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Respiratory muscle oxygenation is not impacted by hypoxia during repeated-sprint exercise

Ramón F. Rodriguez[a], Nathan E. Townsend[b], Robert J. Aughey[a], François Billaut[a,c,*]

[a] Institute for Health and Sport, Victoria University, Melbourne, VIC, Australia
[b] Aspetar, Doha, Qatar
[c] Département de kinésiologie, Université Laval, Québec, QC, Canada

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ABSTRACT

This study aimed to investigate whether exercise hyperpnoea contributes to an impairment of locomotor muscle oxygenation and performance during repeated-sprint exercise in normoxia and hypoxia. Subjects performed ten 10-s sprints, separated by 30 s of passive rest while breathing either a normoxic (21% O_2) or hypoxic (15% O_2) gas mixture. Muscle oxygenation of the vastus lateralis and intercostal muscles was examined with near-infrared spectroscopy. Sprint and recovery vastus lateralis deoxyhaemoglobin was elevated in hypoxia by 9.2% (90% confidence interval 0.2 to 18.0) and 14.1% (90% CL 4.9 to 23.3%) compared to normoxia, respectively. There were no clear differences in respiratory muscle deoxyhaemoglobin (−0.1%, 90% CL −2.9 to 0.9%) or oxyhaemoglobin (0.9%, 90% CL −0.8 to 2.6%) between conditions. Maintenance of respiratory muscle oxygenation may contribute to the rise of vastus lateralis deoxyhaemoglobin in hypoxia during intermittent sprint cycling. This manuscript presents data which extends the fact that oxygen competition could be a limiting factor of exercise capacity.

1. Introduction

Repeated-sprint ability describes the capacity to recover from and maintain sprint (≤10 s) performance during subsequent "all-out" efforts (Girard et al., 2013). Phosphocreatine hydrolysis and anaerobic glycolysis are primary sources of rapid adenosine trisphosphate (ATP) replenishment in repeated-sprint exercise (Parolin et al., 1999; Gaitanos et al., 1993). However, the time course of metabolic recovery exceeds the rest period observed during repeated-sprint exercise (Gaitanos et al., 1993; Harris et al., 1976), which leads to a progressive reduction in both peak and mean power output, with a plateau in the later sprints (Gaitanos et al., 1993; Racinais et al., 2007). Resynthesis of phosphocreatine and removal of inorganic phosphates are processes derived exclusively through oxidative processes and are sensitive to muscle oxygen (O_2) availability (Harris et al., 1976; Sahlin et al., 1979; Hogan et al., 1999). Therefore, underpinning the ability to maintain performance over multiple sprints, is the capacity to deliver O_2 to the locomotor muscles between efforts (Billaut and Buchheit, 2013; Buchheit and Ufland, 2011).

The balance of muscle O_2 delivery vs. extraction can be elucidated with the use of near-infrared spectroscopy (NIRS). Concentrations of deoxyhaemoglobin ([HHb]) and oxyhaemoglobin ([O_2Hb]) rise and fall, respectively, proportional to an increase in metabolic activity in the underlying tissue (Subudhi et al., 2007; Grassi et al., 2003). At sprint onset, there is a rapid increase of vastus lateralis [HHb] (deoxygenation) which trends back towards baseline during the rest periods between sprints (reoxygenation) (Racinais et al., 2007; Buchheit et al., 2009; Smith and Billaut, 2010). However, blood flow competition between the locomotor and respiratory muscles can develop (Harms et al., 1997), which may limit the reoxygenation capacity of the vastus

Abbreviations: [HHb], concentration of deoxyhaemoglobin; [HHb_{RM}], respiratory muscle deoxyhaemoglobin; [O_2Hb], oxyhaemoglobin; [O_2Hb_{RM}], respiratory muscle oxyhaemoglobin; [tHb_{RM}], respiratory muscle total haemoglobin; ΔReoxy, reoxygenation; ∫ Pm × ƒR, inspiratory muscle force development; ATP, adenosine trisphosphate; CI, confidence interval CO_2 carbon dioxide; ES, effect size; F_IO_2, fraction of inspired oxygen; ƒR, respiratory frequency; HHb_{VL}, vastus lateralis deoxyhaemoglobin; HR, heart rate; IV, inspiratory volume; NIRS, near-infrared spectroscopy; O_2, oxygen; O_2Hb_{VL}, vastus lateralis oxyhaemoglobin; $P_{ET}CO_2$, end-tidal carbon dioxide; $P_{ET}O_2$, end-tidal oxygen; Pm, mouth pressure; rpm, revolutions per minute; SD, standard deviation; S_PO_2, arterial oxygen saturation (estimated pulse oximetry); $\dot{V}O_2$, pulmonary oxygen uptake; $\dot{V}O_{2peak}$, peak pulmonary oxygen uptake

* Corresponding author at: Department of Kinesiology, Faculty of medicine, University Laval, 2300 rue de la Terrasse, Quebec, QC G1V 0A6, Canada.
E-mail addresses: ramon.rodriguez-anderson@live.vu.edu.au (R.F. Rodriguez), nathan.townsend@aspetar.com (N.E. Townsend), robert.Aughey@vu.edu.au (R.J. Aughey), francois.billaut@kin.ulaval.ca (F. Billaut).

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lateralis. But there is limited work examining if the O_2 cost of exercise hyperpnoea influences repeated-sprint oxygenation trends and performance.

During high-intensity exercise, the O_2 cost of hyperpnoea and cardiac output distribution devoted to the respiratory muscles accounts for 10–15% of total pulmonary O_2 uptake ($\dot{V}O_2$) (Aaron et al., 1992a, b; Harms et al., 1998). To ensure the required O_2 demands are met, sympathetically-mediated vasoconstriction of the locomotor muscles promote blood flow redistribution towards respiratory musculature (Harms et al., 1997; Sheel et al., 2001; St Croix et al., 2000). This mechanism in part contributes to the peripheral muscle fatigue that develops during high-intensity exercise, and is exaggerated by fatiguing contractions of the respiratory muscles (Dempsey et al., 2006). The deletions effects of respiratory muscle work may be more apparent in acute hypoxia. It has been demonstrated that hypoxia increases pulmonary ventilation and work of breathing compared to normoxia (Cibella et al., 1996; Reeves et al., 1994). By alleviating the hypoxia-induced rise in work of breathing, the development of peripheral fatigue can be attenuated (Amann et al., 2007). The associated negative consequence of respiratory muscle work is seemingly amplified in acute hypoxia, likely mediated by a hastening activation of the respiratory muscle metaboreflex (Dempsey et al., 2006; Amann et al., 2007).

Negative effects of hypoxia on exercise performance and the development of peripheral fatigue during repeated sprints is well established (Billaut and Buchheit, 2013; Smith and Billaut, 2010, 2012; Billaut et al., 2013). Arterial hypoxemia specifically limits reoxygenation capacity between sprint efforts to constrain metabolic recovery (Billaut and Buchheit, 2013). However, it is currently unclear what influence acute hypoxia has on respiratory muscle oxygenation, and on the balance between locomotor and respiratory muscle oxygenation. Therefore, the purpose of the present study was to examine the effect of severe acute arterial hypoxemia on respiratory muscle oxygenation during repeated-sprint exercise, and its impact on the performance of the locomotor muscles. Hypoxemia was induced via a reduction in the fraction of inspired O_2 (F_iO_2), and oxygenation of the vastus lateralis and intercostal muscle were simultaneously measured with near-infrared spectroscopy (NIRS). It was reasoned that both vastus lateralis and intercostal muscle oxygenation would be impaired during exercise, mediated by arterial oxygenation and the work of breathing.

2. Methods

2.1. Subjects

Ten male subjects from a verity of athletic backgrounds (team sports, road cycling, strength training) were recruited to participate in this study (age 26.0 ± 3.6 years; body mass 78.6 ± 9.4 Kg; height 178.3 ± 7.5 cm; $\dot{V}O_{2peak}$ 48.42 ± 6.92 mL min^{-1} Kg). These subjects were chosen because they were accustomed to producing "all-out" bouts of exercise. Subjects self-reported to be healthy and with no known neurological, cardiovascular or respiratory diseases. After being fully informed of the requirements, benefits, and risks associated with participation, each subject gave written informed consent. Ethical approval for the study was obtained from the institutional Human Research Ethics Committee and the study conformed to the declaration of Helsinki.

2.2. Experiment design

Subjects visited the laboratory on six separate occasions. In the preliminary visit, subjects were familiarised with an incremental exercise test used in the following session. In the next session, the incremental exercise test was performed to the limit of exercise tolerance. In the following two sessions, subjects completed the same repeated-sprint protocol used in the main testing sessions for familiarisation. The main testing sessions were performed in a randomised order in normoxia and normobaric hypoxia. All exercise testing was performed on an electronically-braked cycle ergometer (Excalibur, Lode, Groningen, The Netherlands) and expired gases collected on a breath-by-breath basis (COSMED Quark CPET; Cosmed, Rome, Italy).

2.3. Incremental exercise testing

An incremental exercise test was performed for the determination of peak pulmonary oxygen uptake ($\dot{V}O_{2peak}$). The test was initiated at a work rate of 0 W for 3 min, followed by an increase in work rate 1 W every 2 s (30 W^{-1} min^{-1}) until volitional exhaustion or until the cadence fell below 10 rpm self-selected pedalling rate. Peak 30-s average was calculated and used to represent $\dot{V}O_{2peak}$.

2.4. Repeated-sprint exercise

Trials were performed in a single-blind random order, ensuring a balanced order of normoxic and hypoxic trials. All testing was conducted within a 23.92 m^2 environmental exercise laboratory. The F_iO_2 was 0.2084 ± 0.005 and 0.1455 ± 0.0031 for normoxia and hypoxia testing session respectively. After arriving at the laboratory, subjects were fitted with NIRS probes and a heart rate monitor. Testing was performed with the cycle ergometer set to isokinetic mode (120 rpm). In this mode, a variable resistance is applied to the flywheel proportional to the torque produced by the subjects to constrain their peddling rate to 120 rpm. Below 120 rpm, no resistance is applied to the flywheel. After a 7-min warm-up consisting of 5 min of unloaded cycling at 60–70 rpm and two 4 s isokinetic sprints (separated by 1 min each), subjects rested for another 2.5 min before the repeat-sprint protocol was initiated. The repeat-sprint protocol was ten 10-s sprints separated by 30 s of passive rest (5.5 min). Subjects were instructed to give an "all-out" effort for every sprint and verbally encouraged throughout to promote a maximal effort. Each sprint was performed in the seated position and initiated with the crank arm of the dominant leg at 45°. Before sprint one, subjects were instructed to accelerate the flywheel to 95 rpm over a 15-s period and assume the ready position 5 s before the commencement of the test. This ensured that each sprint was initiated with the flywheel rotating at ~90 rpm so that subjects could quickly reach 120 rpm. The handlebars and seat were individually adjusted to each subjects' characteristic. Four subjects used their own clipless pedals and cycling shoes, the reaming six had their feet secured using toe cages and retention straps fitted to the ergometer. Crank arm length was standardised to 175 mm. Visual feedback of power output was not available to the subjects during any sprint. The cycle ergometer software provides power and cadence at 4 Hz.

2.5. Metabolic and ventilatory measurements

Subjects wore a silicone facemask which the breath-by-breath gas sampling line and turbine were attached. The analyser was calibrated before each test against known gas concentrations of O_2 and carbon dioxide (CO_2) (normoxia: 16% O_2 and 5% CO_2; hypoxia: 7% O_2 and 5% CO_2) and the turbine volume transducer was calibrated using a 3 L syringe (Cosmed, Rome, Italy). Data was then exported into Excel so that pulmonary oxygen uptake ($\dot{V}O_2$) could be inspected for errant data points. Editing data was performed to remove the occasional errant breaths caused by for example swallowing or coughing which were considered to not be reflective of the metabolic responses to exercise. These errant data points were removed before values grater then 4 standard deviations from the local mean were removed (Rossiter et al., 2000). A 5-breath rolling average was then applied for the calculation of a peak and nadir for every 40-s sprint/recovery period to give a single value for each sprint and recovery phase. Respiratory frequency (f_R) was averaged over the entire sprint protocol to give one vale for each subject per trial. Because the facemask was removed immediately after the tenth sprint, only maximum values were calculated

over the first 10 s. Mouth pressure (P_m) was recorded continuously at 50 Hz with a pressure transducer (Honeywell, New Jersey, United States of America) attached to the saliva port of the non-rebreathing valve via Tygon tubing (Hans Rudolph inc., Kansas, United States of America). Mean inspiratory P_m was then calculated as an index of respiratory muscle work. To account for any change in f_R, an index of inspiratory muscle force development was also calculated for each exercise trial ($\int P_m \times f_R$) (Witt et al., 2007). For statistical analysis, inspiratory P_m was converted to positive values and presented in the results as such. Heart rate (HR) and arterial oxygen saturation (estimated by fingertip pulse oximetry; S_PO_2) was sampled on a breath-by-breath basis integrated into the Cosmed system.

2.6. Near-infrared spectroscopy

Subjects were instrumented with two NIRS probes to assess muscle O_2 status (Oxymon MKIII, Artinis, The Netherlands). The first probe was fixed over the distal part of the vastus lateralis muscle belly approximately 15 cm above the proximal border of the patella. The second was fixed over the sixth intercostal space at the anterior axillary line to assess changes in the intercostal muscles. Probes were held in place with black plastic spacers secured to the skin using double sided stick disks and shielded from light using a black self-adhesive elastic bandage. Optode spacing for was set to 4.5 cm and 3.5 cm for vastus lateralis and respiratory muscles respectably. Skinfold thickness was measured between the emitter and detector using a skinfold calliper (Harpenden Ltd.) to account for skin and adipose tissue thickness covering the muscle. The skinfold thickness for vastus lateralis (1.19 ± 0.69 cm) and respiratory muscles (1.12 ± 0.44 cm) was less than half the distance between the emitter and the detector in every case. A differential pathlength factor of 4.95 was used (Smith and Billaut, 2010). Data were acquired at 10 Hz A 10^{th} order zero-lag low-pass Butterworth filter was applied to the data to remove artefacts and smooth pedalling induced fluctuations; the resulting output was used for analysis (Rodriguez et al., 2018). Vastus lateralis [HHb] was normalised to femoral artery occlusion, so that 0% represented a 5-s average immediately prior the occlusion and 100% represented the maximum 5 s average (HHb_{VL}). Whereas for vastus lateralis [O_2Hb], 0% represented the lowest 5-s average and 100% represented the 5 s immediately prior to the occlusion (O_2Hb_{VL}). Peaks and nadirs were then identified within every 40-s sprint/recovery period to represent each sprint and recovery phase respectively. Reoxygenation capacity (ΔReoxy, %) was also calculated as the change from peak to nadir of HHb_{VL}. The concentration of respiratory muscle oxyhaemoglobin ([O_2Hb_{RM}]), deoxyhaemoglobin ([HHb_{RM}]), and total haemoglobin ([tHb_{RM}]) were expressed as an absolute change from baseline. Baseline was established before warm-up while seated quietly on the cycle. Because there were no clear peaks and nadirs in the respiratory muscle NIRS signal, an average was calculated for of each 40-s sprint/recovery period. Representative data from a single subject is presented in Fig. 1.

2.7. Statistical analysis

Data in text and figures are presented as the mean ± standard deviation (SD). To assess the magnitude of effects between hypoxia and normoxia, custom-made spreadsheets were used (Hopkins, 2006). To assess the difference between trials, the analysis was performed using the post-only crossover spreadsheet. All measures, other than S_PO_2, and vastus lateralis NIRS variables were log-transformed before analysis then back-transformed to express the changes in percent units and standardised effects. Relative changes (%) and effect size statistics are expressed with 90% confidence intervals (90% CI). Practical significance was assessed by calculating Cohen's d effect size (ES) (Cohen, 1988). Standardised effect sizes of < 0.2, > 0.2 to 0.5, > 0.5 to 0.8, > 0.8 were considered to as trivial, small, moderate and large respectively and presented with 90% CL. Probabilities were also calculated to establish if the chance the true (unknown) differences were lower, similar or higher than the smallest worthwhile change (ES = 0.2). Effects were not considered meaningful if there was < 75% probability of being substantially positive/negative relative to the smallest worthwhile change. If the chance of having higher/lower values then the smallest worthwhile change was both > 5%, the true difference was assessed as *unclear*. For clear effects, the likelihood that the true effect was substantial were assessed qualitatively as follows: *likely* (75 to < 95%), *very likely* (95 to 99.5%), *most likely* (> 99%) (Hopkins et al., 2009).

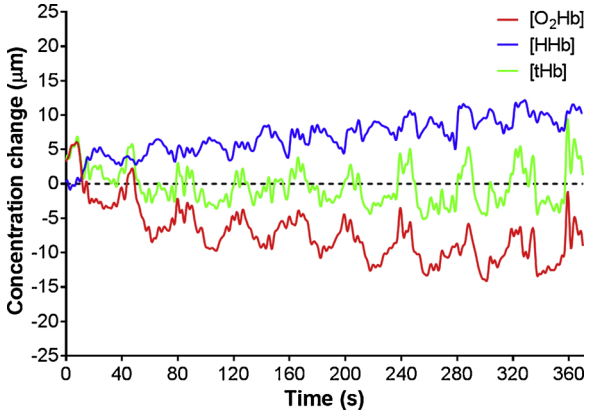

Fig. 1. Representative data of the concentration change from baseline for oxyhaemoglobin ([O_2Hb]), deoxyhaemoglobin ([HHb]) and total haemoglobin ([tHb]) during the repeated-sprint protocol.

Linear mixed models were used to calculate the effect of time (sequential order of sprint and recovery periods) and condition (normoxia and hypoxia) on NIRS and $\dot{V}O_2$ responses to exercise. Subject ID was used as a random effect in all models. Analysis was performed using R Statistical Software (Foundation for Statistical Computing, v3.5.1, Vienna, Austria) and RStudio (RStudio Team, v 1.1.456, Boston, Massachusetts, United States of America). Models were calculated using the R "lme4" package version 1.1-18-1 (Bates et al., 2015). Effect sizes derived from the linear mixed model are presented in Table 3 as regression coefficients and are presented with standard error. The threshold for significance was set at $P < 0.05$. Figures were prepared using GraphPad Prism 6 for Windows (GraphPad Software, La Jolla, California, USA).

3. Results

Mechanical work recorded during the repeated-sprint tests is displayed in Fig. 2. There was no meaningful difference in total work between hypoxia and normoxia (relative difference = −2.9%, 90% CI −7.7 to 2.2%; standardised effect size = −0.21, 90% CI −0.58 to 0.16). There was also a *trivial* difference in peak power during sprint one output between the conditions (−2.1%, 90% CI −7.6 to 3.8%; ES = −0.11, 90% CI −0.43 to 0.20). Physiological responses to exercise are presented in Table 1, and results from the linear mixed model are presented in Table 3. Each sprint and recovery $\dot{V}O_2$ is displayed in Fig. 3. No significant ($P > 0.05$) effect for Time, Condition or Interaction was detected for sprint $\dot{V}O_2$. However overall, sprint $\dot{V}O_2$ in hypoxia was *likely* lower in normoxia compared to hypoxia. During the recovery phases $\dot{V}O_2$ increased over time ($P < 0.0001$), however there were no clear condition effects (Tables 1, and 3).

Respiratory muscle and vastus lateralis NIRS responses are presented in Tables 2 and 3, Figs. 4 and 5. Sprint and recovery O_2Hb_{VL} was *likely* lower in hypoxia compared to normoxia (Table 2), and the linear mixed model reviled that O_2Hb_{VL} was higher in normoxia ($P < 0.01$). The linear mixed model also reviled that O_2Hb_{VL} increased over Time during the sprint phase ($P < 0.0001$). However there was no Condition

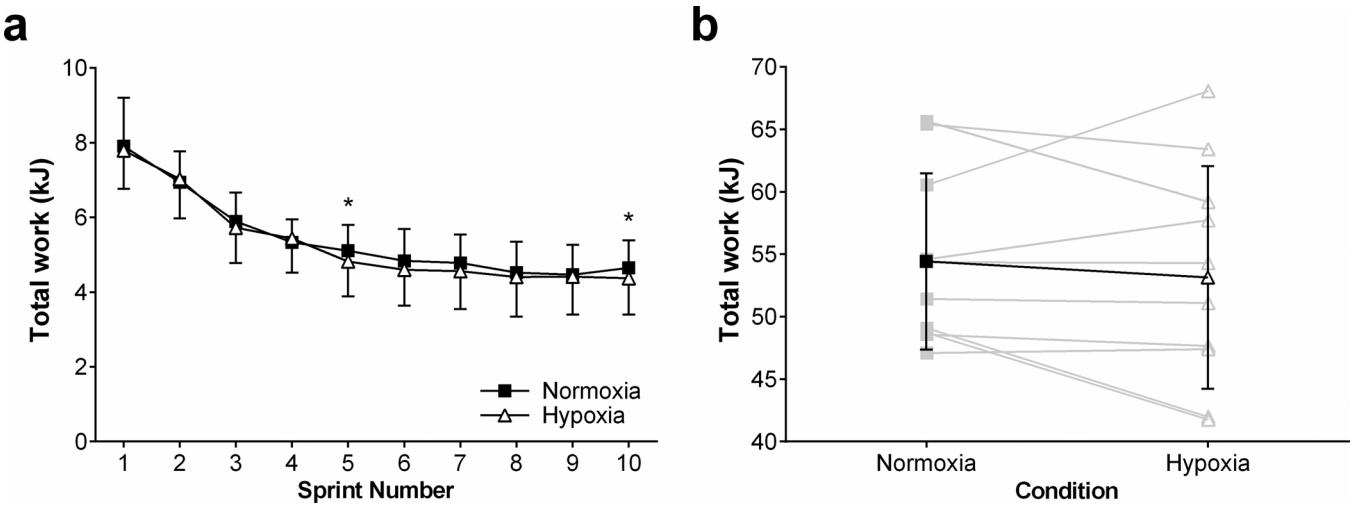

Fig. 2. Total mechanical work completed during repeated-sprint exercise in Normoxia and Hypoxia. (a) Mean total work per sprint and, (b) individual and mean total work completed over the entire protocol. The number of symbols (*); one, two and three denote *likely*, *very likely* and *most likely*, respectively, that the chance of the true effect exceeds a small (−0.2 to 0.2) effect size. Results are presented as mean ± SD.

or Interaction effect. Sprint and recovery HHb_{VL} was *likely* and *very likely* greater in the sprint and recovery periods, respectively, during hypoxic exercise compared to normoxia. A Condition effect for recovery was also reviled ($P < 0.01$). The linear mixed model also showed that HHb_{VL} increase over time for both the sprint ($P < 0.01$) and recovery phases ($P = 0.03$). However, HHb_{VL} during the sprint phase did not increase to the same extent in normoxia as hypoxia (Interaction effect; $P = 0.01$). Differences between the conditions for all of the respiratory muscle derived variables were *unclear* (the chance of having higher/lower values then the smallest worthwhile change [ES = 0.2] was both > 5%), and no significant Condition or Interaction effects were found. However, the linear mixed model revealed that [O_2Hb_{RM}] decreased with time ($P < 0.01$) with no Condition of Interaction

4. Discussion

The present study is the first to report on the influence of a reduction in F_IO_2 on respiratory muscle oxygenation trends during repeated-sprint exercise. Despite substantial arterial hypoxemia (SpO_2 = 87%), respiratory muscle oxygenation was maintained to a similar level to that measured during normoxic exercise. This contrasted with the impairment noted in the vastus lateralis oxygenation during both sprints and recovery periods, as previously demonstrated in previous research (Billaut and Buchheit, 2013). These data extend on what has been demonstrated during continuous high-intensity exercise (Harms et al., 1997; Amann et al., 2007; Turner et al., 2013), that respiratory muscle work could play a role in limiting O_2 transport to the locomotor muscles.

Acute hypoxia typically has no discernible effects on isolated sprint performance (Girard et al., 2017). However, as sprints are repeated, the effects of reduced O_2 availability typically become more apparent (Billaut and Buchheit, 2013; Smith and Billaut, 2012; Billaut et al., 2013). Surprisingly, even if arterial hypoxemia reached 87% in the present study it did not negatively affect total mechanical work to the same extent as in previous research. A non-meaningful -2.9% reduction in total work performed was observed, which was considerably less than previous research using a similar protocol (Billaut and Buchheit, 2013; Smith and Billaut, 2010, 2012). It is probable that there was an element of unconscious pacing from two subjects, despite receiving strong verbal encouragement throughout the sprint exercise. These two subjects were experienced road cyclists (10+ years' experience). Comparing their experimental data to that of the two familiarisation sessions, we believe that these participants just had a bad day performance wise. More typical responses were demonstrated in the other eight subjects. There is the chance that the pacing strategy adopted may have impacted the results. However, this is unlikely because of the more typical responses demonstrated in the other measures.

Consistent with others, sprint HHb_{VL} was higher during the hypoxic trials compared with normoxia, and the difference increased overtime (Billaut and Buchheit, 2013; Bowtell et al., 2014). Similarly, recovery HHb_{VL} was negatively affected by hypoxia. It is plausible that the metabolic demands of exercise were similar between conditions. The

Table 1
Physiological responses to repeated-sprint exercise in normoxia and hypoxia. Abbreviations are: arterial oxygen saturation, SpO_2; heat rate, HR; pulmonary oxygen uptake, $\dot{V}O_2$; end-tidal oxygen, $P_{ET}O_2$; end-tidal carbon dioxide, $P_{ET}CO_2$; inspiratory volume, IV; respiratory frequency, f_R; mouth pressure P_m; inspiratory muscle force development, $\int Pm \times f_R$. The number of symbols (*); one, two and three denote *likely*, *very likely*, and *most likely*, respectively, that the chance of the true effect exceeds a small (−0.2 to 0.2) effect size.

	Normoxia (mean ± SD)	Hypoxia (mean ± SD)	Relative difference (% [90% CI])	Effect size (Cohen's d [90% CI])
S_PO_2 (%)	96 ± 2	87 ± 3	−9.7 [11.4, −8.1]	−5.70 [−6.67, 4,73] ***
HR (bpm) n = 9	151 ± 12	156 ± 11	2.0 [−0.1, 4.2]	0.22 [−0.01, 0.46]
Sprint $\dot{V}O_2$ ($mL\,min^{-1}\,kg^{-1}$)	44.99 ± 5.49	42.65 ± 5.61	−5.3 [−8.4, −2.2]	−0.40 [−0.64, −0.16] *
Recovery $\dot{V}O_2$ ($mL\,min^{-1}\,kg^{-1}$)	37.02 ± 6.19	35.28 ± 6.10	−4.7 [−8.7, −0.6]	−0.27 [−0.50, −0.03]
$P_{ET}O_2$ (mm Hg)	118 ± 3	75 ± 3	−36.5 [−37.6, −35.5]	−17.59 [−18.21, −16.96] ***
$P_{ET}CO_2$ (mm Hg)	34 ± 3	31 ± 3	−7.4 [−9.9, −4.8]	−0.89 [−1.21, −0.57] ***
IV (L)	2.72 ± 0.50	2.70 ± 0.51	−0.6 [−4.0, 2.9]	−0.03 [−0.20, 0.14
f_R ($b\,min^{-1}$)	52.01 ± 15.32	51.40 ± 10.91	0.1 [−5.8, 6.4]	0.00 [−0.21, 0.22]
Inspiratory P_m (cm H_2O)	2.10 ± 0.33	2.20 ± 0.45	3.9 [−3.6, 12.0]	0.22 [−0.21, 0.66]
$\int P_m \times f_R$ (AU)	68.17 ± 13.85	70.48 ± 12.76	3.7 [−4.2, 12.3]	0.17 [−0.20, 0.53]

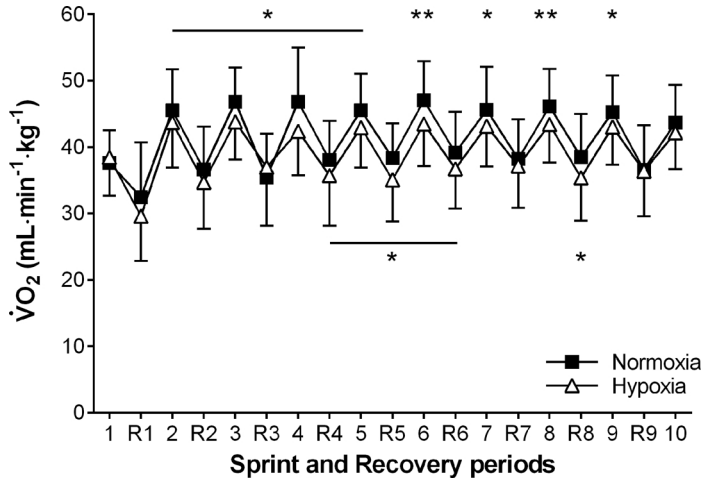

Fig. 3. Sprint and recovery pulmonary oxygen uptake ($\dot{V}O_2$) during normoxia and hypoxia repeated-sprint exercise trials. The number of symbols (*); one, two and three denote *likely*, *very likely* and *most likely* respectively, that the chance of the true effect exceeds a small (−0.2 to 0.2) effect size. Results are presented as mean ± SD.

additive effect of arterial hypoxemia on skeletal muscle O_2 extraction is the likely source of the elevated HHb_{VL} during exercise (Costes et al., 1996). Whereas recovery O_2Hb_{VL} and HHb_{VL} (also to a lesser extent ΔReoxy) is solely linked to limited muscle O_2 delivery between sprint efforts (Billaut and Buchheit, 2013). This is also supported by the evidence that O_2Hb_{VL} was 5% and 9% lower during the sprint and recovery phases respectively. Such changes in muscle oxygenation trends are representative of a mismatch between O_2 supply and extraction. Importantly, vastus lateralis O_2 availability during recovery is a strong determining factor metabolic recovery, and therefore repeated-sprint ability (Parolin et al., 1999; Gaitanos et al., 1993; Harris et al., 1976). Impairment of O_2 transport was also represented by the ~5% reduction in $\dot{V}O_2$ during hypoxic exercise that was shown here (Fig. 3), and similarly by others (Bowtell et al., 2014). It is well established that hypoxia results in a linear decrease in $\dot{V}O_{2peak}$ (Martin and O'Kroy, 1993; Wehrlin and Hallén, 2006). Hence, the lower $\dot{V}O_2$ represented a similar, or even greater fraction of the maximal O_2 utilisation relative to the blunted $\dot{V}O_{2peak}$. Therefore, to meet the metabolic demands of the sprint intervals, non-oxidative ATP resynthesis, specifically PCr hydrolysis, must increase to compensate the energy turnover (Hogan et al., 1999). Contrary to the clear changes in vastus lateralis oxygenation, respiratory muscle oxygenation appears to be unaffected by hypoxia.

Despite clear differences in arterial O_2 saturation (87% vs. 96%), respiratory muscle oxygenation responses were similar between the hypoxic and normoxic exercise trials. Only a relatively small Time effect was shown for $[O_2Hb_{RM}]$ (regression coefficient, -0.15 ± 0.06; $P < 0.01$). This distinct lack of difference suggests that hypoxia did not further compromise O_2 delivery to the respiratory muscles during short intermittent exercise. This contrasts with others who have shown a progressive deoxygenation of the respiratory muscles in response to hypoxia. During voluntary isocapnic hyperpnoea while breathing a hypoxic gas mixture (F_iO_2 = 0.10–0.11), deoxygenation of the strernocleildmastoid and intercostal muscles was exaggerated compared to normoxia (Katayama et al., 2015). Our lack of agreement in these results could be explained by the stark difference in hypoxic gas mixtures used (F_iO_2 = 0.10 vs. 0.15) and the resulting S_PO_2. In the present study, S_PO_2 averaged 87%, compared to the 82% recorded in the previous research (Katayama et al., 2015). There is some evidence in rats that resting respiratory muscle blood flow increases to compensate for arterial hypoxemia, serving to protect O_2 supply (Kuwahira et al., 1993). If arterial hypoxemia was greater, there is the possibility for exaggerated respiratory muscle deoxygenation. However, the degree of arterial hypoxemia induced in the present study does not appear to exaggerate respiratory muscle respiratory deoxygenation compared to similar exercise in normoxia.

Hypoxia is a known potent stimulant of hyperpnoea, which consequently elevates the work of breathing (Cibella et al., 1996; Reeves et al., 1994). As shown by others (Turner et al., 2013), an elevated work of breathing can amplify respiratory muscle deoxygenation. Since there were no meaningful differences in either ventilation patterns (f_R and IV) or inspiratory pressure generation (P_m and $\int P_m \times f_R$) in the present

Table 2
Near-infrared spectroscopy responses to repeated-sprint exercise in normoxia and hypoxia. Abbreviations are: concentration of respiratory muscle oxyhaemoglobin, $[O_2HHb_{RM}]$; concentration of respiratory muscle deoxyhaemoglobin, $[HHb_{RM}]$; concentration of respiratory muscle total haemoglobin, [tHb]; vastus lateralis oxyhaemoglobin, O_2Hb_{VL}; vastus lateralis deoxyhaemoglobin, HHb_{VL}; vastus lateralis reoxygenation, ΔReoxy. The number of symbols (*); one, two and three denote *likely*, *very likely* and *most likely*, respectively, that the chance of the true effect exceeds a small (−0.2 to 0.2) effect size.

	Normoxia (mean ± SD)	Hypoxia (mean ± SD)	Relative difference (% [90% CI])	Effect size (Cohen's d [90% CI])
Respiratory muscles				
$[O_2Hb_{RM}]$ (μm)	−11.07 ± 4.01	−12.07 ± 5.09	−0.1 [−2.9, 0.9]	−0.23 [−0.67, 0.21]
$[HHb_{RM}]$ (μm)	9.48 ± 6.81	10.38 ± 8.04	0.9 [−0.8, 2.6]	0.12 [−0.11, 0.35]
$[tHb_{RM}]$ (μm)	−1.59 ± 4.75	−2.18 ± 7.12	−0.6 [−3.6, 2.4]	−0.11 [−0.69, 0.46]
Vastus lateralis				
Sprint O_2Hb_{VL} (%)	26.11 ± 12.01	20.98 ± 13.76	−5.1 [−10.0, 0.3]	−0.39 [−0.76, 0.02] *
Recovery O_2Hb_{VL} (%)	91.86 ± 9.73	82.83 ± 15.11	−9.0 [−16.7, −1.4]	−0.85 [−1.57, −0.13] *
Sprint HHb_{VL} (%)	75.95 ± 16.85	85.08 ± 9.56	9.2 [0.2, 18.0]	0.50 [0.01, 0.98] *
Recovery HHb_{VL} (%)	25.81 ± 8.28	39.89 ± 14.81	14.1 [4.9, 23.3]	1.55 [0.54, 2.57] **
ΔReoxy (%)	50.14 ± 13.82	45.19 ± 13.05	−5.0 [−10.7, 0.8]	−0.33 [−0.71, 0.05]

Table 3
Linear mixed effects model summary. A positive Estimate vale for Time indicates an increase in that physiological measure as sprints are repeated. For Condition, a positive value indicates that the measure was higher during the normoxia trials. A positive interaction effect indicates that the measure increased overtime to a greater extent in Normoxia compared to hypoxia. Abbreviations are: pulmonary oxygen uptake $\dot{V}O_2$; concentration of respiratory muscle oxyhaemoglobin [O_2Hb_{RM}]; concentration of respiratory muscle deoxyhaemoglobin [HHb_{RM}]; concentration of respiratory muscle haemoglobin [tHb]; vastus lateralis oxyhaemoglobin, O_2Hb_{VL}; vastus lateralis deoxyhaemoglobin, HHb_{VL}. Regression coefficients are presented as value ± standard error. Bold text indicate effects that exceeded the threshold for statistical significance ($P < 0.05$).

Measure		Sprint align = "2pt 0cm; text-align: left"		Recovery align = "2pt 0cm; text-align: left"	
		Regression Coefficient	*P* Value	Regression Coefficient	*P* Value
$\dot{V}O_2$	Time	0.18 ± 0.12	0.13	**0.51 ± 0.13**	**< 0.0001**
	Condition	1.72 ± 1.02	0.09	1.87 ± 1.01	0.07
	Interaction	0.11 ± 0.16	0.50	−0.03 ± 0.18	0.88
$[O_2Hb_{RM}]$	Time	**−0.15 ± 0.06**	**< 0.01**		
	Condition	0.51 ± 0.39	0.20		
	Interaction	−0.02 ± 0.06	0.73		
$[HHb_{RM}]$	Time	7.79 ± 4.50	0.08		
	Condition	32.48 ± 39.49	0.41		
	Interaction	3.00 ± 6.36	0.64		
$[tHb_{RM}]$	Time	0.01 ± 0.05	0.86		
	Condition	0.60 ± 0.40	0.13		
	Interaction	−0.06 ± 0.06	0.38		
O_2Hb_{VL}	Time	**1.21 ± 0.22**	**< 0.0001**	0.27 ± 0.37	0.47
	Condition	2.23 ± 1.92	0.25	**9.37 ± 3.29**	**< 0.01**
	Interaction	0.53 ± 0.31	0.09	−0.06 ± 0.53	0.91
HHb_{VL}	Time	**1.13 ± 0.60**	**< 0.01**	**0.81 ± 0.37**	**0.03**
	Condition	−3.15 ± 2.59	0.22	**−12.40 ± 3.26**	**< 0.01**
	Interaction	**−1.09 ± 0.42**	**0.01**	−0.30 ± 0.53	0.56

study, O_2 cost of hyperpnoea was likely similar between conditions (Aaron et al., 1992a; Dominelli et al., 2014). These results provide evidence that neither the work of breathing nor the O_2 cost of exercise hyperpnoea was significantly influenced by the F_iO_2 during repeated sprints. Intercostal NIRS responses look to only respond proportionally to the metabolic activity of hyperopia, and free from the influence of hypoxemia (Costes et al., 1996; Katayama et al., 2015).

The evidence that the intercostal muscle oxygenation was not influenced by hypoxia, but vastus lateralis oxygenation trends were, has important implications for the ability to perform repeated sprints. It appears that O_2 delivery was preferentially distributed to the intercostal muscles to constrain an excessive decrease in respiratory muscle

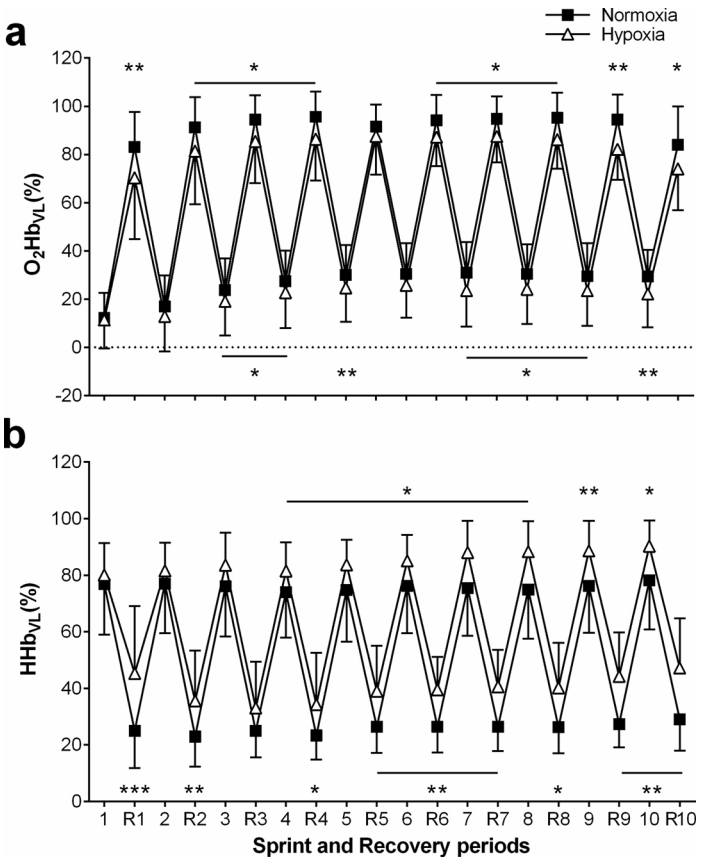

Fig. 4. Vastus lateralis muscle oxygenation trends during repeated-sprint exercise in normoxia and hypoxia expressed as a percentage of arterial occlusion. (a) Change of vastus lateralis oxyhaemoglobin (O_2Hb_{VL}); and (b) vastus lateralis deoxyhaemoglobin (HHb_{VL}). The number of symbols (*); one, two and three denote *likely*, *very likely* and *most likely* respectively, that the chance of the true effect exceeds a small (−0.2 to 0.2) effect size. Results are presented as mean ± SD.

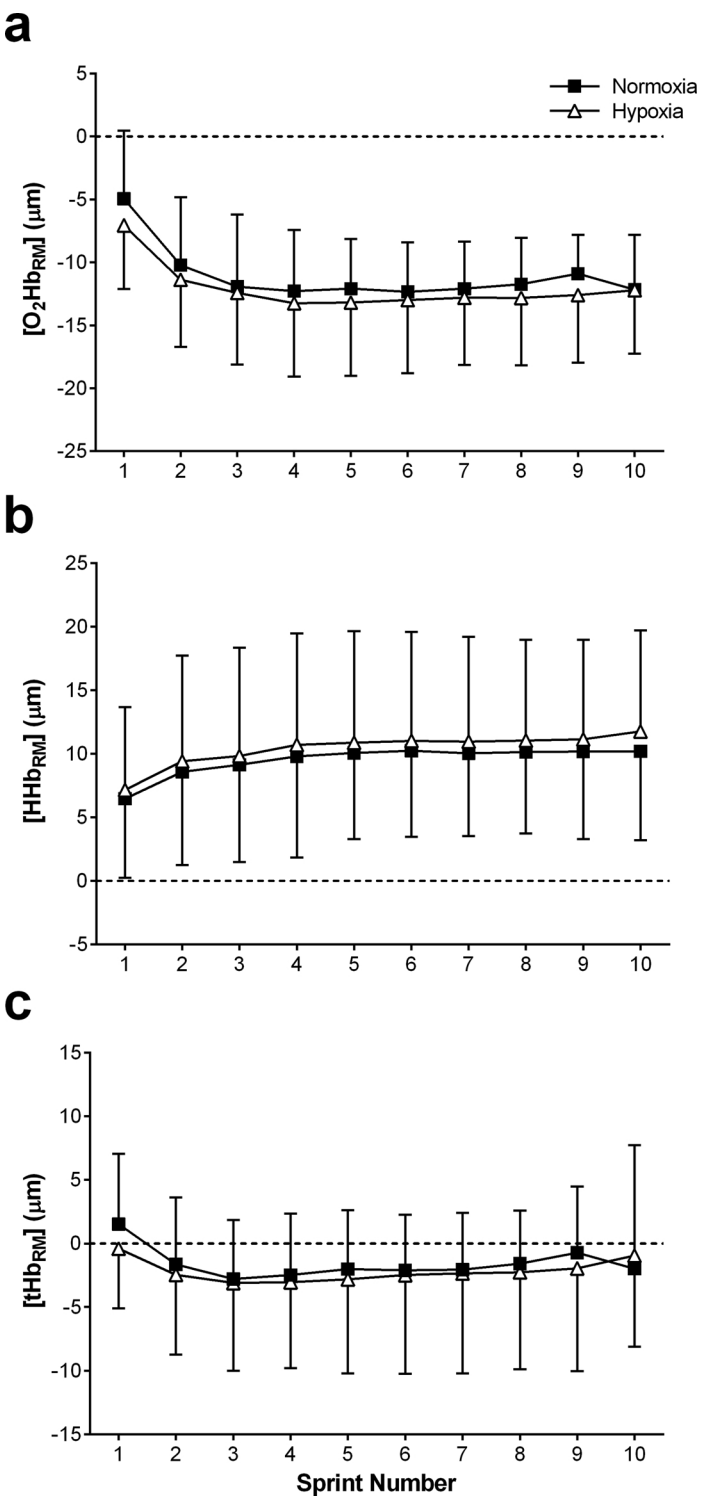

Fig. 5. Respiratory muscle oxygenation trends during repeated-sprint exercise in normoxia and hypoxia expressed as an absolute change from baseline (horizontal line). (a) Concentration change from baseline of respiratory muscle oxyhaemoglobin ($[O_2Hb_{RM}]$); (b) respiratory muscle deoxyhaemoglobin ($[HHb_{RM}]$); and (c) respiratory muscle total haemoglobin ($[tHb_{RM}]$). There was no clear effect of hypoxia on respiratory muscle oxygenation compared to normoxia. Results are presented as mean ± SD.

oxygenation. The work of breathing is estimated to account for 10–15% of total O_2 uptake during high-intensity exercise (Aaron et al., 1992a, b; Harms et al., 1998). To maintain O_2 supply to these essential muscles, blood flow is directed away from the locomotor muscles by the sympathetic nervous system (Harms et al., 1997; Sheel et al., 2001; St Croix et al., 2000; Dempsey et al., 2006). For these reasons, the O_2 cost of exercise hyperpnoea during repeated-sprint exercise could be a contributor of impaired vastus lateralis oxygenation in hypoxia. However, without a direct measurement of blood flow or O_2 uptake, the influence of the work of breathing on O_2 transport per se remains speculative.

Vastus lateralis oxygenation is implicated as an important mediating factor for the metabolic recovery between sprint efforts (Harris et al., 1976; Sahlin et al., 1979; Hogan et al., 1999), and therefore

performance (Billaut and Buchheit, 2013; Buchheit et al., 2009). Reducing the O_2 cost of hyperpnoea is a potential pathway of enhancing blood flow and O_2 availability for the locomotor muscle in hypoxic environments. In fact, there is evidence that inspiratory muscle training attenuates the O_2 cost of voluntary hyperpnoea (Turner et al., 2012), and improves the self-selected recovery time between repeated sprints (Romer et al., 2002). Competition for available O_2 is potentially minimised by the brief periods of passive rest between sprints. As theorised by Dempsey, Romer (Dempsey et al., 2006), locomotor muscle fatigue is exacerbated when a high work of breathing is accompanied by sustained high-intensity exercise. More work exploring the role of hypoxia in locomotor and respiratory muscle oxygenation trends is still needed, especially in the real context of competition when team-sport players spend a longer time running on the field.

5. Conclusion

According to previous research, vastus lateralis deoxygenation during repeated-sprint exercise was exaggerated in hypoxia in the present study. However, with a similar level of inspiratory pressure and force development, hypoxia did not affect intercostal muscle oxygenation. This suggests that blood flow could be preferentially distributed to the respiratory muscles to maintain O_2 delivery proportional to metabolic activity even during intermittent sprints. Reducing the O_2 cost of exercise hyperpnoea may have beneficial effects for O_2 delivery to the locomotor muscles when exercising in hypoxia.

Author contributions

RFR, NET, RJA and FB conceptualised and designed the research project; RFR acquired the data and conducted the statistical analysis; RFR interpreted results with assistance from NET, RJA and FB; RFR wrote the manuscript with revisions from NET, RJA and FB. All authors reviewed and agreed upon the final manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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