Published in final edited form as:
Respir Physiol Neurobiol. 2011 January 31; 175(1): 176–180. doi:10.1016/j.resp.2010.09.011.

Cerebral Blood Flow and Oxygenation at Maximal Exercise: The Effect of Clamping Carbon Dioxide

J. Tod Olin, MD[a,b], **Andrew C. Dimmen, MS**[a], **Andrew W. Subudhi, PhD**[a,c], and **Robert C. Roach, PhD**[a]

[a] Altitude Research Center, Department of Emergency Medicine, University of Colorado Anschutz Medical Campus Altitude Research Center, F524 - PO Box 6508, 12469 East 17th Place, Aurora, CO 80045-0508, USA

[b] Department of Pediatrics, The Children's Hospital and University of Colorado School of Medicine, Aurora, CO The Children's Hospital, 13123 East 16th Avenue, Box B-395, Aurora, CO 80045, USA

[c] Department of Biology, University of Colorado at Colorado Springs 1420 Austin Bluffs Parkway, Colorado Springs, CO 80918, USA

Abstract

During exercise, as end-tidal carbon dioxide (PET_{CO_2}) drops after the respiratory compensation point (RCP), so does cerebral blood flow velocity (CBFv) and cerebral oxygenation. This low-flow, low-oxygenation state may limit work capacity. We hypothesized that by preventing the fall in PET_{CO_2} at peak work capacity (W_{max}) with a newly-designed high-flow, low-resistance rebreathing circuit, we would improve CBFv, cerebral oxygenation, and W_{max}. Ten cyclists performed two incremental exercise tests, one as control and one with PET_{CO_2} constant (clamped) after the RCP. We analyzed PET_{CO_2}, middle cerebral artery CBFv, cerebral oxygenation, and cardiopulmonary measures. At W_{max}, when we clamped PET_{CO_2} (39.7 ± 5.2 vs. 29.6 ± 4.7 mmHg, $P<0.001$), CBFv increased (92.6 ± 15.9 vs. 73.6 ± 12.5 cm/s, $P<0.001$). However, cerebral oxygenation was unchanged (ΔTSI -21.3 ± 13.1% vs. -24.3 ± 8.1%, $P=0.33$), and W_{max} decreased (380.9 ± 20.4 vs. 405.7 ± 26.8 Watts, $P<0.001$). At W_{max}, clamping PET_{CO_2} increases CBFv, but this does not appear to improve W_{max}.

Keywords

cerebral blood flow; cerebral oxygenation; exercise; carbon dioxide clamp; transcranial Doppler; near infrared spectroscopy

Correspondence and request for reprints should be addressed to: Andrew Subudhi, PhD, Department of Biology, University of Colorado at Colorado Springs1420 Austin Bluffs Parkway, Colorado Springs, CO 80918, USA asubudhi@uccs.edu (00 1) 303-724-1675 (office) (00 1) 303-724-1660 (fax). Tod Olin: olin.tod@tchden.edu; Andrew Dimmen: andrew.dimmen@ucdenver.edu; Robert Roach: Robert.roach@ucdenver.edu.

A preliminary report on data contained in this manuscript was presented in abstract form at the 2010 American College of Sports Medicine Conference.

1. Introduction

During intense aerobic exercise, hyperventilation reduces the arterial partial pressure of carbon dioxide (Pa_{CO_2}). As a result, cerebral blood flow (CBF) and oxygenation may fall below a critical level needed to maintain motor output (Nybo and Rasmussen, 2007). Several studies have demonstrated that increasing inspired oxygen (PI_{O_2}) can improve cerebral oxygenation and prolong intense aerobic exercise (Amann et al., 2007; Nielsen et al., 1999; Subudhi et al., 2008), but no studies have been conducted to maintain CBF as a method of improving cerebral oxygenation and exercise performance.

Since CBF is highly dependent on Pa_{CO_2} during exercise (Ogoh and Ainslie, 2009), we sought to maintain CBF by precisely controlling end-tidal carbon dioxide levels (PET_{CO_2}). While rebreathing devices (Banzett et al., 2000; Slessarev et al., 2007) can be used to hold PET_{CO_2} constant (clamp) and thus control CBF at rest, they are not engineered for high-intensity exercise. We felt that by modifying the circuit designed by Banzett et al.(Banzett et al., 2000) to accommodate elevated minute ventilation and CO_2 production, we could clamp PET_{CO_2} and study the effect of CBF on cerebral oxygenation and peak work capacity (W_{max}).

The primary intent of this study was to determine whether the new rebreathing circuit could be used to clamp PET_{CO_2} at exercise intensities above the respiratory compensation point (RCP). To evaluate the circuit's performance, we conducted a pilot study to test the hypothesis that clamping PET_{CO_2} after the RCP would increase CBF, cerebral oxygenation, and W_{max}.

2. Methods

2.1 Sequential gas delivery circuit

To clamp PET_{CO_2} we designed a rebreathing circuit conceptually similar to the one designed by Banzett et al. (Banzett et al., 2000), with modifications to accommodate variable CO_2 production and high ventilatory volumes at maximal exercise. On the inspiratory limb, there is an investigator-controlled supply of compressed air with adjustable flow, a 14 liter reservoir, and a one-way, low-resistance valve connected to a T connector (Hans Rudolf, model 2700; Figure 1). We did not include a heated humidifier as used in the Banzett circuit. On the expiratory limb (Figure 1), there is a 3 meter section of 3.3 cm internal diameter tubing, acting as an inspiratory reservoir of exhaled gas, which then divides into five limbs capped with positive end expiratory pressure (PEEP) valves (Instrumentation Industries, Inc, Bethel Park, PA). Two low-resistance expiratory valves were placed in close proximity to the PEEP valves. We calibrated the PEEP valves to approximately 2-3 cmH_20 and aligned them so that air would be drawn into the circuit from the room if there was negative pressure in the tubing, which occurs only when rebreathing exhaled gas. We used the multiple limb design to provide high peak inspiratory flows and low resistance needed during maximal exercise.

We controlled the relative amounts of inspired gas from the inspiratory and expiratory limbs, in response to real-time PET_{CO_2} measurements, by manually adjusting the flow rate of compressed room air into the inspiratory limb. Rebreathing occurred when the compressed air flow rate was less than alveolar ventilation (Banzett et al., 2000).

2.2 Experimental testing of rebreathing circuit

In this single-blind, crossover experiment approved by the Colorado Multiple Institutional Review Board, 10 male competitive cyclists (professional or category 1), who provided written informed consent, performed two sequential maximal exercise tests, with and

without externally-controlled PET_{CO_2} after the RCP, in a randomly assigned and counterbalanced order. Each participant, instrumented with a custom fit headset with a transcranial Doppler (TCD, Spencer ST3) probe and multichannel NIRS (Oxymon MkIII, Artinis) sensors, completed a 25-30 min self-paced warm up on a Velotron Elite cycle ergometer (Racermate, Seattle, WA). After a brief rest to optimize sensor placement, we increased the workrate from 25 W at 25 W/min until volitional exhaustion or pedal cadence dropped below 50 rpm. Subjects repeated the protocol after 35 min of self-paced recovery. We minimized sensor placement and replacement errors by leaving all instrumentation in place, making fine adjustments between trials.

2.3 RCP selection and PET_{CO_2} clamping

We identified the RCP in the control trials post hoc by averaging three trained investigators' analyses of the PET_{CO_2} and ventilatory equivalent trends. Due to the random assignment of trial order, we were unable to prospectively identify the RCP during the clamped trials. Instead, we clamped PET_{CO_2} (defined as keeping PET_{CO_2} levels within ± 2 mmHg) during moderate exercise at the point we observed the first decrease in PET_{CO_2} levels of 2mmHg lasting greater than 10 s.

2.4 Measurements of cerebral oxygenation, cerebral blood flow, and metabolism

A multichannel, continuous wave NIRS instrument calculated a tissue saturation index (TSI) from measured oxygenated (O_2Hb), de-oxygenated (HHb), and total hemoglobin (THb, O2Hb+HHb). We expressed changes (Δ) in TSI, O_2Hb, HHb, and THb relative to the start of each test. Transcranial Doppler, ipsilateral to the NIRS probes, monitored middle cerebral artery blood flow velocity (CBFv) at depths between 40 to 54 mm. We processed NIRS, transcranial Doppler, and cardiopulmonary variables as previously reported.(Heine et al., 2009; Subudhi et al., 2009)

2.5.1 Calculations and analyses—All measurements were averaged over 15-s intervals. Oxygen consumption (VO_2) could only be determined during the control trial when a mixing chamber was utilized instead of the rebreathing circuit. We used the ratio of CBFv to PET_{CO_2} as a cerebral vasomotor reactivity index and the ratio of CBFv/ABP to PET_{CO_2} as a cerebrovascular conductivity index across workrates to further investigate the effect of the rebreathing circuit on cerebral hemodynamics during exercise.

2.5.3 Statistical analyses—Data from specific 15-s intervals of interest corresponding to 0, 25, 50, 75, and 100 percent of maximal power were analyzed with 2 × 5 repeated-measures ANOVA to evaluate effects of trial across workrate. Criterion for significance was set at $P < 0.05$. Post hoc, pairwise comparisons were made using paired t-tests with Holm's sequential method to control for type I error. Values are means ± standard deviation.

3. Results

3.1 Subject characteristics

Ten subjects (age: 25.9 ± 7.7 years, weight: 71.2 ± 7.7 kg, height: 181.1 ± 7.4 cm) completed the control and intervention trials.

3.2 Control trial

During the control trial, we observed the expected decrease in PET_{CO_2} after the RCP (29.6 ± 4.7 mmHg at W_{max} vs. 36.8 ± 6.6mmHg at RCP, $P<0.001$) without a significant decrease in CBFv (73.6 ± 12.5 cm/s at W_{max} vs. 76.7 ± 15.4 cm/s at RCP, $P=0.86$). ΔTSI decreased after the RCP as expected (-21.3 ± 13.1% at W_{max} vs. -9.7 ± 6.7% at RCP, $P=0.008$). The

RCP occurred at 79.5 ± 4.7 %W_{max}. W_{max} (405 ± 26.8 W), ventilation (200.8 ± 24.6 L/min), and cardiopulmonary responses (VO_{2max} 4.8 ± 0.6 L/min) were representative of altitude specific values for well-trained cyclists (Subudhi et al., 2007).

3.3 Clamp trial

In the clamp trial, we successfully maintained PET_{CO_2} after the RCP (39.7 ± 5.2 mmHg at W_{max} vs. 40.1 ± 4.9 mmHg at RCP, P=0.63). PET_{CO_2} at W_{max} was higher than that in the control trial (P<0.001; Figure 2a). In contrast to the control trial, CBFv continued to increase after the RCP, ending 26% higher (92.6 ± 15.9 cm/s at W_{max} vs. 85.0 ± 17.9 cm/s at RCP, P=0.001,Figure 2b), despite a lower W_{max} (380.9 ± 20.4W, P<0.001). ΔTSI decreased after the RCP (-24.3 ± 8.1 at W_{max} vs. -12.8 ± 10.7 at RCP, P=0.005), but values at W_{max} were similar between trials (P= 0.33; Figure 2c). At W_{max}, there were no differences in heart rate, blood pressure, ventilation, tidal volume, respiratory rate, or oxygen saturation (Table 1). There were no differences at any point across trials in NIRS measurements (ΔO2Hb, ΔHHb, ΔTHb) on either the cerebral cortex or vastus lateralis (muscle data not shown).

3.5 Vasomotor reactivity and conductivity

The reactivity index ($CBFv \cdot PET_{CO_2}^{-1}$) gradually increased across workrates to exhaustion and was not affected by clamping PET_{CO_2} (Figure 2d). When we adjusted the reactivity index for changes in ABP, the resulting conductivity index ($CBFv \cdot ABP^{-1} \cdot PET_{CO_2}^{-1}$) was unchanged across workrates (Figure 2e).

4. Discussion

In this study, we designed a novel rebreathing circuit and successfully clamped PET_{CO_2} during incremental exercise to maximal exertion. Using this circuit, we showed that a relative increase in PET_{CO_2} at W_{max} causes an increase in CBFv. However, despite the increased CBFv, neither cerebral oxygenation nor W_{max} were elevated.

4.1 Success and limitations of rebreathing circuit

With our circuit we achieved our primary goal of clamping PET_{CO_2}, holding levels within ± 2 mmHg, despite rapidly changing minute ventilation and CO_2 production. We accommodated tidal volumes ranging from 0.7 to 4L and minute ventilations in excess of 200 L/min. Minute ventilation was unchanged versus control values and the circuit did not affect heart rate or blood pressure. Because cardiac parameters were unchanged across trials, we believe that the circuit provides a novel approach to isolating and controlling PET_{CO_2}, and thus to manipulate CBF.

We noticed a few technical limitations with the circuit, however. We used several branches on the expiratory limb to minimize inspiratory resistance, but all subjects noted a degree of inspiratory resistance at maximal exercise. This did not appear to cause a distinct ventilatory ceiling in tidal volume or minute ventilation statistically, but the effort required to ventilate may have been higher in the clamped trial. Additionally, rebreathing exhaled gas limits the amount of oxygen available for diffusion. As detailed by Slessarev et al., (Slessarev et al., 2007) the fraction of gas inhaled from the expiratory limb is physiologically neutral and does not participate in diffusion of oxygen or carbon dioxide. Despite this, we did not see differences in oxygen saturations or NIRS measurements across trials, and values were similar to those seen in maximal exercise tests under similar ambient conditions. Calculations of oxygen uptake and carbon dioxide production with this rebreathing system were not possible as the content of inspired gas varied both between and within breaths. This limitation does not affect our conclusions, and technical improvements will make these calculations possible in future studies.

Additionally, because we randomly assigned the order of the trials, we were unable to prospectively identify the RCP for use in the clamp trial. We instead clamped PET_{CO_2} levels when we observed the first sustained decrease, generally in the 250-300 Watt range. For this reason, the RCP identified post hoc in the control trial may not precisely correlate with the work rate at which clamping began in the intervention trial. However, the conclusions of this trial are based on responses at maximal exertion, and are unlikely to be affected by the precise start time of clamping. Despite the above concerns, subjects reached near-maximal values for all cardiopulmonary measures in the clamped trial (Table 1), which demonstrates that our rebreathing circuit can be an effective tool in the study of CBF during high intensity exercise.

4.2 Cerebrovascular implications of clamped PET_{CO_2}

Utilization of the rebreathing circuit to clamp PET_{CO_2} revealed some interesting cerebrovascular physiology which has not been previously described. As expected, during the control condition, PET_{CO_2} fell at very high workrates. However, we did not observe a parallel decrease in CBFv, which would be expected if PET_{CO_2} was the sole regulator of CBFv. Previously, we have questioned the absolute strength of relationship between PET_{CO_2} and CBFv during high intensity exercise since not all subjects show a decrease in CBFv with PET_{CO_2} (Heine et al., 2009). The circuit gives us new insight into the regulation of CBFv after the RCP, and our findings validate recent studies on the determinants of CBF in exercise at submaximal and maximal exertion (Nybo and Rasmussen, 2007). When we clamped PET_{CO_2}, CBFv continued to increase to values 26% higher than control values, demonstrating that while PET_{CO_2} is a key regulator, it is not the only variable exerting an influence on CBFv.

We used a reactivity index to evaluate the relationship further. Results demonstrate that the inherent relationship between PET_{CO_2} and CBFv was similar in the control and rebreathing conditions (Figure 2d). However, since the reactivity index ($CBFv \cdot PET_{CO_2}^{-1}$) increases with exercise intensity, other factors must exert an effect. Evaluation of the conductivity index ($CBFv \cdot ABP^{-1} \cdot PET_{CO_2}^{-1}$) suggests that the increase in ABP may be the key response driving CBFv after the RCP because correcting the reactivity for ABP resulted in a conductivity graph that did not change across workrates (Figure 2e).

We hypothesized that an increase in CBFv would increase cerebral oxygenation, but found evidence to the contrary. The increase in CBFv after the RCP did not affect frontal cortex oxygenation. We have previously noted an imperfect correlation between CBFv and oxygenation (Heine et al., 2009), but were surprised that there were no measurable effects of elevated CBFv on frontal cortex oxygenation in the present study. Since the manipulation of did not affect oxygen saturation, it is possible that our analogous measurements of TSI in the brain were also unaffected. Additionally, because increased middle cerebral artery flow is not all directed to the frontal cortex, expected increases in THb may have been blunted by regional autoregulatory mechanisms.

Finally, our data refute the hypothesis that increased CBFv would improve W_{max}. In fact, W_{max} was significantly impaired (< 20 watts) in the clamped trial. We suspect that the subjective sensations of dyspnea and increased inspiratory resistance led to early termination of exercise with clamped PET_{CO_2}. However, we cannot exclude the possibility that increased PET_{CO_2} influenced the decision to stop exercising via other physiologic mechanisms which were not measured in this pilot study, such as changes in sympathetic activity, acid/base balance, and distribution of cardiac output.

5. Conclusions

In conclusion, we developed a novel rebreathing circuit that allows us to clamp PET_{CO_2} at exercise intensities above the RCP in well-trained athletes. We showed that the circuit provides a means of manipulating CBFv and that CBFv increases relative to PET_{CO_2} at maximal exertion. Despite increases in CBFv, we did not observe similar increases in cerebral oxygenation or W_{max}. Based on these preliminary findings, we believe that the circuit may be used to control PET_{CO_2} in future studies of cerebral blood flow during exercise and offer new insights into factors limiting maximal exercise performance.

Acknowledgments

The authors wish to thank the cyclists who made this study possible and Ruth Johnson, Nick Robbins, Alison Anderson, Megan Wilson, and Barbara Lommen for technical and administrative assistance. This study was supported, in part, by NIH HL-070362, the Altitude Research Center, the Altitude Research Center Foundation, and the Cystic Fibrosis Foundation (OLIN08B0).

Sources of support: This research was supported by the National Institutes of Health (RO1 HL-070362), the Cystic Fibrosis Foundation (OLIN08B0), and the pediatric pulmonology fellowship at the University of Colorado.

References

Amann M, Romer LM, Subudhi AW, Pegelow DF, Dempsey JA. Severity of arterial hypoxaemia affects the relative contributions of peripheral muscle fatigue to exercise performance in healthy humans. J Physiol. 2007; 581:389–403. [PubMed: 17317739]

Banzett RB, Garcia RT, Moosavi SH. Simple contrivance “clamps” end-tidal PCO(2) and PO(2) despite rapid changes in ventilation. J Appl Physiol. 2000; 88:1597–1600. [PubMed: 10797118]

Heine M, Subudhi AW, Roach RC. Effect of ventilation on cerebral oxygenation during exercise: insights from canonical correlation. Respir Physiol Neurobiol. 2009; 166:125–128. [PubMed: 19429529]

Nielsen HB, Boushel R, Madsen P, Secher NH. Cerebral desaturation during exercise reversed by O2 supplementation. Am J Physiol. 1999; 277:H1045–1052. [PubMed: 10484427]

Nybo L, Rasmussen P. Inadequate cerebral oxygen delivery and central fatigue during strenuous exercise. Exerc Sport Sci Rev. 2007; 35:110–118. [PubMed: 17620929]

Ogoh S, Ainslie PN. Cerebral blood flow during exercise: mechanisms of regulation. J Appl Physiol. 2009; 107:1370–1380. [PubMed: 19729591]

Slessarev M, Han J, Mardimae A, Prisman E, Preiss D, Volgyesi G, Ansel C, Duffin J, Fisher JA. Prospective targeting and control of end-tidal CO2 and O2 concentrations. J Physiol. 2007; 581:1207–1219. [PubMed: 17446225]

Subudhi AW, Dimmen AC, Roach RC. Effects of acute hypoxia on cerebral and muscle oxygenation during incremental exercise. J Appl Physiol. 2007; 103:177–183. [PubMed: 17431082]

Subudhi AW, Lorenz MC, Fulco CS, Roach RC. Cerebrovascular responses to incremental exercise during hypobaric hypoxia: effect of oxygenation on maximal performance. Am J Physiol Heart Circ Physiol. 2008; 294:H164–171. [PubMed: 18032522]

Subudhi AW, Miramon BR, Granger ME, Roach RC. Frontal and motor cortex oxygenation during maximal exercise in normoxia and hypoxia. J Appl Physiol. 2009; 106:1153–1158. [PubMed: 19150853]

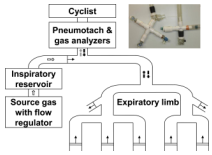

Figure 1.

Rebreathing circuit used to clamp end-tidal carbon dioxide. During the clamped trial, we forced rebreathing from the expiratory limb by limiting the flow of source gas, 21% O_2, 0.03% CO_2. Airflow, denoted by broad arrows, is differentiated as follows: The unbroken hollow arrow indicates continuous inspiratory flow of source gas. The broken hollow arrow indicates cyclic inspiratory flow of source gas. The broken black arrows indicate cyclic inspiratory and expiratory flow of exhaled gas. The broken gray arrow indicates cyclic inspiratory flow of mixed gas.

Valves are denoted by thin arrows with perpendicular bases. Those with a single perpendicular base are near-zero resistance one-way valves. Those with a double perpendicular base are low resistance one-way PEEP valves.

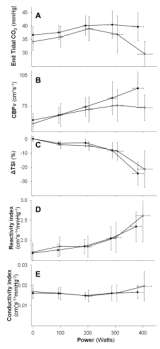

Figure 2.

The effect of clamping carbon dioxide on cerebral blood flow and cerebral oxygenation. A. PET_{CO_2} was effectively clamped at maximal exercise. B. Middle cerebral artery blood flow velocity continually increased to maximal exertion with PET_{CO_2} clamped. C. Frontal cortex tissue saturation index was unchanged with PET_{CO_2} clamped. D. Cerebral vasomotor reactivity index was not affected by PET_{CO_2} clamping. E. Cerebral conductivity showed minimal change across workrates in both trials. (control, ○; clamped, •) Values are means ± SD.

Table 1

Comparison of metabolic, respiratory, cerebral blood flow, and cerebral oxygenation responses to incremental exercise with unclamped (control) and clamped end-tidal carbon dioxide.

	control			clamp					
variable	**rest**	**RCP**	**max**	**rest**	**% control**	**RCP**	**% control**	**max**	**% control**
Power, W	0.0 ± 0.0	324.9 ± 33.2	405.7 ± 26.8	0.0 ± 0.0		324.9 ± 33.2	100%	380.9 ± 20.4	94%
HR, beats/min	93.0 ± 15.4	172.9 ± 12.3	191.9 ± 14.7	88.9 ± 14.0	96%	173.5 ± 12.5	100%	184.1 ± 9.5	96%
BP, mmHg	102.1 ± 11.2	132.8 ± 10.4	135.9 ± 17.8	104.6 ± 5.5	102%	132.4 ± 14.2	100%	139.9 ± 11.4	103%
MV, L/min_{BTPS}	21.3 ± 4.0	129.7 ± 17.6	200.8 ± 24.6	26.2 ± 6.5	123%	157.3 ± 27.3*	121%	192.2 ± 37.2	96%
f, breaths/minute	15.7 ± 5.8	40.9 ± 8.9	62.0 ± 10.9	18.3 ± 7.5	117%	48.3 ± 7.4*	118%	61.3 ± 9.3	99%
Tidal volume, L	1.4 ± 0.5	3.3 ± 0.7	3.3 ± 0.4	1.5 ± 0.3	107%	3.2 ± 0.6	97%	3.1 ± 0.5	94%
$P_{ET}CO_2$, mmHg	34.2 ± 3.2	36.8 ± 6.6	29.6 ± 4.7	36.7 ± 3.1	107%	40.1 ± 4.9	109%	39.7 ± 5.2†	134%
VE/VCO_2	29.2 ± 5.5	20.0 ± 2.6	23.3 ± 2.1						
O_2 uptake, L/min	0.5 ± 0.1	4.0 ± 0.4	4.8 ± 0.6						
MCA V_{mean}, cm/s	58.0 ± 6.4	76.7 ± 15.4	73.6 ± 12.5	61.5 ± 6.4	106%	85.0 ± 17.9	111%	92.6 ± 15.9†	126%
O_2 saturation, %	97.0 ± 1.0	90.6 ± 3.8	87.7 ± 5.5	95.8 ± 2.4	99%	89.0 ± 3.4	98%	85.1 ± 4.7	97%
cerebral oxygenation									
ΔTSI, %	0.3 ± 1.6	-9.7 ± 6.7	-21.3 ± 13.1	0.3 ± 1.9		-12.8 ± 10.7		-24.3 ± 8.1	
ΔO2Hb, μM	0.7 ± 1.2	0.6 ± 6.9	-3.4 ± 10.0	1.4 ± 3.1		1.6 ± 8.9		-1.8 ± 8.7	
ΔHHb, μM	0.2 ± 0.7	7.0 ± 3.6	14.1 ± 6.2	0.6 ± 0.9		8.6 ± 2.5		15.9 ± 4.9	
ΔTHb, μM	1 ± 1.1	7.6 ± 7.8	10.7 ± 12.7	2.0 ± 3.7		10.2 ± 8.7		14.1 ± 11.3	

Data are means ± SD

All NIRS values expressed relative to the start of each test

*P=0.002 vs. control by ANOVA

†P<0.001 vs. control by ANOVA