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Influence of muscle metaboreceptor stimulation on middle cerebral artery blood velocity in humans

Igor D. Braz, Clare Scott, Lydia L. Simpson, Emma L. Springham, Beverly W.L. Tan, George M. Balanos, & James P. Fisher

School of Sport, Exercise & Rehabilitation Sciences, College of Life & Environmental Sciences, University of Birmingham, Edgbaston, Birmingham, UK

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Please send correspondence to:

James P. Fisher, PhD School of Sport, Exercise & Rehabilitation Sciences College of Life & Environmental Sciences University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK tel: +44 (0)121 414 8011 fax: +44 (0)121 414 4121 email: j.p.fisher@bham.ac.uk

NEW FINDINGS

What is the central question of this study?

Is the ability of metabolically sensitive skeletal muscle afferents (muscle metaboreceptors) to increase cerebral perfusion, masked by a hyperventilation-mediated reduction in the partial pressure of arterial carbon dioxide (indexed using $P_{ET}CO_2$), and hence cerebral vasoconstriction, during post-exercise muscle ischemia?

What is the main finding and its importance?

We found that cerebral perfusion is elevated during post-exercise muscle ischemia when $P_{ET}CO_2$ is clamped at baseline, but when $P_{ET}CO_2$ is permitted to fluctuate no such elevation occurs. These findings indicate that muscle metaboreceptors increase cerebral perfusion when the confounding effects of changes in $P_{ET}CO_2$ are obviated.

ABSTRACT

Regional anesthesia to attenuate skeletal muscle afferent feedback abolishes the exercise induced increase in middle cerebral artery mean blood velocity (MCA V_{mean}). However, such exercise related increases in cerebral perfusion are not preserved during postexercise muscle ischemia (PEMI) where the activation of metabolically sensitive muscle afferents is isolated. We tested the hypothesis that a hyperventilation-mediated decrease in the partial pressure of arterial carbon dioxide (CO₂), and hence cerebral vasoconstriction, masks the influence of muscle metaboreceptor stimulation on MCA V_{mean} during PEMI. Ten healthy men $(20 \pm 1 \text{ yr})$ performed two trials of fatiguing isometric handgrip followed by PEMI, under control conditions and with end-tidal CO₂ (P_{ET}CO₂) clamped at ≈ 1 mmHg above the resting partial pressure. In the control trial, P_{ET}CO₂ decreased from rest during handgrip and PEMI, while MCA V_{mean} was unchanged from rest. By design, P_{ET}CO₂ remained unchanged from rest throughout the clamp trial, while MCA V_{mean} increased during handgrip (+10.6 \pm 1.8 cm.s⁻¹) and PEMI (+9.2 \pm 1.6 cm.s⁻¹; P<0.05 vs. rest and control trial). Increases in minute ventilation and mean arterial pressure during handgrip and PEMI were not different in the control and P_{ET}CO₂ clamp trials (P>0.05). These findings indicate that metabolically sensitive skeletal muscle afferents play an important role in the regional increase in cerebral perfusion observed in exercise, but that influence can be masked by a decrease in P_{ET}CO₂ when they are activated in isolation during PEMI.

<u>Abbreviations</u>: CO₂, carbon dioxide; CVCi, cerebrovascular conduction index; HR, heart rate; MAP, mean arterial pressure; MCA V_{mean} , middle cerebral artery mean flow velocity; MVC, maximum voluntary contraction; PEMI, post-exercise muscle ischemia; P_{ET}CO₂, partial pressure of end-tidal carbon dioxide; V_E, minute ventilation.

INTRODUCTION

The brain accounts for $\approx 2\%$ of the human body mass and yet its blood flow equates to $\approx 15\%$ of cardiac output (Lassen, 1959). Although global brain blood flow is reportedly unchanged from rest during exercise (Scheinberg *et al.*, 1954; Hedlund *et al.*, 1962; Globus *et al.*, 1983), localized increases in perfusion occur in regions such as the motor-sensory cortex and supplementary motor area (Olesen, 1971; Orgogozo & Larsen, 1979; Colebatch *et al.*, 1991; Hiura *et al.*, 2014). While indicative of the important coupling between central neuronal activity and local perfusion during muscular contractions, several other factors acting independently and interactively contribute to the regulation of the cerebral blood flow during exercise. These include the arterial tension of carbon dioxide (P_aCO₂), cardiac output, cerebral autoregulation and neural factors (Ide & Secher, 2000; Querido & Sheel, 2007; Willie *et al.*, 2014).

A role for sensory feedback from group III and IV skeletal muscle afferents in evoking the exercise-induced increase in cerebral perfusion has been indicated (Friedman *et al.*, 1991; Friedman *et al.*, 1992; Jorgensen *et al.*, 1993), although this remains controversial (Pott *et al.*, 1997; Vianna *et al.*, 2009). These thin fiber afferents are responsive to mechanical (mechanoreceptors) and metabolic (metaboreceptors) perturbation within the contracting muscle and are recognized for their role in modulating the autonomic response to exercise (Coote *et al.*, 1971; Mitchell *et al.*, 1983; Stebbins *et al.*, 1988; Fisher *et al.*, 2013a). Administration of local anesthesia to the brachial plexus in order to block skeletal muscle afferent feedback during handgrip attenuates the normal increase in regional cerebral perfusion (Friedman *et al.*, 1991; Friedman *et al.*, 1992; Jorgensen *et al.*, 1993). However, the role for such sensory feedback in eliciting an exercise-induced increase in cerebral perfusion is questioned by the observation that it remains at baseline during the isolated activation of the muscle metaboreceptors during a period of post-exercise muscle ischemia (PEMI)

(Jorgensen *et al.*, 1992; Pott *et al.*, 1997; Williamson *et al.*, 2003; Vianna *et al.*, 2009). A potential explanation for these equivocal findings is that during PEMI, a confounding reduction in P_aCO_2 occurs secondary to hyperventilation, which may evoke cerebral vasoconstriction and obscure the effect of sensory feedback from metabolically sensitive skeletal muscle afferents on cerebral perfusion. Reductions in P_aCO_2 have been observed during PEMI (Jorgensen *et al.*, 1992), and while debated, a role for group III and IV skeletal muscle afferents in the increase in ventilation during steady-state exercise (Dempsey *et al.*, 2014) and PEMI (Duncan *et al.*, 1981; Adams *et al.*, 1987; Piepoli *et al.*, 1999; Scott *et al.*, 2002) has been demonstrated.

The aim of our study was to determine the influence of metabolically sensitive skeletal muscle afferents on cerebral perfusion. To achieve this middle cerebral artery mean velocity (MCA V_{mean}), an index of cerebral perfusion, was measured during fatiguing handgrip followed by PEMI with partial pressure of end-tidal carbon dioxide ($P_{ET}CO_2$; a surrogate for P_aCO_2) clamped at baseline and under control conditions where $P_{ET}CO_2$ fluctuated normally. We tested the hypothesis that a hyperventilation-mediated decrease in $P_{ET}CO_2$ evokes a cerebral vasoconstriction that masks the effect of muscle metaboreceptor stimulation on cerebral perfusion during PEMI, and that when $P_{ET}CO_2$ is clamped at baseline an increase in MCA V_{mean} would be revealed during PEMI.

METHODS

The study was approved by the Health, Safety & Ethics committee of the School of Sport, Exercise & Rehabilitation at the University of Birmingham, UK and was undertaken in accordance with the Declaration of Helsinki. After receiving a detailed verbal and written explanation of the experimental protocol, written informed consent for participation was provided by each of the 10 male subjects (age, 20 ± 1 years; weight, 72 ± 13 kg; height, 178 ± 7 cm: mean \pm SD). Subjects were free from cardiovascular, respiratory, neurological, renal or metabolic diseases and were not using any prescription or over-the-counter medication. Abstinence from the ingestion of caffeine, alcohol or performance of strenuous physical activity 24 h prior to the experiment was requested. Room temperature was kept constant at 20-22 °C and external stimuli were kept to a minimum. A familiarization visit was conducted 24-48 h prior to the experimental visit where the entire protocol was conducted.

Measurements

Heart rate (HR) was monitored using a lead II electrocardiogram and beat-by-beat mean arterial pressure (MAP) obtained from the middle finger of the left hand (Portapres, Finapres Medical Systems, Amsterdam, The Netherlands). To verify Portapres measures brachial artery blood pressure was measured from the left arm using an automated sphygmomanometer (Omron, Matsusaka, Japan). A 2 MHz pulsed wave transcranial Doppler ultrasound system (Doppler Box X, Compumedics DWL, Germany) was used to insonate the middle cerebral artery via the temporal window above the zygomatic arch and ascertain MCA V_{mean} . After a satisfactory signal was found at a depth of 50-65 mm, the probe was fixed in place with a headband and ultrasonic gel. The left MCA V_{mean} was interrogated as it supplies the cortical areas associated with the right forearm muscles (Linkis *et al.*, 1995). Cerebral vascular conductance index (CVCi) was calculated as MCA V_{mean} divided by the mean BP.

Subjects wore a mouthpiece and nose clip to permit breath-by-breath determination of minute ventilation (V_E) via a turbine volume transucer (VMM400, Interface Associates, CA, USA), $P_{ET}O_2$ and $P_{ET}CO_2$ (Moxus Modular, AEI Technologies, 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).

Protocol

While seated comfortably, subjects held a custom handgrip dynamometer in their right hand. The maximal voluntary contraction (MVC) was taken as the highest force produced during 3-5 maximal handgrip efforts each separated by at least 1 min. Following instrumentation, respiration was monitored for 10 min to determine baseline P_{ET}CO₂. Subjects then performed two trials of fatiguing handgrip at 40 % MVC each proceeded by an additional 3 min rest period. Subjects were provided with a visual display of the force exerted for guidance. Task failure (fatigue) was defined as the inability to maintain 90 % of the target force for 2 s despite strong verbal encouragement. At task failure a tourniquet cuff around the upper right arm was inflated to 200 mmHg, while the subject continued to perform handgrip for a further 5 s. This PEMI period was maintained for 3 min, after which the cuff was deflated and a 3 min recovery period conducted. According to a randomized single-blind design, two handgrip trials were performed; 1) a control trial where $P_{ET}CO_2$ was not manipulated and fluctuated normally while subjects breathed medical grade air, and; 2) with $P_{ET}CO_2$ clamped at ≈ 1 mmHg above the resting partial pressure using a dynamic end-tidal forcing system. The system uses a prediction-correction system, whereby P_{ET}CO₂ is controlled at the desired level by altering the composition of the inspired gas on a breath-bybreath basis (Robbins et al., 1982). Trials were conducted and separated by 30 min and a rating of perceived exertion (RPE) obtained for each using a 6-20 scale (Borg, 1970).

Statistical analysis

Values are reported as mean \pm SEM. Rest, PEMI and recovery values were calculated by averaging over the 3 min phase. Handgrip values were calculated as a 15 s average at 25, 50, 75 and 100 % of handgrip duration. Main effects of phase (rest, 25, 50, 75 and 100 % of handgrip duration, PEMI, recovery), trial (control, P_{ET}CO₂ clamp) and interaction (phase x trial) were investigated using two-way repeated measures analysis of variance (ANOVA) and Holm-Sidak post hoc tests. Comparisons of changes from rest at 100 % of handgrip duration and PEMI were also made using two-way repeated measures ANOVA and Holm-Sidak post hoc tests. Normality was assessed using Shapiro-Wilk tests and data that were not normally distributed underwent log₁₀ transformation prior to analysis. Ratings of perceived exertion for each trial were compared using Wilcoxon signed rank test and the effect of condition on handgrip duration was examined using a paired t-test. Statistical analyses were undertaken using SigmaPlot 12, Systat Software Inc, UK. Statistical significance was set at *P*<0.05.

RESULTS

There was no significant difference observed in time to fatigue (118 ± 7 vs. 128 ± 11 s; P=0.12) and RPE (median, 18.5 [interquartile range, 17-19] vs. 18 [17.75-19]; P=1.00) between the control and P_{ET}CO₂ clamp trials, respectively.

In the control trial, $P_{ET}CO_2$ decreased progressively from rest during handgrip (by - 12.3 ± 3.7 % at 100 % handgrip duration) and remained below rest during PEMI (-16.9 ± 3.74 %; Figure 1a). By design, $P_{ET}CO_2$ remained unchanged from rest throughout the $P_{ET}CO_2$ clamp trial. Compared to the control trial, $P_{ET}CO_2$ was greater in the $P_{ET}CO_2$ clamp trials at 75 % and 100 % of handgrip, PEMI and recovery. In the control trial, V_E was increased from rest after 50 % of handgrip (by +10.7 ± 3.4 l·min⁻¹ at 100% of handgrip; P<0.05), and remained elevated during PEMI (+6.5 ± 2.1 l·min⁻¹; *P*<0.05 vs. rest; Figure 1b). No significant differences in V_E were observed between the control and $P_{ET}CO_2$ clamp trials (P=0.189).

Resting MCA V_{mean} was not different between trials (Figure 2a). However, whereas in the control trial MCA V_{mean} was unchanged from rest during handgrip, PEMI or recovery (P<0.05), MCA V_{mean} was significantly elevated from rest at 75 % of handgrip (+13.4 ± 2.1 %), 100 % of handgrip (+16.2 ± 2.9 %) and PEMI (+14.4 ± 2.7 %; P<0.05 vs. rest). Compared to the control trial, MCA V_{mean} was greater during the $P_{ET}CO_2$ clamp trial at 25 % (+5.0 ± 1.3 cm·s⁻¹), 75 % (+6.2 ± 2.2 cm·s⁻¹), 100 % of handgrip (+10.6 ± 4.1 cm·s⁻¹) and PEMI (+15.9 ± 2.6 cm·s⁻¹: P<0.05 vs. control). No significant differences in mean BP were found in the control and $P_{ET}CO_2$ clamp trials, and similar elevations from rest noted at 25 %, 50 %, 75 % and 100 % of handgrip and PEMI (P<0.001 vs. rest; Figure 3a). A decrease in CVCi at 75 %, 100 % of handgrip and PEMI was observed during the control trial (-0.11 ± 0.02, -0.16 ± 0.04 and -0.17 ± 0.04 cm·s⁻¹·mmHg⁻¹, respectively; P<0.05 vs. rest), whereas in the P_{ET}CO₂ clamp trial, a decrease in CVCi was only observed at 75 % and 100 % of handgrip (-0.10 \pm 0.02 and -0.11 \pm 0.03 cm·s⁻¹·mmHg⁻¹ respectively; P<0.05 vs. rest; Figure 2b). HR increased to a similar extent during handgrip in both trials (P<0.05 vs. rest), and returned to rest during PEMI (Figure 3b).

DISCUSSION

We sought to determine the influence of metabolically sensitive skeletal muscle afferents on cerebral perfusion. The major novel finding of this study is that only when $P_{ET}CO_2$ is clamped at baseline does MCA V_{mean} , an index of cerebral perfusion, increase during fatiguing isometric handgrip and isolated muscle metaboreceptor activation with PEMI. These findings support our initial hypothesis and suggest that muscle metaboreceptors increase cerebral perfusion during fatiguing isometric contraction of the forearm muscles and PEMI, but their influence is normally obscured by reductions in $P_{ET}CO_2$.

Increases in regional cerebral perfusion noted during handgrip are attenuated following axillary blockade of the exercising limb (Friedman et al., 1991; Friedman et al., 1992; Jorgensen et al., 1993). While indicative of the important role of sensory feedback from group III and IV skeletal muscle afferents in evoking the cerebral perfusion responses to exercise, these studies did not determine the relative contribution made by metabolically and mechanically sensitive muscle afferents. However, neither the selective activation of mechanically sensitive muscle afferents, using static or rhythmic passive calf stretch (Vianna et al., 2009), nor selective activation of metabolically sensitive muscle afferents, using PEMI, evokes an increase in cerebral perfusion (Jorgensen et al., 1992; Pott et al., 1997; Vianna et al., 2009). The latter may be interpreted as evidence that the muscle metaboreceptors do not influence cerebral perfusion, although an alternative explanation for these findings is that the PEMI maneuver evokes a confounding hyperventilation and reduction in P_aCO₂. Indeed, we observed that during PEMI following fatiguing handgrip V_E was elevated (by +6.5 \pm 2.1 1 min^{-1}) whilst P_{ET}CO₂ was decreased (by -6.6 ± 1.4 mmHg). Such findings are in accordance with Duncan et al (1981) and Piepoli et al (1999), the latter noting a 25 % increase in V_E during PEMI following fatiguing handgrip at 50% MVC. In addition, Jorgensen et al (1992) reported cerebral perfusion to be unchanged from rest during PEMI whilst PaCO2 and PETCO2 were decreased from 5.1 to 4.3 kPa and 4.5 to 3.9 kPa respectively. Importantly, when we clamped $P_{ET}CO_2$ during PEMI a notable increase of 14.4 ± 2.7 % in MCA V_{mean} was observed and CVCi was unchanged from rest, whereas when $P_{ET}CO_2$ fluctuated normally no change from rest in MCA V_{mean} was found and CVCi decreased by 22.9 ± 6.1 %. These findings support the concept that the activation of metabolically sensitive skeletal muscle afferents can increase cerebral perfusion during PEMI, but this may be masked by the confounding effects of a hyperventilation-induced reduction in $P_{ET}CO_2$ and consequent cerebral vasoconstriction.

Low to moderate intensity dynamic exercise is well established to induce regional increases in cerebral perfusion, however the effects of isometric exercise are equivocal with both increases (Orgogozo & Larsen, 1979; Vianna et al., 2009) and no change (Jorgensen et al., 1992) reported. One factor that may have contributed to these confounding reports is a hyperventilation-induced reduction in P_aCO₂. Indeed, unlike low to moderate intensity dynamic exercise, the respiratory response to isometric contraction of a small muscle mass is inappropriately high and a subsequent reduction in P_aCO₂ may be evoked (Duncan et al., 1981; Imms & Mehta, 1989). Imms et al (1998) found that MCA V_{mean} increased by 17 % during 2 min of isometric handgrip at 40% MVC in eucapnic subjects, whereas in subjects who hyperventilated during an identical handgrip protocol (P_{ET}CO₂ reduced by 8.2 to 15.1 mmHg), no increase in MCA V_{mean} was observed. This suggests that reductions in P_aCO₂ during isometric exercise could mask the exercise-induced increases in cerebral perfusion. During dynamic exercise at workloads corresponding to >60% maximal oxygen consumption a parallel reduction in P_{ET}CO₂ and MCA V_{mean} is observed (Moraine et al., 1993; Hellstrom et al., 1996; Ogoh et al., 2005; Fisher et al., 2008; Fisher et al., 2013b). However, if PETCO2 is clamped at during incremental maximal exercise not only is the drop in cerebral perfusion prevented but it increases in an exercise-intensity dependent manner (Olin et al., 2011; Subudhi *et al.*, 2011; Fluck *et al.*, 2014). Similar to dynamic exercise, when $P_{ET}CO_2$ is maintained at rest during fatiguing isometric handgrip an enhanced hyperemic response is observed, indicating that normal fluctuations in $P_{ET}CO_2$ restrain the cerebral perfusion responses. Compromised cerebral blood flow and oxygen delivery during exercise can reduce central motor drive causing premature fatigue (Rasmussen *et al.*, 2010b). However, fatiguing handgrip duration was not significantly prolonged when we clamped $P_{ET}CO_2$, suggesting that the hyperventilation-induced fall in cerebral perfusion did not contribute to the task failure. This is consistent with studies showing that while clamping $P_{ET}CO_2$ at resting values during incremental maximal cycling may increase cerebral perfusion and oxygen delivery, exercise performance is not enhanced (Olin *et al.*, 2011; Subudhi *et al.*, 2011; Fluck *et al.*, 2014).

Approximately 95 % of the handgrip-induced increase in MCA V_{mean} during the $P_{ET}CO_2$ clamp trial was preserved during PEMI. This suggests that muscle metaboreceptor stimulation has a major influence on the increase in MCA V_{mean} observed during handgrip. This may be due to a direct neural-vascular coupling effect or secondary to an increase in BP. Muscle metaboreceptor stimulation evokes a robust increase in sympathetic nerve activity to the heart and peripheral vasculature (skeletal muscle, splanchnic, renal), which increases BP secondary to an increase in vascular resistance and/or cardiac output (Murphy *et al.*, 2011; Fisher, 2014; McNulty *et al.*, 2014). The contribution of the sympathetic nervous system and BP to the exercise-induced increase in cerebral perfusion is controversial (Querido & Sheel, 2007; Strandgaard & Sigurdsson, 2008; van Lieshout & Secher, 2008). A key piece of evidence for this argument has been that muscle sympathetic nerve activity and BP remain elevated during PEMI while cerebral perfusion is at baseline (Ide & Secher, 2000; Querido & Sheel, 2007; Secher *et al.*, 2008). However, as we have demonstrated, the confounding effect of changes in $P_{ET}CO_2$ should be taken into account when examining the mechanisms responsible for changes in cerebral perfusion during PEMI. We cannot exclude a direct

contribution from the sympathetic nervous system to the cerebrovascular responses we observed during handgrip and PEMI, but the observation that the reduction in CVCi during PEMI was abolished when $P_{ET}CO_2$ was clamped at baseline suggests that its role is less significant than that of CO₂. Lucas et al., (2010) reported a 0.82 % change in MCA V_{mean} per mmHg change in mean BP, within the autoregulatory range and when P_aCO_2 was controlled. Given that mean BP was elevated by \approx 20 mmHg during PEMI, it is likely that this made a major contribution to the 14 % elevation in MCA V_{mean} we observed at this time.

We have employed the transcranial Doppler technique as an index of cerebral blood flow with the assumption that the diameter of the middle cerebral artery is constant (Willie *et al.*, 2014). Of note, exercise-induced increases in MCA V_{mean} are accompanied by increases in ipsilateral internal carotid artery blood flow (Hellstrom *et al.*, 1996), the 'initial slope index' of the ¹³³Xenon clearance method (Jorgensen *et al.*, 1992) and positron emission tomography evaluation of cerebral blood flow (Poeppel *et al.*, 2007). In addition, several previous reports have used transcranial Doppler ultrasonography measures of MCA V_{mean} during handgrip exercise (Giller *et al.*, 2000; Seifert *et al.*, 2010; Vianna *et al.*, 2012). We have also utilized P_{ET}CO₂ as a surrogate for P_aCO₂. While this may underestimate P_aCO₂ at rest (Robbins *et al.*, 1990) it is strongly correlated with P_aCO₂ at a range of tidal volumes and dead space (McSwain *et al.*, 2010), making it suitable for use in the present study.

Heightened muscle afferent activity has been identified in a number of patient populations (Sterns *et al.*, 1991; Piepoli *et al.*, 1996; Guazzi *et al.*, 2006; Houssiere *et al.*, 2007; Delaney *et al.*, 2010) and our findings suggest that as a consequence increases in cerebral perfusion may be attenuated in these groups during exercise. This may foster exercise intolerance via a central mechanism (Rasmussen *et al.*, 2010a) and diminish the cerebrovascular benefits of exercise, which are in part believed to be mediated by an exercise-induced increase in cerebral perfusion and shear stress (Davenport *et al.*, 2012;

Bolduc *et al.*, 2013; Joyner, 2014). Our observations also imply that $P_{ET}CO_2$ should be controlled in future studies examining regional cortical and brainstem activity during muscle metaboreflex isolation using PEMI.

In conclusion, these findings indicate that metabolically sensitive skeletal muscle afferents play an important role in the regional increase in cerebral perfusion observed in exercise, however a decrease in $P_{ET}CO_2$ secondary to hyperventilation can mask their influence when they are activated in isolation during PEMI.

COMPETING INTERESTS

None

AUTHOR CONTRIBUTIONS

Conception and design of the research was undertaken by JPF, data collection and analyses was undertaken by IDB, CS, LLS, ELS, BWLT and GMB, the manuscript was drafted by IDB and JPF, and all authors (IDB, CS, LMS, ELS, BWLT, GMB and JPF) contributed to data interpretation, editing and revision of manuscript, and approved the final version.

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FIGURE LEGENDS

Figure 1. Partial pressure of end-tidal carbon dioxide (P_{ET}CO₂; Panel A) and minute ventilation (Panel B) responses to handgrip and isolated muscle metaboreceptor activation with post-exercise muscle ischemia (PEMI). Rec, Recovery; * P<0.05 vs. Rest, † P<0.05 vs. Control trial.

<u>Figure 2.</u> Middle cerebral artery velocity (MCA V_{mean}; Panel A) and cerebral vascular resistance index (CVCi; Panel B) responses to handgrip and isolated muscle metaboreceptor activation with post-exercise muscle ischemia (PEMI). Rec, Recovery; * P<0.05 vs. Rest, † P<0.05 vs. Control trial.

Figure 3. Mean arterial pressure (MAP; Panel A) and heart rate (HR; Panel B) responses to handgrip and isolated muscle metaboreceptor activation with post-exercise muscle ischemia (PEMI). Rec, Recovery; * P<0.05 vs. Rest.

FIGURES



Figure 1.



Figure 2.



Figure 3.

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