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CO_2 supplementation dissociates cerebral oxygenation and middle cerebral artery blood velocity during maximal cycling

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Abstract

This study evaluated whether the reduction of prefrontal cortex oxygenation (ScO_2) during maximal exercise depends on the hyperventilation-induced hypocapnic attenuation of middle cerebral artery blood velocity (MCA V_{mean}). Twelve endurance-trained males (age: 25 ± 3 years, height: 183 ± 8 cm, weight: 75 ± 9 kg; mean ± SD) performed in three separate laboratory visits, a maximal oxygen uptake (VO_2max) test, an isocapnic (end-tidal CO_2 tension ($PetCO_2$) clamped at 40 ± 1 mmHg), and an ambient air controlled-pace constant load high intensity ergometer cycling to exhaustion, while MCA V_{mean} (transcranial Doppler ultrasound) and ScO_2 (NIRS) were determined. Duration of exercise (12 min 25 s ± 1 min 18 s) was matched by performing the isocapnic trial first. Pulmonary VO_2 was 90 ± 6% vs. 93 ± 5% of the maximal value ($P = 0.012$) and $PetCO_2$ 40 ± 1 vs. 34 ± 4 mmHg ($P < 0.05$) during the isocapnic and control trials, respectively. During the isocapnic trial MCA V_{mean} increased by 16 ± 13% until clamping was applied and continued to increase (by 14 ± 28%; $P = 0.017$) until the end of exercise, while there was no significant change during the control trial ($P = 0.071$). In contrast, ScO_2 decreased similarly in both trials (-3.2 ± 5.1% and -4.1 ± 9.6%; $P < 0.001$, isocapnic and control, respectively) at exhaustion. The reduction in prefrontal cortex oxygenation during maximal exercise does not depend solely on lowered cerebral blood flow as indicated by middle cerebral blood velocity.

Key words: middle cerebral artery blood velocity, cerebral oxygenation, end-tidal PCO_2, controlled-pace exercise

1. **Introduction**

Cerebral blood flow (CBF) is tightly coupled to cerebral metabolism with a "surplus" increase in flow reflected in the blood oxygenation level-dependent signal[1] and in the near-infrared spectroscopy (NIRS) detected oxygenation.[2] Low to moderate intensity dynamic whole body exercise elevates CBF as evaluated by ^{133}Xe clearance,[3,4] middle cerebral artery mean flow velocity (MCA V_{mean}),[4,5,6] and evaluation of flow in the internal carotid and vertebral arteries.[7] The increase in MCA V_{mean} during low to moderate exercise is attributed, at least in part, to the initial increase in arterial carbon dioxide tension ($PaCO_2$) in response to exercise metabolism. Yet, as exercise intensity progresses, MCA V_{mean} is either plateuing, or reduced towards the resting value, dependent on the magnitude of hyperventilation-induced hypocapnia,[3,4,8] that may reduce $PaCO_2$ to as low as ~29 mmHg.[9] A similar response pattern is described for the NIRS determined frontal lobe oxygenation (ScO_2) with an increase during low intensity exercise and then a gradual decrease when exercise intensity approaches a maximal level and exercise-induced hypocapnia develops.[9] This parity in the responses of ScO_2 and MCA V_{mean} during low to moderate exercise has been linked to the neuronal activation[2], while as exercise intensity progresses humoral factors (f.x., hyperventilation-induced hypocapnia) are suggested to have a more prominent role.[10]

The relationship between ScO_2 and MCA V_{mean} and its relevance for exercise performance has been addressed by preventing the exercise hyperventilation-induced hypocapnic reduction in CBF with CO_2 supplementation during incremental[11-14] and time trial cycling exercise[15]. CO_2 supplementation enhances cerebral oxygen delivery without affecting systemic oxygen delivery and thus, allows the exclusive evaluation of the effect of cerebral oxygenation on exercise performance. However, even though clamping the end-tidal PCO_2 ($PetCO_2$) increased MCA V_{mean} and attenuated the decrease in ScO_2, exercise performance was not enhanced.[11,13,14] During both maximal incremental exercise[12] and time trial cycling exercise at ~65-85% of maximal oxygen uptake (VO_2max)[15] the increase in MCA V_{mean}, besides not enhancing exercise performance, did not attenuate the decrease in ScO_2, i.e. suggesting a seemingly mismatch between cerebral activation and cerebral oxygenation[16]. Since controlled-pace exercise may impose higher metabolic demands than self-paced exercise,[17] it was considered that increased afferent stimulation during controlled-pace exercise may impose an even greater metabolic demand on the prefrontal cortex[18] than incremental and self-paced exercise and thus provoke higher cerebral (de)oxygenation.[19] The cerebral (de)oxygenation and MCA V_{mean} responses during

controlled-pace maximal exercise have not being evaluated and we hypothesized that during controlled-pace maximal exercise, the parity beetween ScO_2 and MCA V_{mean} would be challenged.

In order to test this hypothesis we evaluated ScO_2 and MCA V_{mean} during controlled-pace high-intensity (90-95% VO_2max) combined with a final all-out sprint to exhaustion simulating, e.g. short cycling time trials.[20] Considering that hyperventilation-induced hypocapnia during high intensity exercise attenuates MCA V_{mean}, we clamped $PetCO_2$ at 40 mmHg with CO_2 supplementation. In order to evaluate whether a similar effort was required with and without CO_2 supplementation, we assessed motor activation of the inspiratory muscles by measuring maximal inspiratory pressure (MIP) before and after exercise, as has been evaluated in other skeletal muscle groups following exhaustive leg (e.g.[21]) and handgrip exercise.[22] During high intensity whole body endurance exercise large ventilatory demand may provoke respiratory fatigue even in endurance athletes.[23]

2. Methods

2.1. Subjects and study design

Thirteen healthy endurance trained males (VO_2max: 62 ± 6 ml/kg/min), following informed written and oral consent, volunteered to participate in the study as approved by the Ethical Committee of Copenhagen (H-16035280) and confirming to the *Declaration of Helsinki*, except for registration in a database. Sample size estimation was based on ScO_2 during exercise with and without a CO_2 clamp[15] and power of 0.90 with an α of 0.05. One subject was excluded from analysis due to inadequate Doppler signal during exercise, thus results from 12 subjects are reported (age 25.3 ± 2.7 years, height: 183 ± 8 cm, body mass: 75 ± 9 kg).

The subjects visited the laboratory at the same time of the day on three occasions after having refrained from caffeine, alcohol, and strenuous exercise for at least 24 h. At the first visit, the subjects were familiarized with inhalation of CO_2-enriched air and then performed incremental cycling (Monark 839E, Varberg, Sweden) to exhaustion for determination of VO_{2max}. Following a 10 min warm-up at 100 W and 2 min recovery, the subjects cycled at 200 W with the workload increased by 40 W every 2 min until exhaustion. The maximum workload was calculated as $W_{max} = W_{completed} + 40\ W * (t/120)$, where $W_{completed}$ is the workload of the last completed step and t the seconds at the uncompleted step. On the two following visits, constant-load (~90-95% VO_{2max}) cycling to exhaustion was carried out while breathing either ambient air (control trial), or ambient air and a mixture of ambient and CO_2-enriched air (10% CO_2, 21% O_2, 69% N_2; isocapnic trial).

The workload during the two trials was determined based on that associated with 90% VO_{2max} during the VO_{2max} test. The targeted workload was maintained for 10-12 min, depending on the subject's capacity, and when the subjects approached fatigue they were exposed to a ~20% increase in power output in an all-out sprint to exhaustion. The rationale for the final all-out sprint was both to maximize cerebral metabolic demand and, to simulate the finishing sprint of most cycling races. The subjects received visual feedback of exercise duration and encouragement to provide an all-out effort, while the pedaling frequency was freely chosen. During the isocapnic trial, $PetCO_2$ was monitored visually and maintained at 40 mmHg by manually administering the CO_2-enriched air to an open-ended reservoir that served as mixing chamber with ambient air [24, Fig.1, Clamp). To partially control for the potential respiratory acidosis and increased ventilatory drive induced with CO_2 supplementation, we clamped $PetCO_2$ only when subjects began developing hyperventilation-induced hypocapnia (i.e. $PetCO_2$ falling below 40 mmHg). In a pilot study, $PetCO_2$ could be maintained within ± 1.7 mmHg (reliability, coefficient of variation < 4%). Considering that supplementation of CO_2 during the isocapnic trial could increase ventilation (V_E) and thus, possibly limit exercise performance,[11] the isocapnic trial was carried out first so that the total work performed could be matched in the control trial. Matching the work performed in the two trials allows for a comparison of the cerebrovascular responses to assumed comparable cerebral neural stimulation while the humoral stimulus from the hyperventilation-induced hypocapnia is diminished.

2.2. Cerebral blood velocity and oxygenation

Trans-cranial Doppler ultrasound (TCD; 2 MHz probe; Multidop X; DWL, Sipplingen, Germany) determined MCA V_{mean} and used as an index of CBF, since changes in MCA V_{mean} reflect those of CBF during dynamic exercise.[25,26] MCA V_{mean} was 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. MCA was located unilaterally by insonation through the temporal ultrasound window and the position with the highest signal to noise ratio (depth 48-60 mm) marked. The probe was fixed to a headband with adhesive sonography gel and data sampled at 100 Hz with an AD converter (Chart v5.2 and Powerlab; ADInstruments, Bella Vista, NSW, Australia) and analysed offline (Labchart ver. 7.3, AD Instruments, Colorado Springs, CO, USA). ScO_2 was evaluated using NIRS (INVOS 5100C, Somanetics, Troy, MI, USA) with a sampling frequency of 0.3 Hz. The 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 10-20 system to avoid influence from the frontal and sagittal sinus[26] and ipsilateral to the Doppler probe. Considering the wide intra-individual baseline variability (coefficient of variation for absolute baseline values of approximately 10%, [28]) of cerebral oximetry, we used NIRS data as a trend monitor. It was considered that there are no differences between the two hemispheres during cycling[9,13] and the same placement of the TCD probe and NIRS optode was applied in both trials.

2.3. Ventilatory variables

$PetCO_2$, V_E, respiration frequency (R_F), tidal volume (V_T), CO_2 production (VCO_2), VO_2, and ventilatory equivalents for CO_2 (V_E/VCO_2) and O_2 (V_E/VO_2) were measured breath-by-breath by a metabolic cart (Quark CPET, Cosmed, Rome, Italy). Calibration before each trial was according to the manufactures guidelines and VO_{2max} was taken as the average VO_2 over 30 breaths.[13]

2.4. Cardiovascular variables

Heart rate (HR) was measured with a belt (Garmin, Olathe, Kansas, USA) and mean arterial pressure (MAP) and cardiac output (CO) using finger plethysmography with a cuff placed at the middle phalanx of the third finger connected to a non-invasive monitor (Nexfin: BMEYE, Model 2, Amsterdam, The Netherlands). Values were corrected for the influence of hydrostatic pressure with a height sensor fixed at the estimated level of the heart. In case of inadequate signal, the finger was warmed.[29]

2.5. Blood sampling

$PaCO_2$ and arterial oxygen saturation (SaO_2) were evaluated in two subjects by samples drawn from a line in the right brachial artery under local anaesthesia (lidocaine 2%). The blood samples were drawn at rest and every 2 min during both exercise trials with the last sample immediately before and after (n = 1) exhaustion and analyzed immediately (ABL 800 FLEX Radiometer, Copenhagen, Denmark).

2.6. Motor activation

MIP was evaluated by a maximal voluntary inspiratory maneuver (Mueller) using a handheld mouth pressure meter (POWERBreathe KH1, Warwickshire, England). The subjects were familiarized with the maneuver by performing an inspiratory warm up that increases reliability.[30] Each maneuver was initiated from residual volume (RV) by exhaling slowly until the subjects felt their lungs "empty" and they were then encouraged to inhale with a maximal effort for 2-3 s. Before the exercise trials, the subjects performed the inspiratory warm up and subsequently 3 MIP maneuvers with 1 min recovery between efforts and the most negative pressure over 1 s noted as

MIP_{pre}. A pilot study indicated that the mean between-day CV and within-day CV for MIP_{pre} was 3.5% and 4.5%, respectively. Immediately after completion of the exercise trials, another MIP was determined and noted as MIP_{post}.

2.7. Statistical methods

All variables were recorded continuously, analyzed with a custom-made program in MatLab R2016b (MathWorks, Natick, Massachusetts, USA), and presented as mean ± SD. In order to account for the different sampling frequencies between the NIRS and TDC devices, NIRS data were interpolated to the same length as the TCD data (100 Hz), averaged over 15 s and time aligned according to the conditions established during the trials: 1) immediately before the warm-up (Rest), 2) time of $PetCO_2$ clamping during the isocapnic trial (Clamp) and time that $PetCO_2$ dropped below 40 mmHg during the control trial (No-clamp), and 3) in the last 15 s of intense exercise (end-exercise). Variables are reported as absolute values, and for MCA V_{mean} and ScO_2, also as percentage of values at rest. Comparisons of data at Clamp vs. end-exercise during the isocapnic trial, at No-clamp vs. end-exercise during the control trial, and between trials, allowed for evaluation of the effect of high intensity exercise to exhaustion on MCA V_{mean} and ScO_2. A two-way ANOVA for repeated-measurements was used to evaluate differences between (control and isocapnic) and within (Noclamp/Clamp and at end-exercise) trials with Bonferroni corrected p-values according to the number of comparisons between and within trials. Similarly, differences between MIP_{pre} and MIP_{post}, and between baseline values were tested with paired and independent Student's t-tests. Association between variables was evaluated with Pearson's correlation coefficient. The statistical significance level was set to $P < 0.05$ and analysis performed using SPSS Statistics 24 (IBM, Armonk, New York, USA).

3. Results

VO_{2max} was 62 ± 6 ml/kg/min and W_{max} 371 ± 43 W. Although, the two exercise trials were matched for duration and power output (12 min 25 s ± 1 min 18 s and 286 ± 33 W, respectively) there was a small difference in % VO_{2max} between the isocapnic (90 ± 6%) and control trial (93 ± 5%; $P = 0.012$, Table 1). The duration of the controlled-pace and all-out sprint stages was 11 min 28 s ± 54 s and 48 s ± 28 s, respectively.

3.1. Respiratory variables

During the isocapnic trial, $PetCO_2$ was maintained at 40 ± 1 mmHg from Clamp until end end-exercise, whereas during the control trial $PetCO_2$ decreased from 40 ± 1 at No-clamp to 34 ± 4 mmHg at end-exercise ($P < 0.001$, Fig. 2C). One subject had a high resting $PetCO_2$ (44 mmHg) and thus the targeted value during the isocapnic trial was adjusted accordingly. There was no difference between the two trials at rest and Clamp or No-clamp (40 ± 2 and 40 ± 1 mmHg). During both trials, V_E increased from Clamp, or No-clamp, to end-exercise (129 ± 12 to 176 ± 19, isocapnia and 125 ± 11 to 167 ± 18 l/min, control; $P < 0.001$, respectively) but there were no differences in V_E between trials at any time.

There was no change in VO_2 from Clamp to end-exercise during the isocapnic trial (4.24 ± 0.4 to 4.32 ± 0.4 l/min), while VO_2 increased from No-clamp to end-exercise during the control trial (4.28 ± 0.4 to 4.53 ± 0.4 l/min; $P = 0.015$), but there were no significant differences between the two trials at any time. VCO_2 increased during both trials from Clamp or No-clamp to end-exercise (4.65 ± 0.4 to 5.28 ± 0.8 and 4.55 ± 0.4 to 4.88 ± 0.6 l/min; $P = 0.018$ and 0.028, respectively). At end-exercise VCO_2 was higher during the isocapnic compared with the control trial (5.28 ± 0.8 vs. 4.88 ± 0.6 l/min; $P = 0.03$).

V_E/VO_2 increased from Clamp, or No-clamp to end-exercise during both trials (isocapnic 30.6 ± 2.3 to 41.0 ± 5.7; control 29.4 ± 1.9 to 37.0 ± 4.2; $P < 0.001$). V_E/VO_2 was higher at Clamp and No-clamp during the isocapnic compared with the control trial (30.6 ± 2.3 vs. 29.4 ± 1.9; $P = 0.007$) and at end-exercise (41.0 ± 5.7 vs. 37.0 ± 4.2; $P < 0.001$, respectively), Similarly, V_E/VCO_2 increased equally from Clamp or No-clamp to end-exercise (isocapnic 27.8 ± 1.4 to 33.6 ± 3.7; control 27.6 ± 1.1 to 34.4 ± 3.7; $P < 0.001$) with no difference between trials.

3.2. Cardiovascular variables

There was no difference in HR, MAP ($n = 11$, no evaluation at rest for one subject), or CO ($n = 4$) at any time.

3.3. Blood gas variables

The arterial blood samples (n = 2) confirmed elevation of $PaCO_2$ during the isocapnic compared to the control trial. The correlation between $PetCO_2$ and $PaCO_2$ was highest in the control trial (r = 0.80 vs. r = 0.60).

3.4. Cerebrovascular variables

During the isocapnic trial MCA V_{mean} increased by 16 ± 13% until the time that clamping was applied and continued to increase (by 14 ± 28%; P = 0.017, Table 1) until the end of exercise, while MCA V_{mean} did not change significantly during the control trial (P = 0.071, Fig. 2A). In contrast to the disparity in MCA V_{mean} response, ScO_2 (n = 11) decreased similarly from Clamp, or No-clamp to end-exercise (-3.2 ± 5.1% and -4.1 ± 9.5%, isocapnic and control, respectively; P < 0.001) during both trials, while there was no difference between trials (Table 1, Fig. 2B).

3.5. Motor activation

MIP decreased by 12% following the isocapnic (P < 0.001) and 7% following the control trials (P = 0.025) with no difference between the two trials.

4. Discussion

This study evaluated the relationship between ScO_2 and CBF during controlled-pace high-intensity exercise to exhaustion and found that clamping of $PetCO_2$ elevated MCA V_{mean} but did not prevent the reduction in ScO_2 established at exhaustion following comparable efforts, as evaluated by the similar decline in inspiratory muscle strength during the isocapnic and control trials. The finding of a mismatch between changes in ScO_2 and MCA V_{mean} is in aggreement with observations during maximal incremental exercise[12] and and self-paced cycling performance.[15] Our hypothesis that the parity between ScO_2 and MCA V_{mean} would be challenged during controlled pace exercise was confirmed. However, despite the assumed elevated cerebral metabolic demands during controlled pace exercise, cerebral de(oxygenation) at the end of both trials was comparable with previous reports,[12,15,] even in hypoxia,[13] and MCA V_{mean} at the end of the isocapnic trial was ~15% lower than previously reported.[12] Our data suggest physiological "uncoupling" between prefrontal cortex oxygenation and CBF during high-intensity exercise to exhaustion.

At exhaustion in the control trial, $PetCO_2$ fell below the resting value, while MCA V_{mean} did not change in parallel, providing support to reports questioning the relationship between MCA V_{mean} and $PetCO_2$ during high intensity exercise.[31,12] In addition, during the isocapnic trial, when $PetCO_2$ was clamped, MCA V_{mean} continued to increase until exhaustion (by 14%), rather than being maintained, as would be expected if $PaCO_2$ was the sole determinant of CBF. Taken together, these findings indicate that while $PaCO_2$ is a critical regulator, it is not the only factor affecting cerebral perfusion during intense exercise, in confirmation of findings during submaximal[32] and incremental exercise.[12] The continuous increase in MCA V_{mean} during the isocapnic trial, despite a similar MAP, CO and SaO_2 in the two trials (Table 1), suggests that this progressive rise in cerebral blood velocity cannot be accounted by changes in perfusion pressure, or circulating systemic stimuli (such as arterial hypoxemia). The increase in MCA V_{mean} is likely driven by increased neural activity and the cerebral metabolic demand associated with high intensity exhaustive exercise. Considering that the cerebral metabolic demands due to neural activity were similar between the two trials (suggested by the matched workload and time to exhaustion) it can be inferred that the regional cerebral blood velocity response to exercise is increasing linearly with exercise intensity but that hyperventilation-induced hypocapnia imposes cerebral vasoconstriction and is masking this response as illustrated in the control trial.

Dissociation between oxygenation and CBF has been indicated from a weak association between the two variables after exercise-induced hyperventilation sets in.[31]

Dissociation between cerebral perfusion and ScO_2 revealed with $PetCO_2$ clamping, alluding to a seemingly surplus in cerebral perfusion, may be reflective of the structure of the cerebral vasculature. Cerebral capillaries are not different from those in other vascular beds, but while the capillaries within skeletal muscle are positioned in direct contact to the muscles cells, within the brain there is the blood-brain barrier between the capillaries and the neurons making the diffusion distance for O_2 critical when cerebral activity is elevated. Also, while skeletal muscles increase capillary recruitment in order to establish the O_2 gradient for adequate O_2 diffusion, the lack of capillary recruitment in the brain may necessitate an increase in CBF for that purpose.[26] An alternative explanation for the lack of enhancement of prefrontal cortex oxygenation despite the increased MCA V_{mean} could be because middle cerebral artery flow is not all directed to the prefrontal cortex but also to the lateral surface of the temporal and parietal lobes. Support for such cerebral blood flow redistribution and variable proportional blood flow contribution of cerebral arteries is provided by the attenuation of flow in the internal carotid but not in the vertebral artery during graded dynamic exercise. [7]

A limitation is that CBF was assessed by determination of MCA V_{mean} that measures flow velocity rather than volume flow[33] and it is critical for the validity of TCD in reporting flow changes that the vessel diameter remains constant. Even though the MCA diameter increases with marked elevation in $PetCO_2$, it remains stable within ± 7.5 mmHg of the resting value,[34] which is within the limits observed in the present study ($PetCO_2$ at Clamp and No-clamp: 40 ± 2 and 40 ± 1 mmHg, and at end exercise: 40 ± 1 vs. 34 ± 4 mmHg, isocapnic and control trial, respectively). Another consideration is vasoconstriction of large cerebral arteries during exercise-induced sympathetic activation.[35] However, a strength of this study is that workload was matched and, the inspiratory muscle deficit developed in the two trials was similar, suggesting that the sympathoexcitation elicited was also similar. Another consideration with the sequential execution of the two trials is a possible confounding influence of an order effect. However, since the purpose of the study was to evaluate the relationship between ScO_2 and MCA V_{mean} during maximal controlled-pace exercise and not cycling performance per se, the order of the trials is not considered to have had influenced the cerebrovascular response.

During exercise with a small muscle mass (handgrip) muscle fatigue has been related to reduced cerebral oxygenation[22] and thus, it is considered that reduced prefrontal cortex oxygenation rather than CBF per se could be important for development of fatigue also during whole body exercise. A decline in frontal lobe cerebral oxygenation from submaximal to maximal

exercise intensity is a consistent finding.[36] Prefrontal cortex oxygenation is a regional measurement and may not be reflective of the global value, although that is debated.[36] It can be argued that evaluation of motor cortex oxygenation would be more relevant to exercise, but there is agreement between oxygenation recorded by NIRS from the prefrontal cortex and a calculation of cerebral capillary oxygenation.[22] Furthermore, during maximal cycling exercise changes in NIRS-determined cortical oxygenation are similar in the prefrontal, premotor and motor regions[37] and thus, are probably indicative of elevated oxygen demands, as shown by the increased cerebral metabolic rate for oxygen.[38] It is also considered that assessment of cerebral oxygenation by NIRS may be affected by changes in skin blood flow[39] that would be elevated by the end of exercise as body temperature increases. The ambient temperature and relative humidity during the two trials were similar (23 ± 1 C°, 23 ± 4% and 23 ±1 C°, 26 ± 4%, isocapnic and control, respectively), indicating that the possible influence of skin blood flow was similar in the two trials, and thus it is not expected to affect our interpretation. Nevertheless, even if we consider that skin blood flow may have affected our ScO_2 values such possibility would only strengthen our argument for dissociation between ScO_2 and MCA V_{mean} because that would mean that the extend of the dissociation can be even larger than our observation. Another consideration is that the ScO_2 provided by the NIRS device only reflects the relative change in the ratio of oxyhaemoglobin and total haemoglobin, begging the question whether there could have been a stronger association between MCA V_{mean} and total haemoglobin during the exercise trials. However, since the focus of the paper is the possible dissociation between cerebral blood flow and cerebral tissue oxygenation, the ScO_2 measurement is useful for the evaluation.

Even though we succeeded to clamp $PetCO_2$ with very low variability, yet we clamped $PetCO_2$ rather than the arterial $PaCO_2$. The $PetCO_2 – PaCO_2$ gradient changes during exercise,[40] and thus it should be taken into account that $PetCO_2$ may not have reflected $PaCO_2$, especially because CO_2 supplementation can exert an independent effect of $PetCO_2$ in overestimating $PaCO_2$.[41] However, even if we consider that the reported $PetCO_2$ may overestimate $PaCO_2$, alluding that hypocapnic vasoconstriction was not completely abolished, our interpretation of increased MCA V_{mean} during the isocapnic trial still holds. Blood data from albeit only 2 subjects showed a high correlation between $PetCO_2$ and $PaCO_2$. In support, during high-intensity exercise $PaCO_2$ and $PetCO_2$ are similar when corrected for blood temperature.[42] Supplementation of CO_2 during the isocapnic trial could have exaggerated metabolic acidosis and, via the Bohr effect, provoked a rightward shift on the oxyhaemoglobin curve and thus decreased SaO_2, which

might have contributed to the decrease in ScO_2 from Clamp to end-exercise despite the increase in MCA V_{mean}. Yet, arterial oxygen saturation if anything, was higher during isocapnic compared to control (Table 1).

5. Perspective

Even though exercise performance was not evaluated in the present study, the development of prefrontal cortex deoxygenation at exhaustion provides support to the postulate of cerebral deoxygenation as a limiting factor for exercise performance. The reduction of ScO_2 during controlled-pace high-intensity exercise to exhaustion, while MCA V_{mean} is enhanced with CO_2 supplementation, argues for regional heterogeneity and compartmentalization of function and metabolism in the brain. It is suggested that the "seemingly" surplus in MCA V_{mean} is not all directed to the prefrontal cortex but also to the lateral surface of the temporal and parietal lobes. The all-out finishing sprint complicates precise coupling between cerebral blood flow and prefrontal cortex oxygenation, but a discrepancy between ScO_2 and MCA $V_{mean.}$developed even during the controlled cycling stage. Also, it should be considered that cerebral blood flow during maximal exercise, rather than being regulated to deliver oxygen, could be regulated to maintain fuel availability (e.g., glucose and/or lactate delivery; [43]).

Disclosure statement

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Table. Variables at rest and during the control and isocapnic exercise trials

	Control trial			Isocapnic trial		
	Rest	No-clamp	End-exercise	Rest	Clamp	End-exercise
Power, W		286 ± 33	343 ± 39	-	286 ± 33	343 ± 39
MCA V_{mean}, cm/s	65.0 ± 14.0	71.0 ± 22.2	67.0 ± 23.4	63.32 ± 11.5	74.0 ± 15.6	82.9 ± 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	40 ± 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*
R_F, 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*
SaO_2, %	97.8 ± 0.6	91.6 ± 0.4	91.5 ± 0.3	97.5 ± 0.4	93.2 ± 0.4	93.4 ± 0.2
VO_2, l/min	0.51 ± 0.2	4.28 ± 0.5	4.53 ± 0.4	0.51 ± 0.1	4.24 ± 0.4	4.31 ± 0.5
VCO_2, ml/min	0.42 ± 0.1	4.55 ± 0.4	4.88 ± 0.6	4.44 ± 0.8	4.65 ± 0.4	5.28 ± 0.8†
V_E/VO_2	26.4 ± 2.2	2.4 ± 1.9	37.0 ± 4.2*	27.4 ± 3.5	30.6 ± 2.3†	41.0 ± 5.7*†
V_E/VCO_2	31.7 ± 2.7	27.6 ± 1.1	34.4 ± 3.7*	31.6 ± 2.9	27.8 ± 1.4	33.6 ± 3.7*
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
MIP, cm H_2O	159 ± 24	- -	148 ± 24*	163 ± 27	- -	144 ± 21*

Power, power output; MCA V_{mean}, mean cerebral blood flow velocity in the middle cerebral artery; ScO_2, cerebral oxygenation; $PetCO_2$, end-tidal carbon dioxide pressure; V_E, ventilation; R_F, respiratory frequency; V_T, tidal volume; SaO_2, arterial oxygen saturation; VO_2, oxygen consumption; VCO_2, carbon dioxide output; V_E/VO_2, ventilatory equivalent for oxygen; V_E/VCO_2, ventilatory equivalent for carbon dioxide; MAP, mean arterial pressure; CO, cardiac output; HR, heart rate; MIP, maximal inspiratory pressure. Values are means ± SD; n = 12 subjects, except ScO_2 and MAP n = 11, CO n = 4 and SaO_2 n = 2; *, difference from No-clamp or Clamp to end-exercise; †, difference between isocapnic and control trials ($P < 0.05$).

Figure legends

Figure 1. End-tidal PCO_2 clamp. The operator visually monitors breath-by-breath $PetCO_2$ and manually adds CO_2-enriched air to an open-ended inspiratory reservoir to maintain $PetCO_2$ (Adopted from Ref.[24]).

Figure 2. Cerebrovascular and respiratory variables at rest and during the isocapnic and control exercise trials. A, MCA V_{mean}, middle cerebral artery mean blood flow velocity. B, ScO_2, cerebral oxygenation. C, $PetCO_2$, end tidal carbon dioxide pressure. Because Clamp, No-clamp and exhaustion occurred at different times for each subject exercise time was expressed in iso-times. Values are means ± SD; *, difference from No-clamp or Clamp to end-exercise; †, difference between isocapnic and control exercise trials, (n = 12, $P < 0.05$).

Figure 1

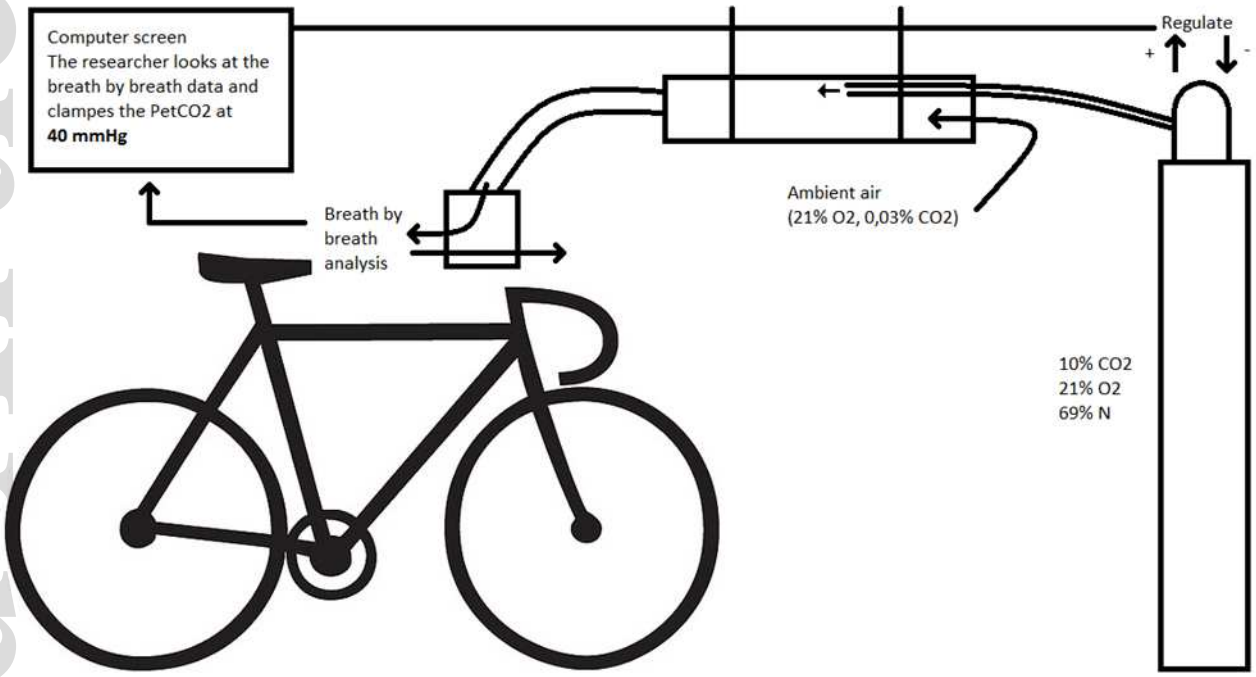

Figure 2