

EFFECT OF INTENSE EXERCISE HYPERPNEA ON CEREBRAL BLOOD FLOW AND OXYGENATION

A MASTER THESIS

by

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Abstract

Introduction: Evidence indicates that the cerebral vessels is subjected to sympathetic nerve activity (SNA), yet whether or not human cerebral blood flow (CBF) is affected by SNA remains controversial. It has been observed that high-intensity exercise induced fatigue of the respiratory muscles can initiate a metaboreflex, causing sympathoexcitation and vasoconstriction in the limbs, thereby limiting limb blood flow and O₂ delivery to both the resting and exercising locomotor muscles. However, it is not known whether fatigue of the respiratory muscles can induce vasoconstriction of the cerebral vasculature and thus attenuate CBF and cerebral oxygenation. The aim of the present study was to control the influence of the arterial partial pressure of CO₂ on CBF, by clamping end-tidal PCO₂ (PETco₂) to isocapnic levels during high-intensity exercise, in order to examine the possible influence of a respiratory metaboreflex on CBF and cerebral oxygenation.

Methods: Twelve endurance-trained males participated in this cross-over study. Subjects visited the laboratory 3 times in total; 1 for familiarization including determination of maximal oxygen consumption (VO_{2max}), and 2 for either a control (CON) or isocapnic trial (ISO), consisting of constant load high-intensity ($\geq 85\%$ VO_{2max}) cycling to exhaustion. Mean blood flow velocity in the middle cerebral artery (MCA v_{mean}) and frontal lobe cerebral oxygenation (ScO₂) were measured during both trials with transcranial Doppler ultrasound and NIRS, respectively. To confirm the presence of respiratory muscle fatigue, maximal inspiratory pressure (MIP) was measured pre- and post-exercise.

Results: Mean exercise duration and intensity during the trials were 12 min 24.9 s \pm 1 min 18 s and 91 \pm 5 %VO_{2max}, respectively. During ISO Petco₂ was successfully clamped at 40 \pm 1 mmHg. The presence of respiratory muscle fatigue was confirmed by a 12% (P < 0.001^B) decrease in ISO and a 7% (P = 0.025^B) decrease in CON of MIP. MCA v_{mean} increased 12% in ISO from clamping to end-exercise (P = 0.017^B), while it remained unchanged during CON. Similar, at end-exercise MCA v_{mean} was higher during ISO than CON (P < 0.002^B). ScO₂ decreased in both ISO (P = 0.001^B) and CON (P < 0.001^B) at end-exercise, with no difference between trials.

Discussion: Although respiratory muscle fatigue was induced, we could not confirm the presence of a respiratory muscle fatigue induced decrease in MCA v_{mean} during ISO. In contrast, a decrease in ScO₂ was observed despite the increased MCA v_{mean}. Thus, it cannot be excluded that a SNA induced decrease in CBF did occur in other arteries, but that it remained undetected, since only flow velocity in MCA was measured. In conclusion, the presence of a respiratory muscle fatigue induced attenuation of CBF during high-intensity exercise could not be confirmed. Because the regulation of CBF is multifactorial, and this study only measured MCA v_{mean}, and controlled Paco₂ by means of clamping PETco₂, it cannot be excluded that a respiratory metaboreflex contributes to an overall SNA-induced regulation of CBF.

Introduction

Sympathetic nerve activity (SNA) in humans has been evaluated in various tissues, including the skin, lungs, kidneys, cardiac and skeletal muscles, and splanchnic circulation (Esler et al., 1984). With respect to the brain, even though it is recognised that the human cerebral vessels are innervated by sympathetic nerve fibers (Seifert & Secher, 2011; Lieshout & Secher, 2008), the functional consequences of this innervation is yet to be elucidated (Seifert & Secher, 2011). A possible role of the sympathetic nervous system in the regulation of cerebral blood flow (CBF) has been reported in animals (Cassaglia et al., 2008). Moreover, using the noradrenaline spillover technique, Mitchell et al. (2009) demonstrated in humans that the cerebral vessels are exposed to SNA. In addition, it has been shown that when cardiac output (CO) is restrained by a beta-1 adrenergic blockade, the observed decrease in CBF, measured as middle cerebral artery mean blood flow velocity (MCA v_{mean}), is eliminated when SNA is inhibited by stellate ganglion blockade, suggesting a role of the sympathetic nervous system in CBF regulation, at least during conditions of restrained CO (Ide et al., 2000). Nevertheless, despite the above indications, it is still a matter of discussion whether or not human CBF is affected by SNA (Strandgaard & Sigurdsson, 2008; Lieshout & Secher, 2008).

It has been observed that when performing whole body endurance exercise at intensities ≥ 80 -95% of maximal oxygen uptake ($VO_{2\text{max}}$), fatigue of the inspiratory muscles can occur in well-trained endurance athletes (Babcock, Pegelow, Johnson, & Dempsey, 1996; Johnson, Babcock, Suman, & Dempsey, 1993). Furthermore, it has been shown that this respiratory muscle fatigue provokes a metaboreflex, causing sympathoexcitation (St. Croix et al., 2000) and vasoconstriction in the limbs, thereby limiting limb blood flow and O_2 delivery to resting (Sheel et al., 2001) and exercising locomotor muscles (Harms et al., 1997). The significance of respiratory fatigue for exercise performance has been demonstrated with mechanical unloading of the respiratory muscles during high-intensity exercise, where exercise performance was enhanced compared with control high-intensity exercise and the associated high ventilatory demands (Harms et al., 2000).

It is well established that during exercise, vasodilation in the contracting skeletal muscles is critical in order to match O_2 delivery and demand (Joyner & Casey, 2015; Volianitis & Secher, 2016). However, during high-intensity whole body exercise, when the maximal

pumping capacity of the heart is approached, an increase in SNA-induced vasoconstriction is necessary for the preservation of arterial blood pressure (Calbet et al., 2004; Mortensen et al., 2008; Volianitis & Secher, 2002; Volianitis et al., 2003) and for redistribution of the available CO to the skeletal muscles (Calbet et al., 2007; Kagaya et al., 1994). This blood flow regulation is in line with the observation that locomotor muscle blood flow is reduced during exercise-induced respiratory muscle fatigue, indicating a redistribution of CO away from the limb muscles to the respiratory muscles in an attempt to “defend breathing” instead of locomotion (Harms et al., 1997). However, it is not known whether fatigue of the respiratory muscles can induce vasoconstriction of the cerebral vasculature and thus attenuate CBF and cerebral oxygenation. As exercise increases brain metabolism, cerebral vasoconstriction could compromise cerebral oxygen delivery and oxygenation, and thus induce so-called “central fatigue” and lead to the termination of exercise (Kayser, 2003; Nybo & Rasmussen, 2007), or in case of severe CBF reduction, loss of consciousness (Immink et al., 2014). In support of this notion, Rasmussen et al. (2010) found that cortical motor output decreased in parallel with cerebral deoxygenation during high-intensity exercise, indicating a relationship between cerebral oxygenation and central fatigue.

One main determinant of CBF is the arterial pressure of CO₂ (Paco₂) (Secher et al., 2008; Willie et al., 2014; Ainslie & Duffin, 2009). Light, and moderate dynamic exercise is associated with an increase in Paco₂ (Jørgensen et al., 1992a, 1992b) with a parallel increase in MCA v_{mean} (Jørgensen et al., 1992a, 1992b; Moraine et al., 1993). However, during conditions of intense hyperpnea, as during high-intensity whole body exercise, the associated hyperventilation reduces Paco₂ and provokes constriction of the cerebral vessels (Nybo & Rasmussen, 2007) with an associated decrease in MCA v_{mean} (Moraine et al., 1993), and a compromised cerebral O₂ delivery (Rasmussen et al., 2010).

In order to investigate the possible independent effect of a respiratory muscle fatigue induced metaboreflex on CBF it is critical to preserve Paco₂ at isocapnia, since exercise-induced hyperventilation lowers Paco₂, and thus brain perfusion. Previously, Siebenmann et al. (2013) examined the effects of hypocapnia and isocapnia on cerebral oxygenation, ventilation, and VO_{2max} during exercise. They executed two incremental cycle tests at 3454 m altitude with and without end-tidal CO₂ pressure (PETco₂) clamped at 40 mmHg. Even though the supplementation of CO₂ increased MCA v_{mean} and attenuated the reduction in cerebral

oxygenation, it did not influence VO_{2max} , indicating that the compromised cerebral oxygenation that resulted from the hypocapnia did not restrict maximal exercise capacity in hypoxia. Additionally, Fan et al. (2013) executed a cycle time trial in normoxia and hypoxia with $PETCO_2$ clamped at 45 mmHg. They observed an increase in MCA v_{mean} with CO_2 clamping in both hypoxia and normoxia, yet it did not affect the near-infrared spectroscopy (NIRS) determined cerebral oxygenation, or performance (Fan et al., 2013).

However, in the study by Siebenmann (2013), respiratory muscle fatigue most likely did not occur, as the exercise protocol was incremental and consisted of only a short duration of high-intensity work (~2 min above 200W). Similarly, in the study by Fan et al. (2013), the workload (initial workload of ~63 to ~78% VO_{2max} at the 14th km, for a 15 km cycle time trial) was not sufficient in eliciting a ventilatory response that could provoke respiratory muscle fatigue. Thus the question remains whether high-intensity exercise-induced respiratory muscle fatigue can reduce blood flow to the brain.

The aim of the present study was to control the influence of $Paco_2$ on CBF in order to examine the possible independent effect of a respiratory metaboreflex on CBF and cerebral oxygenation. We hypothesized that at end-exercise a decrement in MCA v_{mean} and cerebral oxygenation could be observed during $PETCO_2$ clamping, indicating a possible influence of respiratory muscle fatigue on CBF.

Methods

Subjects

Thirteen healthy endurance trained males were recruited for the present study following oral and written informed consent. The study was approved by the local ethics committee in accordance with the Declaration of Helsinki (H-16035280, Copenhagen, Denmark). One subject was excluded from analysis due to inadequate data, i.e. the loss of the MCA v_{mean} signal during the end of trial 2 (n=12, age: 25.3 ± 2.7 years, height: 183.3 ± 7.5 cm, body mass: 75.3 ± 9.2 kg, $\text{VO}_{2\text{max}}$: 61.7 ± 5.9 ml/kg/min and W_{max} : 371 ± 42.6 W).

Protocol

The subjects visited the lab at the same time of the day on three separate occasions. The subjects were asked to refrain from caffeine, and alcohol as well as any additional strenuous exercise at least 12 and 24 hours, respectively, prior to the visits. The first visit consisted of familiarization to the protocol and the execution of an incremental exercise test to exhaustion to determine $\text{VO}_{2\text{max}}$. The two following visits consisted of a constant-load exercise trial at $\sim 85\text{-}95\%$ of $\text{VO}_{2\text{max}}$ until exhaustion where the subjects either breathed ambient air (control trial, CON), or a mixture of ambient and CO_2 -enriched air (isocapnic trial, ISO) using a crossover design. All trials were conducted on an electronically braked cycle ergometer (Monark 839E, Varberg, Sweden). Visual feedback on exercise time and revolutions per minute (rpm) was given and the subjects were encouraged verbally to provide an all-out effort throughout all trials.

Familiarization: The subjects were familiarized with the maximal inspiratory pressure (MIP) maneuver and the inhalation of mixed ambient and CO_2 -enriched air. MIP was measured using a handheld mouth pressure meter to confirm if any respiratory muscle fatigue developed during the constant-load trials. The highest MIP obtained under the familiarization trial was noted and used to calculate the resistance for the inspiratory warm up in the following visits. Furthermore, the subjects' $\text{VO}_{2\text{max}}$ was determined using an incremental step test until exhaustion. After a light 10 min warm up at 100 W and a 2 min break, the subjects began the incremental test at an intensity of 200 W. The intensity was increased with 40 W every 2 min until the subjects stopped voluntarily. The maximal workload was calculated as $W_{\text{max}} = W_{\text{completed}} + W_{\text{increment}} * (t/120)$ where $W_{\text{completed}}$ was the workload of the last completed step, $W_{\text{increment}}$ was

the workload increment per step, and t was the number of seconds in the uncompleted step.

MIP measurements: Before the initiation of the constant-load trials, the subjects completed a standardized inspiratory warm up (2 sets of 30 repetitions with 40% of the MIP obtained during the familiarization, with 3 min rest between sets; Volianitis et al., 2001) using an inspiratory muscle trainer (POWERBreathe KH1, Warwickshire, England). After a 3 min break, 3 MIP maneuvers were performed. The highest of these 3 maneuvers (i.e. the most negative pressure obtained during 1 s) was reported as MIP_{pre} . Each maneuver was initiated from residual volume (RV) by exhaling slowly and completely until the lungs felt empty and the subjects were encouraged to inhale maximally for 2-3 s. One min recovery was provided between each maneuver. This approach of measuring MIP has been demonstrated as reliable with a mean between-day coefficient of variation (CV) and within-day CV of 3.5 and 4.5%, respectively (Hansen et al., unpublished data). One min after the completion of the constant-load exercise, another MIP measurement was performed, in order to confirm respiratory fatigue. Similar to pre-exercise, 3 maneuvers were performed of which the highest value was denoted as MIP_{post} . In order to evaluate, the effectiveness of the respiratory warm-up MIP was also measured immediately before initiation of the respiratory warm-up and compared with MIP_{pre} .

Exercise trials: Each trial started with a 3 min rest period where the subjects sat still on the bike while baseline measurements were collected. After this resting period, the subjects completed a 10 min warm up starting at 100 W increasing 10 W each min followed by a 2 min break and were then quickly (i.e. over a 1 min period) brought up to a workload corresponding to ~85-95% of VO_{2max} (~77% of W_{max}). The subjects maintained this workload for 10-12 min before transitioning into an all-out effort with increased intensity (i.e. a 20% increase in W) until voluntary exhaustion, breathing either ambient air (CON) or a mixture of ambient air and a CO_2 enriched gas (10% CO_2 , 21% O_2 69% N) (ISO). To compare the possible effect of respiratory muscle fatigue on CBF and cerebral oxygenation, the same exercise work had to be performed. Based on the assumption that ISO would increase the ventilatory response due to the supplementation of CO_2 thus decreasing total exercise time compared CON, and previous observations of decreased exercise performance during CO_2 supplementation (Subudhi et al.,

2011), ISO was executed first. Consequently, CON was executed last and followed the exact same protocol thus replicating the same absolute work (exercise duration and power output) as during ISO, i.e. with the only difference being the absence of any supplementation of the CO₂ enriched gas. During both trials, rpm was chosen freely.

In order to control PaCO₂ a PETco₂ clamp was constructed (Olin et al., 2012). During ISO, when PETco₂ decreased below 40 mmHg, the CO₂ enriched mixture was manually titrated into an open-ended inspiratory reservoir, by one of the investigators in response to online feedback from a computer screen (Fig. 1). The titration of the CO₂ enriched mixture allowed the PETco₂ to be clamped to isocapnia, thus preventing the wash-out of CO₂ caused by hyperventilation. We have previously demonstrated that it was possible to clamp PETco₂ within ± 1.7 mmHg during exercise with good reliability (CV < 4%) using this clamping procedure (Hansen et al., unpublished data).

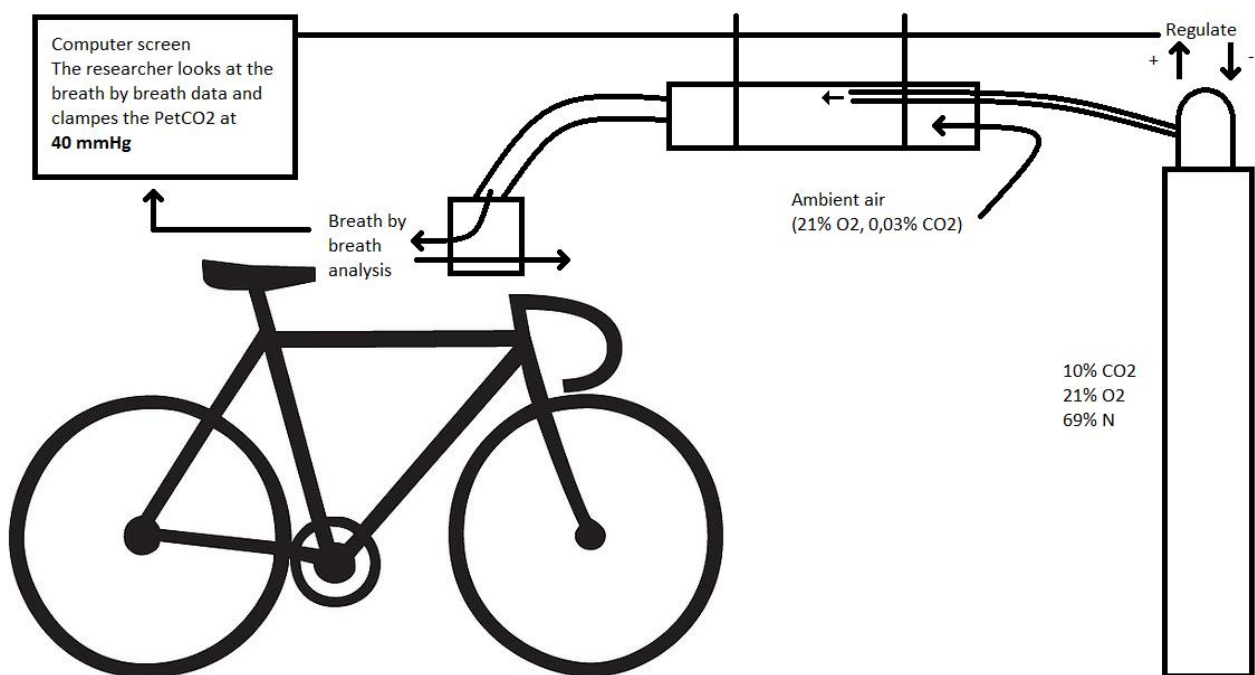


Fig. 1. End-tidal pressure of carbon dioxide (PETco₂) clamp. The operator visually monitors breath-by-breath measurements of PETco₂ by adding a fraction of CO₂ to an open-ended inspiratory reservoir in order to maintain isocapnia. Adopted from: Olin et al., 2012.

Cerebral blood flow and cerebral oxygenation

To evaluate CBF during ISO and CON, MCA v_{mean} was used as an index for CBF, as changes in MCA v_{mean} have been reported to reflect changes in CBF (Jørgensen, 1995; Secher et al., 2008). MCA v_{mean} was determined and calculated as the mean velocity of the time-averaged maximal velocity over the cardiac cycle derived from the envelope of the maximum frequencies from the Doppler spectra. Before the initiation of the constant load trials, the location of MCA of the subjects was found by insonation through the temporal window with trans-cranial Doppler ultrasound (2 MHz probe; Multidop X; DWL, Sipplingen, Germany). The position that elicited the highest signal to noise ratio (depth range: 48-60 mm) was marked and the probe was subsequently fixed to a custom-made headband and fastened with adhesive sonography gel. Data from the TCD were sampled at 100 Hz, and stored for offline analysis (Chart v5.2 and Powerlab; ADInstruments, Bella Vista, NSW, Australia).

To evaluate cerebral oxygenation (ScO_2), NIRS was used. The NIRS (INVOS 5100C, Somanetics, Troy, MI, USA) optode (3 and 4 cm emitter detector separation, wavelength 730 and 808 nm) was placed over the prefrontal cortical area between Fp1 and F3, or Fp2 and F4, according to the landmarks of the international 10-20 system, to avoid influence from the frontal and sagittal sinus (Perrey, 2008). Additionally, the NIRS optode was placed ipsilateral to the Doppler probe as it was expected that there were no differences between the two hemispheres (Siebenmann et al., 2013). The same unilateral placements of the Doppler probe and NIRS optode were repeated in both constant-load trials for each subject.

Ventilatory variables

During the experimental trials, the subjects wore a mask covering their nose and mouth (Hans Rudolf, Shawnee, Kansas, USA). Ventilatory variables (PETco_2 , ventilation (V_E), respiration frequency (RF), tidal volume (V_T), CO_2 production ($V\text{CO}_2$), O_2 consumption ($V\text{O}_2$), $V_E/V\text{CO}_2$ and $V_E/V\text{O}_2$) were measured breath-by-breath by a metabolic cart (Quark CPET, Cosmed, Rome, Italia). $V\text{O}_{2\text{max}}$ was calculated as the highest $V\text{O}_2$ obtained during 30 consecutive breaths (Siebenmann et al., 2013).

Cardiovascular variables

Heart rate (HR) was measured with a heart rate monitor belt (Garmin, Olathe, Kansas, USA). To measure arterial blood pressure and CO, a non-invasive photoplethysmography device was used (Nexfin: BMEYE, Model 2, Amsterdam, The Netherlands). A finger cuff was placed at the middle phalanx of the third finger. To adjust for the influence of hydrostatic pressure, a height sensor was fixated at heart level. In case of no signal, efforts were made to rewarm the fingers of the participants (Monnet et al., 2012).

Blood values

Additionally, for confirmation of PETco₂ as a surrogate measure of Paco₂, arterial blood samples were drawn from two subjects (Fig. 2) from the right brachial artery under cover of local anaesthesia (lidocaine 2%). The catheter (20G Arterial Cannula, BD, UT, USA) was sutured to the skin and flushed with saline after each blood sample. The blood samples were drawn during both ISO and CON at rest and every 2 min during exercise with the last measurement immediately before (n=1) and after (n=1) exhaustion. Each blood sample was approximately 5 mL (4 ml discarded, and 1 ml analyze) and total blood withdrawn was approximately 45 ml. The blood samples were analyzed using a blood-gas analyzer (ABL 800 FLEX Radiometer, Copenhagen, Denmark) for Paco₂ and lactate.



Fig. 2. An arterial blood sample is drawn from a subject during one of the experimental trials.

Data analysis and statistics

All descriptive data are presented as mean \pm SD. Cardiorespiratory variables were recorded continuously during both trials. All data from the metabolic cart, NIRS, TCD and Nexfin were exported to Microsoft Excel 2016 (Microsoft, Microsoft Redmont campus, Redmont, Washington, United States), then imported, processed, and analyzed with a custom-made program in MatLab R2016b (MathWorks, Natick, Massachusetts, United States). During both ISO and CON, all data were time aligned and averaged over a 15 s window immediately before the initiation of warm-up (representing rest), clamping (ISO) or $PET_{CO_2} < 40$ mmHg (CON), and end-exercise. $MCA v_{mean}$ and ScO_2 were calculated and reported as both absolute values and as percentage of rest, whereas all other variables were expressed in absolute values.

The objective of the present study was to test the effect of clamping PETco₂ at 40 mmHg on MCA v_{mean} and ScO₂ during conditions of respiratory muscle fatigue. Accordingly, MCA v_{mean} and other variables during ISO was compared at the point just before the initiation of clamping (i.e. when a drop in PETco₂ < 40 mmHg could be observed) to that at end-exercise (maximal fatigue), and these responses were then compared with CON at the same physiological time points (i.e. PETco₂ < 40 mmHg and end-exercise, respectively). These points were identified in each subject through a breath-by-breath analysis of PETco₂, and by the termination of exercise (same time point as ISO). Accordingly, to test for any significant differences at end-exercise compared to when PETco₂ dropped below 40mmHg (defined as 'pre-clamping' during ISO, and 'PETco₂ < 40mmHg' during CON), and between CON and ISO, paired student's t-tests were performed, corrected for multiple comparisons by use of the Bonferroni procedure, noted as ^B. Similarly, MIP_{pre} to MIP_{post}, and any baseline differences were tested with paired student's t-tests. The statistical significance level was set to $P \leq 0.05$. The statistical analysis was performed using SPSS Statistics 24 (IBM, Armonk, New York, United States).

Results

Main results are summarized in Table 1. There were no significant differences between ISO and CON on any variables at rest. In Table 1, the time points that were subjected to statistical analysis will be defined as: 'Rest', 'PETco₂ < 40 mmHg' (CON) and 'Pre-clamping' (ISO), and 'End-exercise' for both trials.

Exercise intensity and PETco₂ clamping

Mean exercise duration and intensity during the trials were 12 min 24.9 s ± 1min 18 s and 91 ± 5%VO_{2max}, respectively. Although external power output and duration were similar, a difference (P = 0.012) in %VO_{2max} measured from start to end-exercise was evident between ISO (90 ± 6%VO_{2max}) and CON (93 ± 5%VO_{2max}).

In ISO, PETco₂ was clamped until exhaustion to 40 ± 1 mmHg for 11 subjects and 44 ± 1 mmHg (target 44 mmHg) for one subject due to a higher resting PETco₂.

On average, subjects were PETco₂ clamped at 50% of total exercise time (± 15%) during ISO and went below a PETco₂ of 40mmHg at 46% of total exercise time (± 16%), during CON.

Maximal inspiratory pressure

To confirm the presence of respiratory muscle fatigue, MIP was measured pre- and immediately post exercise. From MIP_{pre} to MIP_{post} a decrease of 12% could be observed in ISO (163 ± 27 cmH₂O vs. 144 ± 21 cmH₂O, P < 0.001^B) and 7% in CON (159 ± 24 cmH₂O vs 148 ± 24 cmH₂O, P = 0.025^B), thereby confirming that the constant load trials were successful in inducing respiratory muscle fatigue. No difference was found between ISO and CON post-exercise.

Effect of respiratory warm-up: At ISO, MIP increased from 152 ± 20 cmH₂O to 163 ± 27 cmH₂O (P = 0.028), when measured before and after the respiratory warm-up. Similarly, during CON, MIP increased from 146 ± 19 cmH₂O to 159 ± 24 cmH₂O after the respiratory warm-up (P = 0.001).

Cerebrovascular variables

Cerebral blood flow velocity: MCA v_{mean} increased from clamping to end-exercise in ISO (74 ± 15.6 to 82.7 ± 23.6 cm/s; $P = 0.017^{\text{B}}$), while in CON, MCA v_{mean} remained unchanged (71 ± 22.2 to 67 ± 23.4 cm/s) from $\text{PETco}_2 < 40$ mmHg to end-exercise. Although there was no difference between ISO and CON at the onset of clamping and $\text{PETco}_2 < 40$ mmHg respectively, MCA v_{mean} was higher at end-exercise during ISO than CON (82.7 ± 23.6 vs 67 ± 23.4 cm/s; $P = 0.001^{\text{B}}$). MCA v_{mean} % change from rest increased by 10% from clamping to end-exercise in ISO (117 ± 14 to $129 \pm 18\%$; $P = 0.038^{\text{B}}$), while in CON, MCA v_{mean} remained unchanged from $\text{PETco}_2 < 40$ mmHg to end-exercise (108 ± 18 to $101 \pm 21\%$) (Fig. 3, A).

Cerebral oxygenation (n=11): ScO_2 decreased in both ISO and CON from clamping or $\text{PETCO}_2 < 40$ mmHg to end-exercise (73 ± 5.3 to $69.8 \pm 4.9\%$ and 75.9 ± 9.8 to $71.8 \pm 9.5\%$; $P = 0.001^{\text{B}}$ and $P < 0.001^{\text{B}}$, respectively). Yet, no differences between ISO and CON were observed at either clamping or $\text{PETco}_2 < 40$ mmHg or at end-exercise. As the signal was lost during exercise for one subject, only 11 subjects were compared.

Respiratory variables

End-tidal carbon dioxide pressure: During ISO, PETco_2 increased from 40 ± 2 mmHg at clamping to 41 ± 1 mmHg at end-exercise ($P = 0.005^{\text{B}}$), whereas PETco_2 decreased from 40 ± 1 to 34 ± 4 mmHg during CON ($P < 0.001^{\text{B}}$). There was no difference between ISO and CON at clamping or $\text{PETco}_2 < 40$ mmHg (40 ± 2 and 40 ± 1 mmHg), respectively. However, in CON, PETco_2 was lower at end-exercise compared to ISO (41 ± 1 vs 34 ± 4 mmHg; $P < 0.001^{\text{B}}$) (Fig. 3, B).

Ventilation: During both ISO and CON, V_E increased from clamping or $\text{PETco}_2 < 40$ mmHg to end-exercise (129.1 ± 11.6 to 175.6 ± 19.4 vs 125.4 ± 10.8 to 167.1 ± 18.3 l/min; $P < 0.001^{\text{B}}$). No differences were found between ISO and CON at any time point (Fig. 3, C).

Respiratory frequency: RF increased from clamping or $\text{PETco}_2 < 40$ mmHg in ISO and CON, respectively, to end-exercise (42 ± 8 to 62 ± 9 and 39 ± 7 to 59 ± 11 breaths/min; $P < 0.001^{\text{B}}$). At the onset of clamping or $\text{PETco}_2 < 40$ mmHg, ISO and CON respectively, ISO was higher than CON (42 ± 8 vs 39 ± 7 ; $P = 0.02^{\text{B}}$) while there was no difference at end-exercise.

Tidal Volume: V_T decreased in both ISO and CON from clamping or $\text{PETco}_2 < 40$ mmHg respectively, to end-exercise (3.2 ± 0.6 to 2.9 ± 0.7 and 3.3 ± 0.6 to 2.9 ± 0.6 l/breaths; $P = 0.004^{\text{B}}$

and $P < 0.001^B$, respectively). No differences were found between ISO and CON at any time point.

Oxygen Consumption: There was no change in $\dot{V}O_2$ from clamping to end-exercise during ISO (4244 ± 419 to 4316 ± 488 ml/min), while it increased from $PET_{CO_2} < 40$ mmHg to end-exercise during CON (4282 ± 467 to 4532 ± 432 ml/min; $P = 0.015^B$). Moreover, there were no differences between ISO and CON at any time point.

Carbon dioxide output: $\dot{V}CO_2$ increased in both ISO and CON from clamping or $PET_{CO_2} < 40$ mmHg, respectively, to end-exercise (4649 ± 403 to 5290 ± 858 and 4555 ± 438 to 4888 ± 574 ml/min; $P = 0.018^B$ and 0.028^B , respectively). No differences were found between clamping and $PET_{CO_2} < 40$ mmHg, while $\dot{V}CO_2$ at end-exercise was higher during ISO (5290 ± 858 vs 4888 ± 574 ml/min; $P = 0.03^B$).

Ventilatory equivalents: Both ISO and CON increased $\dot{V}_E/\dot{V}O_2$ (31 ± 2 to 41 ± 6 and 29 ± 2 to 37 ± 4 ; $P < 0.001^B$) and $\dot{V}_E/\dot{V}CO_2$ (28 ± 1 to 34 ± 4 and 28 ± 1 to 34 ± 4 ; $P < 0.001^B$) from clamping or $PET_{CO_2} < 40$ mmHg respectively, to end-exercise. $\dot{V}_E/\dot{V}O_2$ was higher in ISO than CON at the onset of clamping or $PET_{CO_2} < 40$ mmHg (31 ± 2 vs 29 ± 2 ; $P = 0.007^B$) and at end-exercise (41 ± 6 vs 37 ± 4 ; $P < 0.001^B$), while there was no difference in $\dot{V}_E/\dot{V}CO_2$.

Cardiovascular variables

Mean arterial pressure (n=11): MAP remained unchanged within ISO and CON at both time points (120 ± 17 to 117 ± 24 and 123 ± 10 to 126 ± 22 mmHg), and between clamping or $PET_{CO_2} < 40$ mmHg, and end-exercise. Due to the lack of resting measurements for one subject, only 11 subjects were included in the analysis.

Heart rate: HR increased from clamping or $PET_{CO_2} < 40$ mmHg to end-exercise in both ISO and CON (177 ± 8 to 187 ± 5 and 175 ± 8 to 187 ± 4 beats/min; $P < 0.001^B$). No differences among trials were found at any time point.

Cardiac output (n=4): CO remained unchanged in ISO and CON from clamping or $PET_{CO_2} < 40$ mmHg, respectively, to end-exercise (20.7 ± 2.7 to 23.4 ± 2.4 and 20.9 ± 0.7 to 22.2 ± 2.9 l/min). No differences were found between ISO and CON at any time point. Only 4 subjects were included in the analysis as one or several of the measurements (i.e. $PET_{CO_2} < 40$ mmHg or pre-clamping, and end-exercises) for the rest of the subjects were defined as outliers (range: 2.75 – 11 l/min at a HR of 174-186 bpm).

Table 1. *The effect of PETco₂ clamping throughout constant load exercise*

Variable	Control Trial			Isocapnic Trial		
	Rest	PETco ₂ < 40	End-exercise	Rest	Pre-clamping	End-exercise
Power, W	0 ± 0	286 ± 33	343 ± 39	0 ± 0	286 ± 33	343 ± 39
MCA v _{mean} , cm/s	65.0 ± 14.0	71.0 ± 22.2	67.0 ± 23.4	63.3 ± 11.5	74.0 ± 15.6	82.7 ± 23.6*†
ScO ₂ , %	74.5 ± 7.6	75.9 ± 9.8	71.8 ± 9.5*	72.2 ± 6.7	73.0 ± 5.3	69.8 ± 4.9*
PETco ₂ , mmHg	37 ± 2	40 ± 1	34 ± 4*	37 ± 2	40 ± 2	41 ± 1*†
V _E , l/min	12.9 ± 3.7	125.4 ± 10.8	167.1 ± 18.3*	13.7 ± 2.9	129.1 ± 11.6	175.7 ± 19.4*
RF, breaths/min	15 ± 3	39 ± 7	59 ± 11*	14 ± 4	42 ± 8†	62 ± 9*
V _T , l/breath	0.9 ± 0.3	3.3 ± 0.6	2.9 ± 0.6*	1.1 ± 0.6	3.2 ± 0.6	2.9 ± 0.7*
VO ₂ , ml/min	512 ± 162	4282 ± 467	4532 ± 432	512 ± 78	4244 ± 419	4316 ± 488
VCO ₂ , ml/min	426 ± 142	4555 ± 438	4888 ± 574*	444 ± 82	4649 ± 403	5290 ± 858*†
V _E /VO ₂	26 ± 2	29 ± 2	37 ± 4*	27 ± 3	31 ± 2†	41 ± 6*†
V _E /VCO ₂	32 ± 3	28 ± 1	34 ± 4*	32 ± 3	28 ± 1	34 ± 4*
MAP, mmHg	100 ± 7	123 ± 10	126 ± 22	99 ± 14	120 ± 17	117 ± 24
CO, l/min	5.7 ± 0.7	20.9 ± 0.7	22.2 ± 2.9	5.7 ± 2.7	20.7 ± 2.7	23.4 ± 2.4
HR, bpm	71 ± 14	177 ± 8	187 ± 4*	67 ± 10	177 ± 8	187 ± 5*

Values are means ± SD; n = 12 subjects, except ScO₂ n = 11, MAP n = 11, and CO n = 4. Power, power output; MCA v_{mean}, mean cerebral blood flow velocity of the middle cerebral artery; ScO₂, cerebral oxygenation; PETco₂, end-tidal carbon dioxide pressure; V_E, ventilation; RF, respiratory frequency; V_T, tidal volume; VO₂, oxygen consumption; VCO₂, carbon dioxide output; V_E/VO₂, ventilatory equivalent for oxygen; V_E/VCO₂, ventilatory equivalent for carbon dioxide; MAP, mean arterial pressure; HR, heart rate; CO, cardiac output. *Difference from PETco₂ < 40 mmHg or pre-clamping to end-exercise (P < 0.05). †Difference between isocapnic trial and control trial (P < 0.05)

Blood gas variables

The arterial blood samples (n=2) can be seen in

Table 2. The arterial blood samples confirmed elevation of P_{aCO_2} during ISO compared to CON at end-exercise (35.6 and 34.7 vs 32.2 and 30.5 mmHg, respectively). The correlation between P_{aCO_2} and P_{ETCO_2} were higher in CON ($r = 0.659$ and 0.935) than during ISO ($r = 0.475$ and 0.723).

Table 2. Arterial blood samples from two subjects during rest and throughout constant load exercise

Variable	Control trial								
	Lying rest	Sitting rest	2 min	4 min	6 min	8 min ¹	10 min ²	12 min	End-exercise
P_{aCO_2} (mmHg)	36.4	40.2	42.1	39.1	37.1	35	35	35.3	30.5
	40.4	38.6	49.4	45.9	43.4	38.3	35.6	32.2	ND
Lactate (mmol/l)	0.5	0.5	6.4	8.4	9.8	10.3	10.9	11	13
	1.3	0.8	6.7	9.3	11.3	12.8	14	14.8	ND
Variable	Isocapnic trial								
	Lying rest	Sitting rest	2 min	4 min	6 min	8 min ²	10 min ¹	12 min	End-exercise
P_{aCO_2} (mmHg)	32.2	40.8	41.4	40.8	40	36.5	37.2	34.5	34.7
	42.1	39.2	48.1	44.6	37.5	32.9	33.67	35.6	ND
Lactate (mmol/l)	0.6	0.4	6	7.7	9.7	9.7	10.8	12.2	14.2
	1.3	0.7	6.41	9	10.8	12.7	14.2	16	ND

Values for each blood sample; ND = no data; n = 2 subjects. n = 1 at end-exercise (13:26 min) as one subject terminated exercise immediately after 12 min (12.14 min) and thus had no further blood samples after the 12th min sample. ¹ Subject 1 clamping or $P_{ETCO_2} < 40$ mmHg, ² Subject 2 clamping or $P_{ETCO_2} < 40$ mmHg.

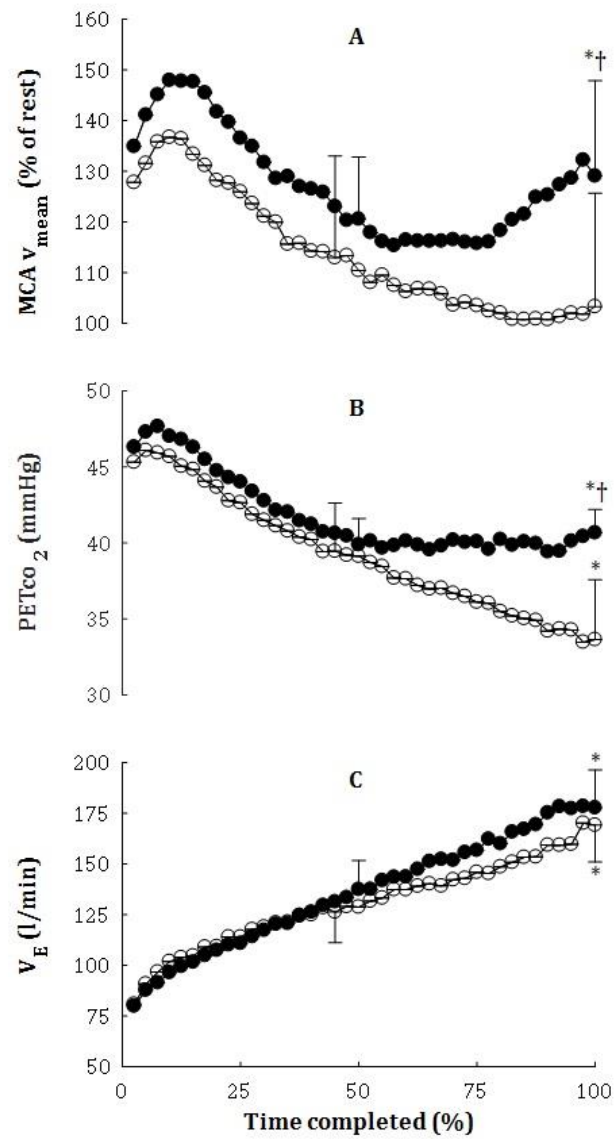


Fig. 3. Control (○) and Isocapnia (●) (n=12). Values are means at 0 to 100% of exercise time completed, and error bars represents SDs at the average time points of the statistical comparisons (pre-clamping and PETco₂ < 40, ISO and CON, respectively). Clamping increased MCA v_{mean}. PETco₂ decreased in control from PETco₂ < 40 to end-exercise and increased in ISO from pre-clamping to end-exercise. Ventilation (V_E) remained unchanged between trials. *Difference from PETco₂ < 40 mmHg or pre-clamping to end-exercise (P < 0.05). †Difference between isocapnic trial and control trial (P < 0.05)

Discussion

Contrary to our hypothesis, we could not confirm the presence of a respiratory muscle fatigue-induced decrease in MCA v_{mean} or cerebral oxygenation during isocapnia.

The respiratory metaboreflex has been shown to decrease blood flow to the resting (Sheel et al., 2001) and exercising (Harms et al., 1997) locomotor muscles during high-intensity exercise and thereby decrease exercise performance (Harms et al., 2000). The purpose of the present study was to investigate if this reflex could also attenuate blood flow to the cerebral vasculature and thus compromise CBF and cerebral oxygenation. Accordingly, to control the influence of P_{aCO_2} on vessel diameter and thereby CBF (Secher et al., 2008; Willie et al., 2014; Ainslie & Duffin, 2009), a P_{ETCO_2} clamp was constructed (Olin et al., 2012) which allowed us to manually titrate supplemental CO_2 whenever a drop below 40 mmHg in P_{ETCO_2} was observed.

Cerebral blood flow

The main findings of the present study were that clamping P_{ETCO_2} at 40 mmHg increased MCA v_{mean} compared to CON at end-exercise and that MCA v_{mean} increased throughout the clamping period in ISO, whereas it remained constant in the same period during CON. The idea of deliberately manipulating P_{ETCO_2} to examine CBF and cerebral oxygenation responses to exercise is in agreement with earlier studies (Subudhi et al., 2011; Siebenmann et al., 2013; Fan et al., 2013). However, this study is the first to examine if exercise-induced respiratory muscle fatigue can decrease indexes of CBF and cerebral oxygenation, i.e. MCA v_{mean} and NIRS-determined frontal lobe oxygenation. Fan et al. (2013) and Siebenmann et al. (2013) measured MCA v_{mean} but found no attenuation of MCA v_{mean} in response to incremental exercise to exhaustion. Yet, it is questionable whether the incremental exercise protocol used in their studies induced respiratory muscle fatigue and provided a strong enough stimulus to redistribute blood flow towards the respiratory muscles (Dempsey et al., 2002). In the present study, respiratory muscle fatigue was illustrated by a 7 – 12% reduction in MIP_{post} in response to the exercise trials. Nonetheless, no decreases in MCA v_{mean} or ScO_2 were observed during ISO, which could have several explanations: It is possible that the cerebral vasculature does not respond to exercise-induced increases in SNA in a manner that can reduce CBF significantly, thus supporting opponents against a functional role of SNA in CBF regulation (Strandgaard & Sigurdsson, 2008).

If a respiratory metaboreflex does in fact act on the cerebral vasculature, it cannot be excluded that its effect on CBF could be “masked” or overruled by other competing demands of the high-intensity exercise trials as we only controlled P_{aCO_2} through the manipulation of P_{ETCO_2} . For instance, Ogoh et al. (2005) demonstrated that increases in CO correlated with increases in MCA v_{mean} during whole-body exercise, indicating a relationship between the ability to increase CO, to support the increased demand for systemic O_2 delivery, and increases in CBF. Although it possibly reflects an underestimation of CO (the quality of our CO data is questionable, see Limitations) compared to previous observations in well-trained subjects at maximal whole-body exercise (Ekblom & Hermansen, 1968), we observed a mean CO of ~ 23 l/min ($n=4$) at end-exercise. Even if we use the conservative estimation of CO that we found in the present study, it is reasonable to assume that CO increased significantly during the trials to parallel the large VO_2 and HR at end-exercise (see Results), and thus according to the findings by Ogoh et al. (2005), could have affected v_{mean} . In addition, Jørgensen et al. (1992b) showed that MCA v_{mean} increased during heavy-exercise but P_{aCO_2} remained constant. Furthermore, they demonstrated that this increase in v_{mean} occurred independently of changes in MAP, and suggested it could be explained by an increase in brain activation and mechanoreceptor feedback. Together, these observations confirm what is already known about CBF, i.e. that multiple factors other than P_{aCO_2} regulate CBF (Willie et al., 2014; Secher et al., 2008). This could suggest that e.g. increases in CO, or increased neuronal activity, to sustain or even increase muscle fiber recruitment as the exercise trials progressed, could explain the increase in MCA v_{mean} in ISO despite the unchanged P_{ETCO_2} in the present study. Thus, the excessive demands from the exercise trials could have increased MCA v_{mean} comparatively more than a potential decrease in v_{mean} caused by a respiratory muscle fatigue-induced cerebral vasoconstriction. If true, most likely these factors and the associated increase in v_{mean} were also present during CON but overruled by the significant decrease in P_{ETCO_2} resulting from normal exercise-induced hyperventilation during maximal exercise.

Although it would violate the TCD-based assumption of a constant diameter, an alternative interpretation for the increase in MCA v_{mean} observed during ISO could be that SNA-induced vasoconstriction reduced MCA diameter, which accordingly would increase v_{mean} during conditions of maintained flow volume. Nevertheless, although the regulation of MCA

diameter during exercise has been debated (Pott, 2004; Willie et al., 2014) consensus seems to be that regulation of vessel diameter occurs in vessels downstream to MCA (Willie et al., 2014).

Besides the effect from respiratory muscle fatigue on sympathetic nerve traffic, respiratory activity per se has been reported to exert substantial influence on SNA (Dempsey et al., 2002; Eckberg et al., 1985), which perhaps could also have “masked” or negatively affected the possibility to detect changes in CBF due to SNA-induced vasoconstriction. For instance, it has been observed that during end-inspiration, when the lungs are maximally inflated, SNA is inhibited and opposite during end-expiration, SNA is augmented (Eckberg et al., 1985). Due to this within-breath variation in SNA, it could suggest that large changes in V_T and RF, as observed in the present study from rest to end-exercise (*Table 1*), could have affected the modulation of SNA during the exercise trials. Indeed, the respiratory modulation of SNA is augmented with increasing V_T beyond eupnea, as during exercise, and SNA is efficiently inhibited during inspiration with a very high V_T (Dempsey et al., 2002). Although no differences were observed in either VE, RF or V_T at end-exercise, the magnitude and the relative contribution of the inhibitory and facilitatory SNA response, and whether these were different between trials or not, remains unclear.

Due to the interaction of both exercise duration and intensity on respiratory muscle fatigue (Romer et al., 2007; Johnson et al., 1993; Babcock et al., 1995), it cannot be elucidated whether either of these parameters were of insufficient magnitude to provoke sufficient respiratory fatigue, thus failing to “unmask” the possible SNA-induced vasoconstriction of the cerebral vasculature. The presence of respiratory muscle fatigue was confirmed, illustrated by relative decrements of 12 and 7% in MIP, during ISO and CON, respectively. Although all the subjects were familiar with maximal exercise, confirmed by their well-trained status, and the fact that they did exercise to exhaustion at ISO, the decreases in MIP are somewhat in the lower boundary of previously reported mean decreases in MIP (8.2 – 28.6%) after whole-body exercise (Janssens et al., 2013). In a study by Babcock et al. (1996), high-fit and fit subjects cycled 15.2 ± 1.7 min and 17.9 ± 2.6 min, respectively, at 92-94% VO_{2max} , and the authors observed a mean decrement in transdiaphragmatic pressure of ~23%. These exercise durations are notably longer than in the present study, indicating an important contribution of exercise duration in order to provoke respiratory fatigue. In support, repeated fatiguing contractions of the respiratory muscles show a time-dependent augmentation of

muscle sympathetic outflow (Dempsey et al., 2002). Accordingly, although the mean exercise intensity and duration in the present study (91.4 %VO_{2max} and 12.4 min, respectively) are in agreement with other studies reporting the coincidence of respiratory muscle fatigue (Babcock et al., 1995, 2002), it cannot be excluded that a longer exercise duration could have increased the respiratory metaboreflex response. With respect to our data, a possible tendency towards a reduction in MCA v_{mean} to the very end of exercise in ISO can be seen in Fig. 3, A, which could represent a tendency to, or perhaps the beginning of, a reduction in MCA v_{mean} . It could be speculated whether this reflected some degree of vasoconstriction in the cerebral vasculature independent from decrements in PaCO₂. However, due to the absence of statistical significance, it should be interpreted with caution.

Perhaps another explanation could be related to the redundancy (Willie et al., 2014) and heterogeneity (Sato et al., 2011) of CBF. For instance, Sato et al. (2011) observed a redistribution of CBF during graded exercise, including a plateau in blood flow in the internal carotid artery supplying MCA with blood. Thus, it cannot be excluded that a SNA induced decrease in CBF did occur in other arteries, but that it remained undetected, since only flow velocity in MCA was measured. This could be supported by the observed decrease in ScO₂ despite the increased MCA v_{mean} during ISO.

Cerebral oxygenation

It has been postulated that maintenance of cerebral oxygenation in order to avoid “central fatigue” is important for exercise performance (Nybo & Rasmussen, 2007; Kayser, 2003; Rasmussen et al., 2010). In a recent study investigating a cohort of world-class Kenyan runners, Santos-Concejero et al. (2017) observed that cerebral oxygenation decreased progressively towards exhaustion during repeated high-intensity intervals. Additionally, they found that the runners who performed best and completed most intervals were also the runners who “defended” their cerebral oxygenation the most (i.e. who best preserved their cerebral oxygenation) indicating the importance of avoiding “central fatigue”. During ISO, a decrease in ScO₂ at end-exercise compared to pre-clamping was observed, however in contrast to CON, this decrease was accompanied by an increase in MCA v_{mean} . Moreover, the ScO₂ decrease in CON and ISO at end-exercise was not significantly different from each other, suggesting a similar decrement in cerebral oxygenation from rest, despite differences in MCA v_{mean} . Given the

proposed relationship between voluntary exhaustion and a decrease in cerebral oxygenation (Santos-Concejero et al., 2017), the fact that ScO₂ was reduced at end-exercise in ISO is not surprising, however the fact that it did not follow MCA v_{mean} is. One explanation for this observation could be that CBF did not increase to the pre-frontal cortex and thus did not affect the NIRS-determined ScO₂. Because this study only measured v_{mean} and ScO₂ in one cerebral artery and in the frontal lobe, respectively, it remains unknown whether cerebral oxygenation increased elsewhere in response to a possible increase in CBF to other parts of the brain, as supported by the findings of Sato et al. (2011) of a heterogenic CBF regulation during exercise. According to this interpretation, it cannot be excluded that changes in CBF and cerebral oxygenation happened elsewhere, which just remained undetected in the present study.

Another suggestion could be that exercise-induced metabolic acidosis, and the corresponding lack of an effect from respiratory compensation due the continuous supplementation of CO₂, resulted in a rightward shift on the oxyhemoglobin curve and thus decreased SO₂. This could also have affected the SO₂ at brain level and might explain the decrease in ScO₂ from pre-clamping to end-exercise despite the increase in CBF. This would be in line with the observed difference in %VO_{2max} between trials (P = 0.012) where ISO did the same external work but with at a significant lower %VO_{2max} compared to CON, perhaps indicating a lower SO₂.

In combination with the above, an alternative speculation could be that a minor redistribution of CO to the brain, away from the active limb muscles, could contribute to the different %VO_{2max} between trials. During exercise, as different organ systems are competing for the available CO (Volianitis & Secher, 2016), less distribution of blood and thus VO₂ to the working limb muscles at the same power output could decrease %VO_{2max}. Although blood samples were only taken from two subjects in the present study, lactate concentrations seemed to be elevated in ISO at end-exercise compared to CON (Table 2) which would be in line with previous reports of increased RPE and/or metabolic acidosis during PETco₂ clamping (Subudhi et al., 2011; Fan & Kayser, 2013). Thus, it could be speculated whether some kind of metabolic acidosis occurred at a higher rate during ISO compared to CON, which perhaps could be due to less CO available for the working muscles, or a rightward shift on the oxyhemoglobin curve, Yet, precaution should be taken because no direct evidence to support these speculations were obtained.

Maximal inspiratory pressure

Fatigue can be defined as *"a loss in the capacity for developing force and/or velocity of a muscle, resulting from muscle activity under load and which is reversible by rest"* (Aubier et al., 1990). This study evaluated the presence of respiratory muscle fatigue by measuring the largest negative pressure that could be generated at the mouth. Although no definite consensus of the optimal protocol to evaluate respiratory muscle fatigue exist (Janssens et al., 2013), this way of measuring the respiratory muscles could have some potential limitations that need to be addressed. Firstly, it relies on the volitional effort of the subjects, thereby relying on subject motivation (Aldrich & Spiro, 1995) and day-to-day variability in strength (Harms et al., 2000). Secondly, it provides a measure of global inspiratory muscle strength (Larson et al., 1993) and thus it does not measure the isolated force generation by the diaphragm as evaluated during the measurement of transdiaphragmatic pressure (Roussos & Macklem, 1977). Thirdly, in line with previous studies (Volianitis et al., 2001; Wen et al., 1997), there was no direct control of when RV was reached. Differences in thoracic gas volume have shown to affect both MIP and voluntary activation, with the highest pressures and voluntary activation obtained at low and high lung volumes, respectively (McKenzie et al., 1996). Accordingly, differences in RV could have affected the subsequent MIP. To overcome this, standardization was made by encouraging subjects to fully empty their lungs and to familiarize them to the procedure before the first trial. Indeed, it has been shown that maximal inspiratory mouth pressure can be reliably measured over consecutive days (Maillard, Burdet, Van Melle, & Fitting, 1998) and that the reliability increases if preceded by an inspiratory warm-up (Volianitis et al., 2001). Data from our laboratory has confirmed the good reliability by demonstrating a mean between-day CV and within-day CV of 3.5 and 4.5%, respectively, using the same MIP protocol as in the present study (Hansen et al., unpublished data). Moreover, even though the present study did not use phrenic nerve stimulation but relied on voluntary effort, it has been shown that mouth measured MIP reflects transdiaphragmatic pressure in response to phrenic nerve stimulation in healthy subjects (Hamnegård et al., 1995), suggesting that MIP can be used as a valid, non-invasive alternative to transdiaphragmatic pressure measurements.

Our own observations during the study supported the importance of measuring MIP immediately after end-exercise, illustrated by MIP measured 1 min post-exercise was significantly lower ($P = 0.013$) than 3 min post-exercise (of note, only the highest of the 3 MIP post-exercise is reported (i.e. MIP_{post}). This is in line with the fast recovery rate after high-

frequency fatigue (Carroll et al., 2017) and earlier observations of fast recovery of respiratory muscle strength after exercise (Coast et al., 1990; Bye et al., 1984). For instance, Bye et al. (1984) observed that after high-intensity cycling, transdiaphragmatic pressure was significantly reduced within 2 min post-exercise, yet after 5 min it was virtually returned to baseline. Similar, Coast et al. (1990) observed a decrease in MIP at 17% and 13%, respectively 60 and 120 s post-exercise, indicating a relatively fast rate of recovery.

As a secondary objective, we evaluated the effect of the respiratory warm-up on MIP and whether the warm-up would increase MIP as demonstrated by others (Volianitis et al., 2001). We observed an increase in MIP during both ISO and CON, confirming that the warm-up worked as intended.

Limitations

Cardiac output and mean arterial pressure

Measurements of finger arterial pressure has been shown to track arterial blood pressure and CO during resting conditions (Bogert & van Lieshout, 2005). Moreover, continuous, beat-to-beat analysis of CO by the Nexfin® device has been recommended as a suitable and non-invasive method to assess CO during exercise (Bartels et al., 2011). However, in this study, the validity of the CO measurements is questionable, indicated by the fact that 8 subjects had measured a CO of <11 l/min at end-exercise, and thus were excluded from analysis. Subudhi et al. (2011) also used the Nexfin® device during exercise to exhaustion, however no results were provided regarding either arterial blood pressure or CO, which could be interpreted as a lack of valid measurements. The questionable use of this device to measure CO during exercise is supported by the observation of a poor estimation of both absolute and relative changes in CO during volume expansion by use of the Nexfin® device (Monnet et al., 2012). With respect to MAP, no differences in MAP were evident within or between ISO and CON, suggesting that arterial blood pressure were maintained despite the increased demand for blood flow to the exercising limbs in response to the demands from the exercise. Nevertheless, because MAP was measured by the same device as CO, we suggest that these values should be interpreted with caution.

Cerebral blood flow velocity

In this study, MCA v_{mean} was used as a surrogate measure for changes in CBF. As this approach measures flow velocity rather than volume flow (Aaslid et al., 1982) it is based on the assumption that the diameter of the insonated vessel remains constant. Even though it has been argued that high levels of P_{aco_2} could jeopardize this premise (Willie et al., 2014), Serrador et al. (2000) found no changes in MCA diameter during hypocapnia, normocapnia or hypercapnia in conscious humans. A study using high-resolution MRI (Verbree et al., 2014) recently confirmed this by observing no change in MCA diameter within ± 7.5 mmHg of resting P_{ETco_2} , which are within the levels measured in the present study. Nevertheless, considering the assumption of a constant diameter for the validity of TCD and the heterogeneity of CBF redistribution, it could be argued that it is inconclusive to evaluate the possible effect of SNA on global CBF with use of a single TCD.

NIRS-determined cerebral oxygenation

It should be considered that NIRS-determined ScO_2 might be affected by changes in skin blood flow (Sørensen et al., 2015). Similar, Davis et al. (2006) observed a good correlation between forearm muscle oxygenation and skin blood flow at different temperatures during conditions of both local and whole-body heating, suggesting a contribution of skin blood flow to the measurements of tissue oxygenation. Although we did not measure it directly, it could be argued that skin blood flow was not significantly different between the two trials, as the same amount of external work was completed. In support for this are the equal ambient temperature and relative humidity during ISO (23 ± 1 C°, $23 \pm 4\%$) and CON (23 ± 1 C°, $26 \pm 4\%$), respectively. Consequently, it is unlikely that differences in skin blood flow could have significantly affected ScO_2 .

PETco₂ as a surrogate for Paco₂

A limitation of the present study is that we clamped P_{ETco_2} rather than P_{aco_2} . Although we succeeded to clamp P_{ETco_2} within 40 ± 1 mmHg, the $P_{\text{ETco}_2} - P_{\text{aco}_2}$ gradient changes during exercise (Jones et al., 1979) and therefore, it is possible that we did not manage to clamp P_{aco_2} per se. To confirm whether the P_{ETco_2} clamp worked as intended, i.e. to maintain P_{aco_2} despite

pronounced exercise induced hyperpnea, arterial blood samples were drawn from two subjects. Data from these subjects confirmed that clamping PETco₂ increased Paco₂ compared to CON (Results), however in line with earlier observations, PETco₂ tended to overestimate Paco₂ during end-exercise (Subudhi et al., 2011), and underestimate Paco₂ during rest (Jones et al., 1979). On the other hand, it has been found that when Paco₂ was corrected for blood temperature, no differences could be observed between Paco₂ and PETco₂ during high-intensity exercise (Losa - Reyna et al., 2015), suggesting that PETco₂ is a useful, non-invasive surrogate for Paco₂. Moreover, controlling Paco₂ during exercise by use of PETco₂ clamping is in agreement with earlier studies (Fan et al., 2013; Fan & Kayser, 2013; Siebenmann et al., 2013; Subudhi et al., 2011) and is to our knowledge the only non-invasive way to monitor Paco₂.

In conclusion, the presence of a respiratory muscle fatigue induced attenuation of CBF and cerebral oxygenation during high-intensity exercise could not be confirmed. However, because the regulation of CBF is multifactorial, and this study only measured MCA v_{mean} , and controlled Paco₂ by means of clamping PETco₂, it cannot be excluded conclusively that a respiratory metaboreflex contributes to an overall SNA-induced regulation of CBF. Indeed, a reduction in frontal lobe oxygenation was observed during PETco₂ clamping, although this was not reflected in an attenuated MCA v_{mean} . In addition, further studies investigating whether a possible dose-response relationship exists between exercise intensity and or duration on CBF regulation, and the use of transcranial magnetic stimulation to evaluate the possible respiratory muscle fatigue-induced contribution to central and peripheral fatigue are needed.

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