

The use of acute oxygen supplementation upon muscle tissue saturation during repeat sprint cycling

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ABSTRACT

This study examined performance and physiological responses (power output, tissue saturation index) to repeat sprint cycling with oxygen supplementation (O₂Supp [fraction of inspired oxygen 1.00]). Fourteen amateur male cyclists took part. Two visits to the laboratory entailed; 15min relative intensity warm-up, 10min of passive recovery, followed by 10x15s repeated sprints, during which air inspired had FiO₂ 1.00 oxygen or normal air. Outcome measures include, mean power (W) and change in Tissue Saturation Index (Δ TSI%). Repeated measures ANOVA were used to examine difference between conditions in mean power output. Paired samples t-tests were used to examine differences between conditions in Δ TSI (%) and rate of muscle reoxygenation and deoxygenation (%·s⁻¹). Mean power output was 4% higher in the oxygen condition compared to normoxia ($p < .01$). There was a significant positive correlation between power output and reoxygenation rate during O₂Supp ($r = 0.65$, $p = .04$). No correlation was seen between power output and reoxygenation rate during normoxia ($r = -0.30$, $p = .40$). A significantly increased deoxy rate was seen in the O₂Supp condition compared to normoxia ($p = .05$). Oxygen supplementation appears to elicit the greatest performance improvements in mean power, potentially facilitated by an increasing muscle reoxygenation rate. This evidences the utility of oxygen as an ergogenic aid to in cycling performance.

Keywords: Hyperoxia; Sports medicine; Physiology; Interval training; Sports performance.

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INTRODUCTION

Hyperoxia occurs when cells, tissues and organs are exposed to a level of oxygen higher than that of sea level (Mach et al., 2011). To create hyperoxic conditions one must breathe medical grade oxygen or an oxygen enriched gas mixture (oxygen supplementation [O₂Supp]). The creation of a hyperoxic condition can be viewed as an ergogenic aid, which is being explored within various sporting populations.

O₂Supp primarily functions on the premise of increasing the supply of muscle oxygen at exercise onset (Vanhatalo et al., 2010). O₂Supp has been shown to aid repeated sprint performance through an increased resynthesis rate of cellular metabolic phosphocreatine (PCr) (Haseler et al., 1999; Hogan et al., 1999). Moreover, O₂Supp (FiO₂ 0.7) has been shown to spare PCr degradation (by up to 55 s) over fixed workloads, compared with normoxia (Vanhatalo et al., 2010). It could be expected that O₂Supp during repeat sprint training would be effective at increasing the resynthesis rate of PCr and muscle oxygenation. Multiple sporting events rely on the ability to replenish and recover intramuscular stores (myoglobin oxygen saturation [MbO₂], PCr, Adenosine Triphosphate [ATP]) between high intensity efforts to enable continued high intensity performance (Glaister, 2005; Haseler et al., 1999).

High Intensity Interval Training (HIIT) simulates the energy demands of intermittent-sprint sports, with high intensity bouts followed by brief periods of recovery (Buchheit and Laursen, 2013). HIIT is categorised by peak ability during the first interval followed by decreasing performance in the subsequent repetitions (Bishop et al., 2011; Girard et al., 2011). Short duration sprints (< 15 s) interspersed with brief recoveries (< 60 s) result in near complete depletion of PCr during work periods, and incomplete resynthesis of PCr during the rest periods. This resynthesis is an oxidative process that requires free oxygen for rapid resynthesis. Performance during acute HIIT is reliant on reducing lactate accumulation and maintaining muscle oxygen status to resynthesise PCr rapidly (McMahon and Jenkins, 2002).

HIIT based research has utilised Near-Infrared Spectroscopy (NIRS) extensively (Gatterer et al., 2018; Jones et al., 2015; Prieur and Mucci, 2013). NIRS provides a non-invasive assessment of muscle oxygenation (tissue saturation) and haemodynamic status (peripheral blood flow) (Boushel and Piantadosi, 2000; Ferrari et al., 2011). Distinct changes in tissue saturation have been observed by NIRS during HIIT, characterised by large desaturation and restoration profiles. Similarly, the recovery time course of muscle oxygenation has been suggested to be correlated to PCr, at least following sub-maximal exercise, and resultantly NIRS is considered a proxy of PCr resynthesis (McCully et al., 1991, 1994). Attenuating a decline in arterial haemoglobin oxygen saturation (S_aO₂) with concurrent O₂Supp can increase full body oxygenation, by as much as 7% (Hogan et al., 1999). Recent evidence suggests that increasing the recovery rate of muscle oxygen can be correlated with improved performance in repeat sprint efforts (Buchheit and Ufland, 2011; Delextrat et al., 2018). These findings highlight the apparent importance of 'enhanced' muscle oxygenation profiles on subsequent performance.

Invasive measures of muscle oxygenation (muscle biopsies) have been previously used in O₂Supp research, however, this methodology has very little ecological application (Cardinale et al., 2019). It is suggested the NIRS technique should be used within O₂Supp research to better characterise the peripheral muscle response to this ergogenic aid and more fully inform exercise practitioners in 'real world' settings.

A primary aim of training modalities such as, HIIT and/or repeat sprint cycle training are to enhance the delivery and utilisation of oxygen (Perrey and Ferrari, 2018). O₂Supp has been shown to aid performance

(Porter et al., 2019) within these exercise disciplines, intuitively NIRS could provide useful novel mechanistic insight for the response to O₂supp.

The aim of this study was to assess whether the NIRS technique can be used within O₂Supp research to better characterise the peripheral muscle response to this ergogenic aid. We hypothesised that both muscle oxygenation and performance (power output) would differ during oxygen supplementation compared with normal air.

MATERIAL AND METHODS

Participants

Fourteen trained male University students volunteered for the study (1.81 ± 0.04 m, 77.7 ± 11.0 kg, 25.9 ± 7.4 years, thigh skinfold 10.5 ± 4.1 mm). Participants were healthy and were not taking any prescribed medications.

Ethical approval for the studies procedure was granted by the University ethics committee in accordance with the Declaration of Helsinki. Participants were informed of the procedure and asked to give written informed consent and complete a health questionnaire (PAR-Q).

Measures

Participants were required to undergo two testing session in the lab over a week period. Sessions were completed with at least 48 h between sessions. Laboratory visits were conducted at the same time of the day (± 2 h) to minimise circadian effects (Brisswalter et al., 2007). Participants were asked to maintain normal activity and sleep pattern prior to and between testing sessions. Participants were requested to arrive at the laboratory adequately hydrated and to abstain from caffeinated products in the preceding 4 h of each visit. Additionally, participants were asked to refrain from strenuous physical activity 24 h prior to participating.

This study was a single-blind, within-participant design comprising two counterbalanced assessments of repeat sprint performance under: O₂Supp (FiO₂ 1.00) or normal air (FiO₂ ~ 0.21). Each of the two sessions participants completed a 15 min cycling warm up, 10 min passive recovery, finishing with 10 x 15 s sprints with 45 s of passive recovery on a Wattbike cycle ergometer. All participants had previous experience with repeat sprint lab testing protocols. Performance measures such as: mean and peak power output were taken, along with NIRS measures of muscle oxygenation (resaturation and desaturation rates), as well as blood lactate concentrations.

Procedures

Participants completed the same procedure on both visits comprising; 15 min warm up 52% of heart rate reserve (Karvonen et al., 1957), 10 min passive recovery, finishing with 10 x 15 s sprints with 45 s of passive recovery, undertaken using a Wattbike Pro (Wattbike Ltd., Nottingham, UK) with the magnetic setting set to zero and air brake set to ten. The protocol was in accordance with pilot testing conducted prior to this study i.e. participants had sufficient load in order to reach peak power and not exceed max cadence.

Wattbike Pro was used to collect performance data (mean power), which was then used to calculate fatigue index (F1%) $((\text{Best sprint} - \text{worst sprint})/\text{best sprint}) * 100$. No prior familiarisation was conducted in the current study as it was established that each participant was familiar with repeat sprint cycling on a cycle ergometer.

Hyperoxic and normoxic gas mixtures were administered throughout the repeated sprints. Gas mixtures were administered via a rig of 4 x 200 L Hans Rudolph Douglas bags connected to a Hans Rudolph mask and head net (Hans Rudolph, Shawnee, KS, USA). O₂Supp was prefabricated using medical grade oxygen cylinder (British Oxygen Company, Surrey, UK) prior to administration. Hyperoxic air was saturated and warmed to room temperature before administration. FiO₂ of 1.00 was used to maximise the effects of oxygen supplementation, even though FiO₂ of 0.3 and above have been shown to be beneficial. Maximum dosage should be used to understand all effects, then a dose response relationship can be established in further research.

Preceding to each trial, a pre-exercise 20 µl capillary blood lactate sample was taken from the right ear lobule. Sample was mixed with haemolysing solution within a 0.5 ml haemolysing solution cup. Subsequent blood lactate samples were taken two minutes apart after the period between the warmup, and during the recovery period of each sprint repetition. All samples were analysed within using a Biosen (EKF diagnostics, Cardiff, UK).

Participants were required to wear a portable NIRS device to monitor oxygen saturation of the *vastus lateralis* muscle tissue (PortaMon, Artinis Medical Systems B.V., Elst, Netherlands). The NIRS device was fixed to the belly of the right *vastus lateralis*. Any bodily hair was removed within the device placement area and cleaned with an alcohol wipe to remove residue. The device was placed 3 cm anterior to the midpoint between the top of the greater trochanter and the lateral epicondyle. The device was taped with an adhesive wrapping and secondly wrapped with a black-out sports strapping to eliminate the entrance of ambient light. The same researcher attached the device on every occasion, ensuring an external pressure of less than 20 mmHg on the device. Indelible ink was used to draw around the device to guarantee accurate NIRS placement during the subsequent visits. Throughout the protocol the NIRS devices were connected to a personal computer via the Bluetooth™ system for data acquisition (10 Hz), and conversion from analogue to digital data.

The tissue haemoglobin saturation index (TSI), expressed in % and calculated as $([O_2Hb]/([O_2Hb] + HHb)) \times 100$ (which demonstrates the O₂ supply and O₂ consumption) (Ferrari and Wolf, 2007) was utilised. TSI was calculated using the Spatially Resolved Spectroscopy (SRS) methodology and was used to assess muscle reoxygenation rate. Reoxygenation rate (reoxy rate) (%·s⁻¹) was calculated as the change in TSI (%) from the end of the sprint by fitting a linear model to the 45 s part of the TSI (%) recovery. The slope of the relationship was retained as an index of reoxygenation rate. Similarly, deoxygenation rate (deoxy rate %·s⁻¹) was calculated as the change in TSI (%) from the beginning to end of the sprint by fitting a linear model to the 15 s part of the TSI (%) decline. Change (Δ) values were obtained during sprint and recovery periods. These were taken as; the difference between the baseline value (start sprint) and the one second average of the maximum value achieved during the sprint period and; the baseline value (end sprint) and the one second average of the maximum value achieved during the recovery period. NIRS data was processed using the methodology suggested in Rodriguez (Rodriguez et al., 2018). A one second moving average was applied to the data to attenuate the “noise” in the signal, whilst maintaining the integrity of the original data.

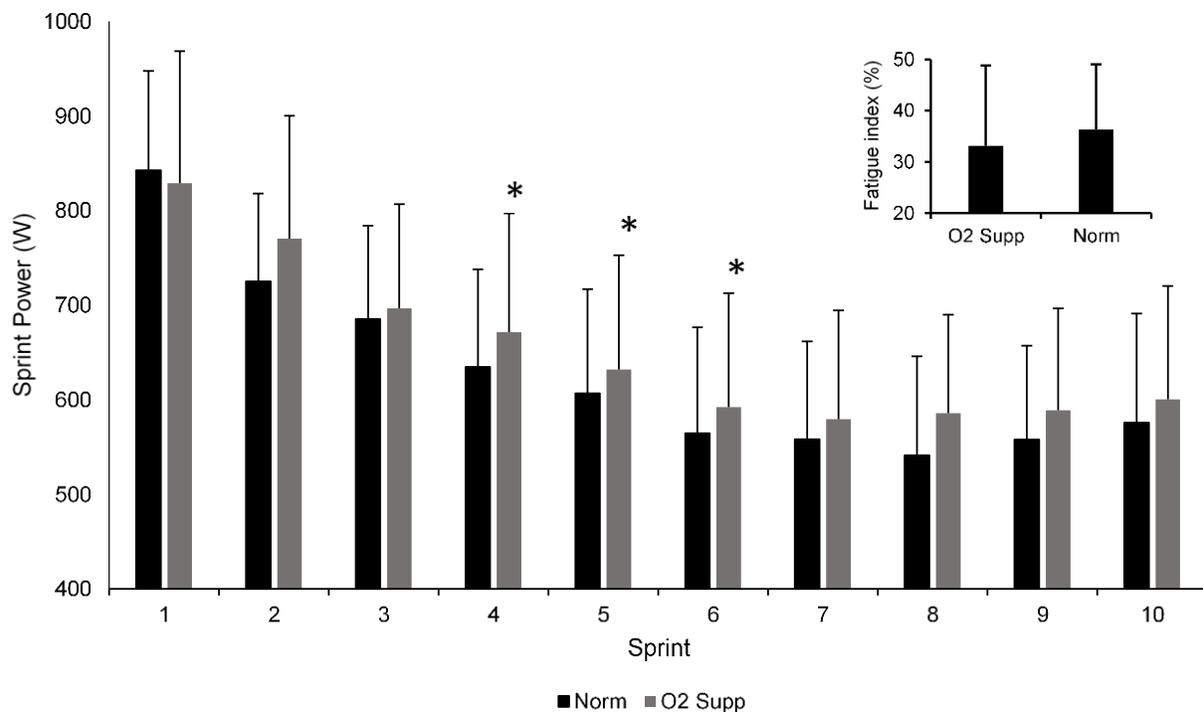
Statistical analysis

An a priori power analysis revealed that 10 participants would provide significant power to detect differences at an α-level of 0.05 for the primary outcome measure (mean power output) (G*POWER 3.1 Software, Düsseldorf, Germany). Statistical analysis was performed using the statistical package, SPSS statistics version 25 for windows (SPSS, Inc, Chicago, IL, USA).

A two-way repeated measure ANOVA (Condition X Sprint) was used to analyse differences in the ten 15 s sprints across the different protocols, followed by Turkey's post hoc tests where appropriate. Paired samples t-tests were conducted to examine the differences between the conditions for; change in tissue oxygenation (Δ TSI %), deoxy rate ($\% \cdot s^{-1}$) and reoxy rate ($\% \cdot s^{-1}$). Pearson's correlations were conducted to test the relationship between tissue oxygenation and power output between the conditions. Shapiro- Wilk normal distribution tests were conducted on all data. Data was screened for outliers outside of two standard deviations from the mean, no data was excluded. Effect size for individual measures were calculated and reported as Cohen's *d* and interpreted using bounds as 0.2, 0.5, > 0.8, where they are small, medium and large respectively. α level set at $p = .05$ for all analyses.

RESULTS

Figure 1. shows mean sprint power output (W) per sprint interval. Repeated measures ANOVA found a significant interaction effect $F(9,13) = 2.66$, $p = .045$, $ES = 0.98$ and post hoc analysis shows a higher mean sprinting power for sprints 4-6. There was a significant increase ($\sim 4 \pm 2.6\%$) in power output (W) in the O₂Supp group mean compared to the norm group ($ES = -0.28$, 95% CI = 0.87 to 2.52; $p < .01$). Figure 1. shows a non-significant; $t(13) = 1.735$, $p = .11$, $ES = 0.23$ reduction in Fatigue index (FI%) following 10 cycling sprints under the two conditions. A 3% reduction in FI (%) is seen in the O₂Supp condition.

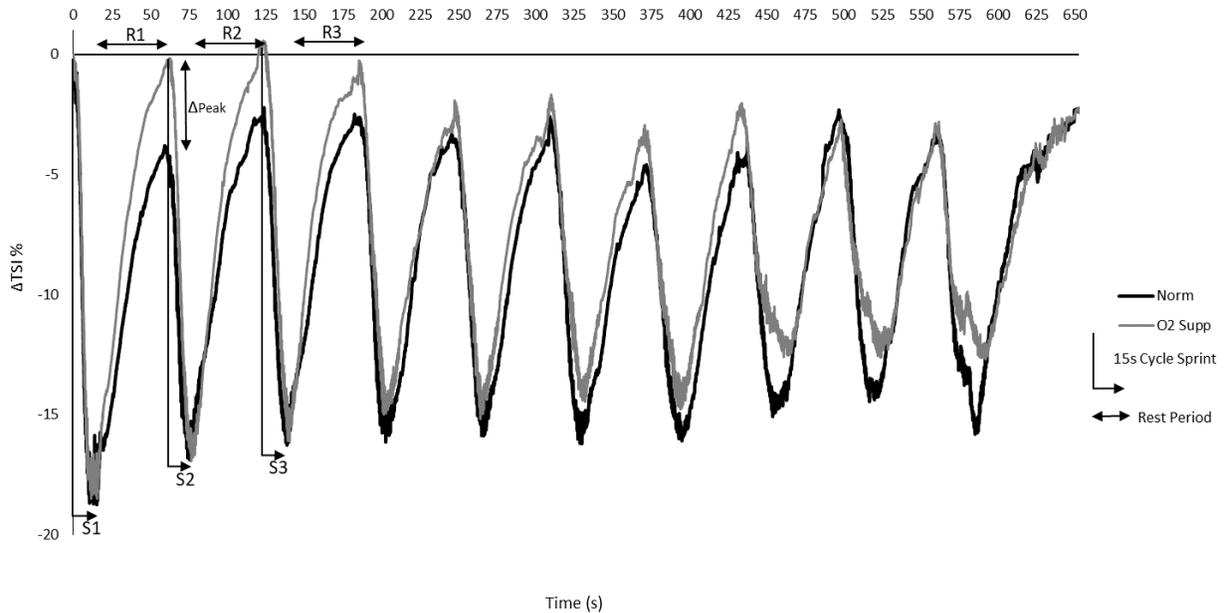


* significant difference between condition (O₂ Supp and norm) ($p < .05$).

Figure 1. Mean sprinting power across 10 sprints (1a) and Fatigue index score under two conditions (1b) ($n = 14$).

Figure 2. displays the group average norm vs. O₂Supp Δ TSI (%) data trace during (10 x 15 s with 45 s recovery) repeat cycling efforts. During the repeat efforts there was a rapid drop in TSI at the onset of each sprint with a nadir achieved approximately 10 s into each sprint. During each 45 s recovery period there was

a trend of a rapid recovery of group TSI (first 20 s), followed by a slowing in the recovery rate (final 25 s). The rate and extent of TSI recovery in the hyperoxic condition is facilitating a quicker oxygen resaturation to baseline; this hyperoxic effect is clear for recovery periods 1-3. This response is attenuated as the sprints progress (sprints 4-10). After sprint 6 TSI fails to return to baseline as the sprints are completed.



Legend. $\Delta Peak$ – Change in peak resaturation, S1- Sprint One, R1- Recovery One.

Figure 2. Tissue Saturation Index (TSI %) characteristic representation data for the group, during the repeat sprint cycling protocol. Norm and O₂ Supp traces are overlaid.

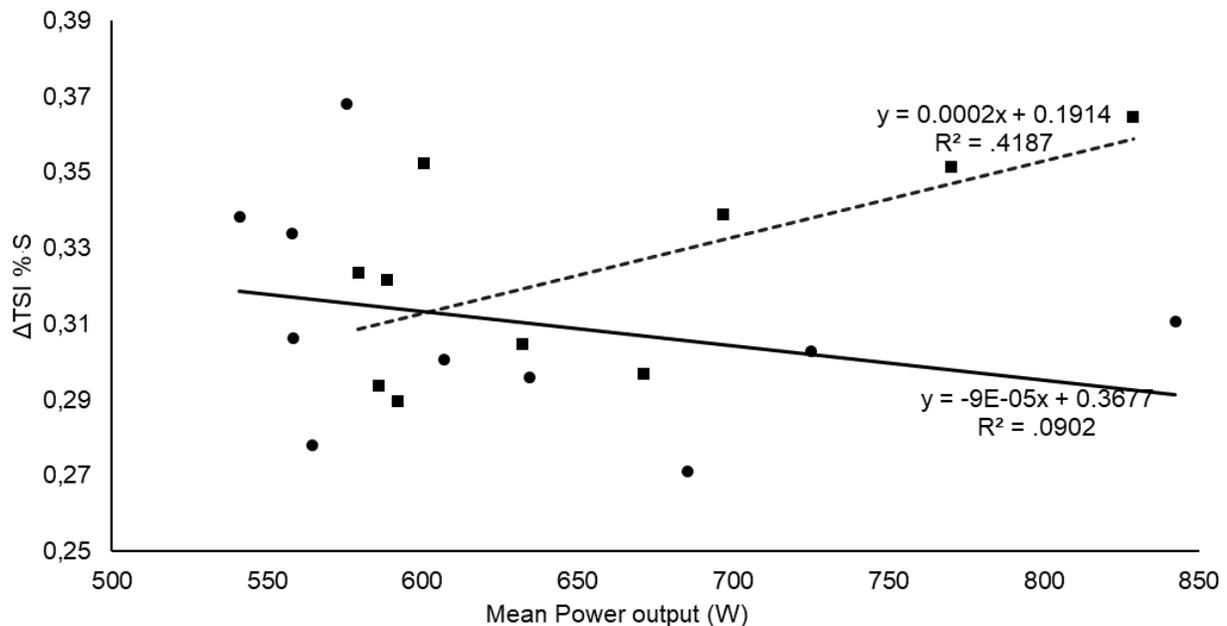


Figure 3. Relationship between muscle reoxygenation rate ($\% \cdot s^{-1}$) and mean cycling power (W) (n = 14). ■ O₂ Supp (---), ● Norm (—).

Mean blood lactate was higher in the O₂Supp condition (9.81 mmol·L⁻¹), albeit by a small margin (0.43 mmol·L⁻¹), $t(9) = 3.36$, $p < .01$, $ES = 0.13$. When comparing sprints directly between conditions, it was only after sprints 4 and 8 that this difference reached significance.

No significant difference was seen in the mean recovery amplitude (i.e. Δ) Δ TSI (%) in the O₂Supp group vs. norm group ($ES = -0.33$, 95% CI = -1.71 to 0.51; $p = .25$). A mean difference in individual sprints Δ TSI (%); 2.4%, 2.2% and 3.0% can be seen in Figure 2, for Sprints 4-6 O₂Supp vs. norm group. No significant difference was seen within the group mean reoxy rate (%·s⁻¹) between the two conditions ($ES = 0.33$, 95% CI = 0.01 to 0.04; $p = .26$). A significantly increased deoxy rate (%·s⁻¹) was seen in the O₂Supp condition compared to norm ($ES = -0.61$, 95% CI = -0.13 to -0.00; $p = .05$).

Figure 3. displays a positive correlation between Reoxy Rate (%·s⁻¹) and mean sprinting power during O₂Supp ($r = 0.65$, $p = .04$). No significant correlation was found between Δ TSI (%) and mean sprinting during normoxia ($r = -0.30$, $p = .40$).

DISCUSSION

The aim of this study was to assess whether the NIRS technique can be used within O₂Supp research to better characterise the peripheral muscle response to the ergogenic aid. It was hypothesised that muscle oxygenation and performance that would differ during oxygen supplementation compared to normal air. This is the first paper of its kind to assess changes in the rate of muscle oxygen resaturation, and desaturation during O₂Supp in repeat sprint cycling, using non-invasive 'real world' measures.

Primary findings revealed changes in muscle oxygen recovery (%·s⁻¹) rates in the presence of extra oxygen (FiO₂ 1.00). This recovery rate was strongly correlated with preceding power output (W) ($r = 0.65$, $p = .04$) (Figure 1 and 2). There was no correlation between recovery rate and power output during normoxia ($r = -0.30$, $p = .40$) (Figure 3). Lactate levels were higher in the O₂supp condition compared to normoxia ($p < .01$). Additionally, there was an increased deoxy rate in O₂Supp compared to normoxia across the 10 sprints ($p = .05$).

The rate of recovery of muscle oxygen is one of most important aspect of fitness to a sprint trained athlete (Glaister, 2005). The ability to flush out fatigue related metabolic by-products and the replenishment of fuel for successive sprints has obvious importance for performance. HIIT is highly reliant on the utilisation of PCr during exercise and exclusively upon aerobic processes (resynthesis) during periods of recovery (McMahon and Jenkins, 2002). O₂Supp has been shown to be beneficial for sprint performance due to the increased resynthesis rate of cellular metabolic PCr (Hogan et al., 1999; Linossier et al., 2000). The significant increase in power output (3.95%), non-significant increase in the extent of Δ TSI (%) recovery and reoxygenation rate (%·s⁻¹) seen here are potentially indicative of an enhanced PCr resynthesis profile (McCully et al., 1994). Though PCr resynthesis was not measured directly, muscle (re)oxygenation has been suggested by others to be representative of this (Buchheit and Ufland, 2011; McCully et al., 1991, 1994). Additionally, the changes seen in performance variables (W) between O₂Supp and normal air, replicated previous research showing increases in mean power output ($\sim 4 \pm 2.6\%$ [30 W]) during O₂Supp (Figure 2).

Blood lactate data from this study have been previously published (Porter et al., 2019). In brief, muscle oxygenation data is supported by the significantly reduced lactate response despite a higher power output. The ability to attenuate an anaerobic metabolic environment during repeat sprint work should allow for enhanced PCr resynthesis. Lactate and muscle oxygenation data highlight that despite higher intensity

exercise (increased power output), there is a higher availability of oxygen. This suggests that in the initial sprints, oxygen availability is the primary mechanism for enhanced performance (Figure 1).

Studies by Jones *et al.*, (Jones *et al.*, 2015, 2018) demonstrated a positive relationship between enhanced muscle oxygenation during recovery and increased subsequent performance following a training programme. Their study shows that a 3 s faster desaturation time contributes to increase in mean cycling performance by as much as 10%. This alteration in desaturation profile is suggested to occur as a result of positive peripheral muscle morphological adaptations such as increased mitochondrial density and efficiency, resulting in enhanced oxygen extraction (Jacobs *et al.*, 2013; Prieur and Mucci, 2013). The findings of the current study reflects potential adaptations due to similar desaturation profile, as seen in Jones *et al.*, (Jones *et al.*, 2015, 2018). These findings also mirror those of Buchheit *et al.*, (Buchheit *et al.*, 2012) who also found that an increased rate of muscle deoxygenation (6%) during SIT elicits metabolic adaptations, such as increased citrate synthase activity, resulting in increased power output. The current study shows a significant increase in deoxy rate and non-significant increase in reoxy rate, similar to the previous studies. Figure 3. highlights that both power and reoxy rate increases with O₂Supp although not significantly different from control, they are significantly positively correlated- as power increases with O₂Supp so does reoxy rate. The increase rate of reoxygenation may likely cause the subsequent increase in performance as additional oxygen is available to the working muscle. This correlation is not evident during the normoxia condition where reoxy rate has a weak negative correlation with power output. The simplest explanation for this is, due to an increased availability of oxygen as a result of the administered O₂Supp and not as a result of morphological adaptation to training.

Higher re-saturation rates occur from the first sprint, but it is not until sprint 4 onwards that performance increases are seen in the O₂Supp condition (Figure 2). The high availability of oxygen allows for rapid resaturation of myoglobin and haemoglobin, meaning subsequent sprints can start from a metabolically advantageous point. Inevitably there comes a point where, despite extra oxygen, recovery is not rapid enough to maintain performance.

Interestingly, the novel findings of this study open further avenues for exploration. Firstly, due to the acute nature (short term effects - minutes) of the current study, the changes in muscle oxygen and power output have only been evidenced acutely. Few long-term or short-term training studies using O₂Supp have examined the effects of 'chronic supplementation' on muscle oxygen response or peripheral muscle composition (increased mitochondrial density, increased baseline utilisation capacity of oxygen). Previous training studies (Burgomaster *et al.*, 2008; Gibala *et al.*, 2006) have demonstrated changes in tissue saturation profile and musculoskeletal adaptation i.e. increased mitochondrial biogenesis/ proliferation, increased enzyme activity (citrate synthases) following SIT protocols. It would appear intuitive for further studies to initially identify the effects of acute O₂Supp administration on performance using NIRS (as shown here). An understanding of the effects of O₂Supp, whether this is duration and/ or dosage dependant, will be key to optimal administration.

Despite equivalent findings between repeat sprints, time trials (TT) and time trials to exhaustion (TTE) using O₂Supp, there is a lack of comparable studies with short duration recoveries following high intensity repeat sprints, even though this training approach is commonly used in sprint training programmes (Buchheit and Laursen, 2013; Glaister, 2005). Utilising TT or TTE themselves as a training approach for intermittent sports is unheard of despite the performance benefits for TT and TTE (Mallette *et al.*, 2018; Wilson *et al.*, 1974). As such the current study should pave the way for further studies exploring optimised training for high intensity repeat sprint training.

Due to a technical issue, S_aO_2 was not ascertained during data collection. Having data on the individual response of S_aO_2 during O_2 Supp, would have allowed the authors to determine whether changes in the muscle oxygenation were related to delivery or utilisation. Furthermore, ventilatory parameters would have better informed the results of this study. Unfortunately, collecting ventilatory gases whilst administering a manipulated gas content, posed logistical constraints that could not be overcome.

Supplementary oxygen elicits meaningful performance improvements whilst increasing muscle reoxygenation rate during repeat cycling sprints as shown in figure 3. This increase in reoxygenation rate may likely cause the subsequent increase in performance. This study looks at the mechanistic approach to O_2 Supp and demonstrates the potential utility of O_2 Supp as an ergogenic aid within cycle and repeat sprint exercise. This study is the first of its kind to demonstrate changes in muscle oxygenation during O_2 Supp using non-invasive 'real world' measures.

CONCLUSIONS

Many cyclists incorporate some form of HIIT in their training, with the aim of improving cycling performance. The results of the current study show that in University level cyclists (amateur) when compared with a sea level condition, O_2 Supp elicits immediate acute enhancements to performance measures via an improved muscle oxygenation status. This allows athletes to experience an immediate enhancement in their training, allowing them to work harder in a single training session, with the aim of inducing greater resultant adaptation. NIRS is a functional tool in which skeletal muscle oxygenation data can be seen in 'real time', complimenting external power data, allowing coaches to make better informed decisions (Perrey and Ferrari, 2018).

AUTHOR CONTRIBUTIONS

Dr. Michael Porter, Dr. Kate Reed and Dr. Ben Jones, all developed the study design. Dr. Michael Porter undertook the data collection with Dr. Kate Reed and Dr. Ben Jones supervising the findings of this work. All authors discussed the results and contributed to the final manuscript.

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DISCLOSURE STATEMENT

The authors have no conflicts of interest that are directly relevant to the content of this manuscript.

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