
*‡
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Rest
Ex
PEI
C
ICA_Q ($ml \cdot min^{-1}$)
350
300
250
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MAP (mmHg)
140
120
100
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0
*
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†
Rest
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PEI