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Oxygen availability affects exercise capacity, but not neuromuscular fatigue characteristics of knee extensors, during exhaustive intermittent cycling

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ABSTRACT

Purpose: To compare the effects of different hypoxia severities on exercise capacity, cardio-respiratory, tissue oxygenation and neuromuscular fatigue characteristics in response to exhaustive intermittent cycling. **Methods:** Eleven well-trained cyclists, repeated supra-maximal cycling efforts of 15 s (30% of anaerobic power reserve, 609±23 W), interspersed with 45 s of passive rest until task failure. The exercise was performed on separate days in normoxia (SL; simulated altitude/end-exercise arterial oxygen saturation = 0 m/~96%), moderate (MH; 2,200 m/~90%) and severe (SH; 4,200 m/~79%) hypoxia in a cross-over design. Neuromuscular tests, during brief (5 s) and sustained (30 s) maximal isometric voluntary contractions of the knee extensors, were performed at baseline and exhaustion. **Results:** Exercise capacity decreased with hypoxia severity (23±9, 16±6 and 9±3 cycle efforts in SL, MH and SH, respectively $P<0.001$; $\eta^2=0.72$). Both cerebral ($P<0.001$; $\eta^2=0.86$) and muscle ($P<0.01$; $\eta^2=0.54$) oxygenation decreased throughout the exercise, independent of condition ($P\geq0.45$; $\eta^2\geq0.14$). Compared to SL, muscle oxygenation was globally lower in MH and SH ($P=0.011$; $\eta^2=0.36$). Cardiovascular solicitation neared maximal values at exhaustion in all conditions. Peak twitch amplitude with single and paired electrical stimuli ($P<0.001$; $\eta^2\geq0.87$), maximal torque ($P<0.001$; $\eta^2\geq0.48$) and voluntary activation measured using transcranial magnetic stimulation ($P\leq0.034$; $\eta^2\geq0.31$) during brief and sustained MVCs were all reduced at exhaustion, independent of condition ($P\geq0.196$; $\eta^2\geq0.15$). **Conclusions:** Despite reduced exercise capacity with increasing severity of hypoxia during exhaustive intermittent cycling, neuromuscular fatigue characteristics were not different at task failure and cardiovascular solicitation neared maximum values.

Keywords: altitude; exhaustion; fatigue; graded hypoxia; intermittent exercise; neuromuscular fatigue; transcranial magnetic stimulation.

ABBREVIATIONS

b*f*	Breathing frequency
EMG	Surface electromyography
FiO_2	Inspired fraction of oxygen
MEP	Motor evoked potential
MH	Moderate hypoxia
MNS	Motor nerve stimulation
MVC	Maximal voluntary contraction
NIRS	Near-infrared spectroscopy
O_2	Oxygen
$PetCO_2$	End-tidal partial pressures of carbon dioxide
$PetO_2$	End-tidal partial pressures of oxygen
RF	Rectus femoris
RMS	Root mean square
SH	Severe hypoxia
SL	Sea level
SpO_2	Pulse oxygen saturation
TMS	Transcranial magnetic stimulation
TSI	Tissue saturation index
VA	Voluntary activation
$\dot{V}CO_2$	Carbon dioxide production
$\dot{V}_E$	Minute ventilation
VL	Vastus lateralis
$\dot{V}O_{2max}$	Maximal oxygen uptake
$\dot{V}_T$	Tidal volume

INTRODUCTION

Reduced oxygen (O_2) availability is detrimental to exercise capacity in closed-loop locomotor tasks, as evidenced by reduction of sustained power output during cycling time trials (Amann et al. 2006; Girard et al. 2016) or repeated cycling sprints (Billaut et al. 2013; Soo et al. 2020). For cycling tasks performed to the limit of tolerance (open-loop design) in hypoxia, an earlier exercise termination is typically seen for intermittent (Flinn et al. 2014) and constant-load (Goodall et al. 2012) high-intensity or maximal incremental (Osawa et al. 2011) tests compared to normoxia. Moderate-to-severe hypoxic conditions (inspired fraction of oxygen or FiO_2 = 0.15-0.11) increase cardio-respiratory requirements, quadriceps muscle recruitment and can exacerbate muscle/cerebral de-oxygenation during a submaximal whole-body exercise as compared to a similar exercise in normoxia (Goodall et al. 2014a). What causes early exercise cessation with augmented hypoxia severity has been hotly debated, yet without reaching consensus (Thomas et al. 2018).

A recently proposed, but debated, hypothesis suggests that the development of peripheral locomotor muscle fatigue from exhaustive efforts is confined to a certain limit (also referred as an "individual critical threshold") that is not exceeded (Amann et al. 2016). In support, completion of high-intensity cycling trials in a wide range of O_2 availability conditions often coincides with a very similar and severe degree of peripheral fatigue (Δ30-60% in twitch torque amplitude from pre-to-post exercise); a threshold beyond which the degree of associated sensory perception would not be tolerable, triggering task disengagement (Hureau et al. 2018). Conversely, based on dissimilar end-exercise levels of peripheral fatigue across a range of normoxic-to-hypoxic conditions, others have questioned the importance of a critical peripheral fatigue threshold in regulating exercise performance (Thomas et al. 2018). These observations arise from constant load cycling at 80% of maximal work rate (Goodall et al. 2012) but also

after either submaximal isometric knee extensions to task failure (Goodall et al. 2010; Froyd et al. 2016) or maximal intermittent dynamic leg extensions (Christian et al. 2014).

By stimulating the motor cortex with transcranial magnetic stimulations (TMS), it is possible to determine the extent of supraspinal fatigue, that is, fatigability caused by a suboptimal output from the motor cortex (Todd et al. 2003). Thus far, TMS has demonstrated declines in voluntary activation (VA) of knee extensor muscles in response to unilateral isometric leg extension until exhaustion (Goodall et al. 2010) and after either short-duration, exhaustive or prolonged (4 h; Jubeau et al. 2017) constant-load cycling tasks (Goodall et al. 2012). Recently, we failed to demonstrate that muscle activation, estimated from peripheral (motor nerve stimulation [MNS]) and cortical (TMS) VA values, was significantly reduced by repeated sprints (10 × 4-s sprints with 30 s of recovery) and/or exposure to hypoxia up to 3,600 m (Soo et al. 2020). Short repeated "all out" (<10 s) and longer sub-maximal intermittent sprints (10-30 s) are two distinct exercise models (Girard et al. 2011), likely inducing different profiles of neuromuscular fatigue. Findings from repeated-sprint studies using a closed-loop design and conducted in hypoxia (Billaut et al. 2013; Soo et al. 2020) may not be extended to an exhaustive intermittent cycling task to explain why exercise performance is impaired in these conditions. Consequently, robust data on the nature of the different components of fatigue and their magnitudes, including the completeness of cortically- and peripherally-derived estimates of VA at elevations higher than 3,600 m simulated altitude, following exhaustive intermittent cycling (i.e., open loop design) is yet to be provided.

This study aimed at examining the effects of hypoxia severity on intermittent cycling responses continued to the limit of tolerance (i.e., until task failure), the associated cardio-respiratory, tissue oxygenation responses and resulting neuromuscular fatigue adjustments. We hypothesized that increasing severity of arterial hypoxemia would (i) reduce exercise capacity, (ii) exacerbate quadriceps muscle recruitment responses and muscle/cerebral deoxygenation

levels (i.e., resulting from an increased relative exercise intensity) (iii) induce a shift towards greater central, relative to peripheral, mechanisms of fatigue experienced by the knee extensors.

METHODS

Participants

Eleven, well-trained, male cyclists (38.1 ± 7.0 years; 182.8 ± 8.6 cm; 82.9 ± 15.9 kg; 7.7 ± 2.6 h cycling per week; 368 ± 47 W and 4.29 ± 0.75 $L \cdot min^{-1}$ for maximal aerobic power and maximal oxygen uptake [$\dot{V}O_{2max}$], respectively) participated in the study. All participants were born and raised at <1,500 m and had not travelled to elevations >1,000 m in the 3 months prior to investigation. Prior to any experimental procedures, participants gave their informed, written consent. All procedures were conducted according to the Declaration of Helsinki for use of Human Subjects and approved by the Ethics Committee of *Shafallah Medical Genetics Center*.

Experimental design

Each participant completed a familiarization session and three experimental trials in a randomized, double-blind, cross-over design. All tests were completed in a normobaric hypoxic chamber (Colorado Mountain Room System; Colorado Altitude Training, Boulder, CO). The experimental trials were separated by at least 5 days, performed at the same time of the day (±2 h), and conducted in temperate ambient conditions (air temperature: 24°C; relative humidity: 40%). Participants were asked to avoid vigorous exercise for 24 h, caffeine for 12 h, and food for 2 h, before each trial. They were permitted to drink *ad libitum* during testing.

Familiarization session

During the first visit, participants were first accustomed with the testing procedures used to assess neuromuscular function. Optimal levels of stimulation intensities were determined for motor cortex and femoral nerve stimuli (see below), and these levels remained constant during the rest of the protocol (i.e., for all neuromuscular assessments). Following ~15 min of rest,

baseline neuromuscular function (Pre-tests) was recorded in normoxia, which served as the Pre-tests assessment for all conditions. Afterwards, participants performed an incremental cycling test on an electromagnetically braked cycle ergometer (Excalibur Sport, Lode, Groningen, The Netherlands), while breathing room air for the determination of maximal aerobic power output and $\dot{V}O_{2max}$. Workload increased at a rate of 25 $W \cdot min^{-1}$ until participants reached exhaustion, as indicated by volitional cessation of exercise, or failure to maintain a pedal cadence of 70 rpm despite strong verbal encouragement. Maximal values for cardiorespiratory function and hemodynamics were taken as the highest 30 s mean value attained prior to the participant's volitional exhaustion. Finally, after 20 min of passive rest, participants performed three single, 10 s cycling sprints (peak power output = 1,254 ± 291 W) against a constant force (0.9 $N \cdot kg^{-1}$ of body mass) with 2 min rest between maximal efforts for the purpose of quantifying individual anaerobic power reserve.

Experimental sessions

The experimental session was conducted as follows: (1) participants were seated for 15 min to rest and allow for instrumentation; (2) standardized warm-up (*i.e.*, 5 min of continuous cycling at 50% of power associated with $\dot{V}O_{2max}$, immediately followed by 2 min at 100% of power associated with $\dot{V}O_{2max}$ [368 ± 47 W]) and, after 2 min of rest, cycling 15 s at 30% of the anaerobic power reserve (644 ± 127 W) at a cadence of 110 rpm in normoxia (Buchheit and Laursen, 2013); (3) climatic chamber entrance and rest for 2-min on the cycle ergometer (wash-in period) prior to the beginning of exercise; (4) exercise protocol *(see below)*; (5) neuromuscular function assessment (post-tests), initiated 7 min after exercise cessation, in normoxia.

Intermittent exhaustive cycling exercise

The exercise protocol consisted of performing intermittent cycling until exhaustion at supra-maximal intensity; i.e.,15 s at 30% of the anaerobic power reserve (644 ± 127 W) with a fixed

pedaling cadence of 110 rpm (visual and verbal feedback and reached after ~2-3 s), interspersed with 45 s of passive rest. This protocol was randomly conducted at sea level (SL; simulated altitude/FiO_2 0 m/~0.21), and at moderate ~2,200 m (MH; FiO_2 ~0.16) and severe ~4,200 m (SH; FiO_2 ~0.12) simulated altitudes. During each cycle effort, participants were instructed to rapidly reach the pedal cadence (within 3-4 s) and exercise was terminated by the investigators when pedal cadence dropped below 70 rpm for > 5 s. Unfinished sprints (participants not being able to turn the pedals) were not taken into consideration.

Responses to exercise

Cardiorespiratory function

Expired gases were collected by a mask enclosing both the mouth and nose and recorded breath by breath using a calibrated analyzer (Cosmed Quark b^2, Rome, Italy) for the calculation of O_2 uptake, carbon dioxide production ($\dot{V}CO_2$), minute ventilation ($\dot{V}_E$), tidal volume (V_T), breathing frequency (bf) and end-tidal partial pressures of oxygen ($PetO_2$) and carbon dioxide ($PetCO_2$). Heart rate, cardiac output and stroke volume were measured using automated impedance cardiography (ICG; Q-Link PhysioFlow PF-07, Manatec Biomedical; France) that has been shown to be both valid and reliable during submaximal exercise (Charloux et al. 2000). Pulse oxygen saturation (SpO_2) was estimated non-invasively via pulse oximetry using a finger probe (Palmsat 2500, NONINMedical Inc., Plymouth, MI, USA) and rating of perceived exertion, obtained using the 6-20 Borg scale, was recorded at exactly 10 s following each exercise bout.

Because the total exercise duration of the intermittent exhaustive cycling test was different from one participant / condition to another, cardio-respiratory data were time normalized over the individual total exercise duration for each trial. For final analysis, data were presented at exercise onset and near exhaustion (1-20 and 81-100% of the time to exhaustion, respectively).

Electromyography, prefrontal cortex and muscle oxygenation responses

For each 15 s cycling effort, surface electromyographic (EMG) signals from *vastus lateralis* (VL) and *rectus femoris* (RF) muscles of the right lower limb were recorded. The root mean square (RMS) of the EMG signal over a 5 s epoch between the 5th and the 10th second of the 15 s cycle effort was calculated for each muscle. EMG data post-processing was performed in Spike2 (Version 3.21; Cambridge Electronic, UK).

Uninterrupted measurements of cerebral and muscle tissue oxygenation trends were obtained via near-infrared spectroscopy (NIRS; Oxymon MkIII, Artinis, The Netherlands). One NIRS emitter-detector pair was placed over the left prefrontal lobe, between Fp1 and F3 (international EEG 10–20 system). A second emitter-detector pair was placed on the distal part of the right VL (approximately 15 cm above the proximal border of the patella). Spacing between optodes was fixed at 45 mm using a black, plastic spacer held in place via double-sided tape. A modified form of the Beer–Lambert Law was used to assess muscle oxygenation as the percentage of tissue saturation index (TSI; oxyhemoglobin/[oxyhemoglobin + deoxyhemoglobin] × 100). Differential path length factors were fixed at 5.93 for cerebral and at 3.83 for muscle tissues. NIRS data were acquired at 10 Hz, and down sampled to 1 Hz for analysis.

For each individual, NIRS and EMG data were time normalized on a scale 0-100% using 20% intervals of total exercise duration. All EMG and NIRS signals were normalized to the first sprint value of each condition, which was assigned the value of 100%.

Neuromuscular function

Neuromuscular test battery

Neuromuscular assessment included six sets (recovery = 1 min) of three brief contractions [~5 s, maximal voluntary contraction (MVC), 50% MVC and 75% MVC, recovery = 6 s] of the knee extensors (Girard et al. 2013). The intensities for the sub-maximal contractions were calculated from the preceding MVC, and the feedback of the target torque was provided via a

computer monitor. During brief contractions, TMS or MNS stimulations were alternatively delivered ~1.5 s after the plateau (3 sets with TMS and 3 sets with MNS). In addition, a potentiated twitch was evoked 5 s after each MVC with MNS. Thereafter, participants performed a 30 s sustained MVC including MNS and TMS, delivered 2 s apart, at the beginning and the end of the sustained MVC (~5 and ~25 s, respectively) (Girard et al. 2013).

EMG and torque recordings

Isometric knee extensor torque of the right leg was measured during both voluntary and evoked contractions on an isokinetic dynamometer (Biodex; Isokinetic Dynamometer, Shirley, NY). Participants were seated with their hip joint angles set at 90° (0° is full extension) and their chest and working leg tightly fixed against the chair. The axis of the dynamometer was aligned with the knee flexion-extension axis, and the lever arm was attached to the shank around the ankle with a strap. Participant position information was recorded to ensure identical positioning for each test occasion.

Surface EMG activity was recorded using bipolar Ag/AgCl electrodes (Ambu Blue sensor T, Ambu A/S, Denmark; diameter = 9 mm; inter-distance electrode = 30 mm) placed according to SENIAM's recommendations and fixed lengthwise over the muscle belly. Before electrode placement, the skin was lightly abraded and washed to remove surface layers of dead skins, hair, and oil. The reference electrode was attached to the right wrist. The myoelectric signal (sampling frequency = 2,000 Hz) was amplified (gain × 1000) and filtered (bandwidth frequency = 30-500 Hz) to minimize extraneous noise and possible movement artefacts in the low-frequency region and to eliminate aliasing and other artefacts in the high-frequency region. EMG signals were recorded by commercially available hardware (Biopac MP35, systems Inc., Santa Barbara, CA) and its dedicated software (Acqknowledge 3.6.7, Biopac Systems Inc., Santa Barbara, CA).

Motor nerve stimulation

Single supramaximal electrical stimuli (max voltage 400 V, rectangular pulse of 200 ms) were delivered to the right femoral nerve using a high-voltage, constant-current, stimulator (Digitimer DS7AH, Welwyn Garden City, Hertfordshire, UK). The cathode ball electrode was manually pressed into the femoral triangle (*i.e.,* 3–5 cm below the inguinal ligament) by the same experimenter and the anode (5 × 9 cm) was located in the gluteal fold opposite the cathode. The intensity of stimulation was determined during the familiarization session by delivering single stimuli with increments of 10 mA until plateaus occurred in twitch amplitude and M-wave. Supramaximal stimulation was ensured by increasing the final intensity by 50% (mean current: 117 ± 56 mA; range: 60–220 mA).

Transcranial magnetic stimulation

A magnetic stimulator (Magstim 200, The Magstim Company, Dyfed, UK) was used to stimulate the motor cortex. A single TMS pulse (1-ms duration) was delivered via a concave double-cone coil (13 cm diameter) maintained manually over the vertex of the scalp. The coil was slightly moved to preferentially activate the left motor cortex (contralateral to the right leg) until eliciting the largest motor evoked potential (MEP) in the *vastus lateralis* during 50% MVC contractions and with a stimulation intensity of 60% of the maximal stimulator power output (Girard et al. 2013). Motor threshold occurred at 41 ± 10% of maximum stimulator output, and during each of the experimental trials TMS was delivered at 140% of the motor threshold (60 ± 9% of maximum stimulator output; range: 49–74%).

Analysis of neuromuscular parameters

Voluntary torque and EMG activity (RMS) were recorded during 1 s of plateau. Raw RMS data were also were normalized to the superimposed M-wave as an index of neural drive (i.e. RMS/M ratio). Voluntary activation was assessed using the twitch interpolation method (Merton, 1954). Briefly, the torque produced during a superimposed twitch during the MVC was compared to the torque produced by the potentiated twitch: VA_{MNS} (%) = (1 –

[superimposed twitch/potentiated twitch]) × 100. Voluntary activation was also assessed using TMS (VA_{TMS}) by measuring the torque responses to motor cortex stimulations during submaximal and maximal contractions (Girard et al. 2013; Goodall et al. 2009): VA_{TMS} (%) was subsequently quantified using the equation: (1 – [superimposed twitch/estimated resting twitch]) × 100.

Muscle contractility was assessed from the electrically-evoked resting twitch as peak twitch amplitude (e.g. the highest value of twitch tension production). The estimated resting twitch evoked by TMS was also used as an index of the torque-generating capacity of the knee extensors.

The peak-to-peak amplitudes of evoked M-wave and MEP were measured offline, and the amplitude of MEP was normalized to that of the M-wave (i.e. MEP/M ratio) elicited at the same torque during sustained 30-s MVCs. The duration of the corticospinal silent period evoked by TMS was determined as the interval from stimulus to return of continuous EMG by visual inspection (Goodall et al. 2010).

Statistical analysis

Values are expressed as means ± SD. All variables were analyzed with a two-way repeated-measure ANOVA [Time (pre/post, or 20/100% or 20/40/60/80/100% of exercise duration) × Condition (SL, MH and SH)]. Data variance was first assess using Mauchly's test of sphericity and a Greenhouse-Geisser correction was applied when required. Post-hoc comparisons were performed using Bonferroni-adjusted *p* values. Partial eta-squared was calculated as measures of effect size with values of 0.01, 0.06 and >0.14 considered as small, medium and large, respectively. A Spearman's rank-order correlation was performed to determine the relationship between 11 participants' SL, MH and SH number of cycle efforts. All statistical calculations were performed using SPSS statistical software V.24.0 (IBM Corp., Armonk, NY, USA). The significance level was set at $P<0.05$.

RESULTS

Intermittent exhaustive cycling exercise *(Figure 1)*

Exercise capacity differed ($P<0.001$; $\eta^2=0.72$) among the three conditions. Compared to SL (23 ± 9), a smaller number of cycle efforts were completed before reaching exhaustion in MH (16 ± 6; $P=0.023$) and SH (9 ± 3; $P<0.001$), with also a difference between MH and SH ($P=0.003$). There was an association between SL with both MH ($r=0.658$; $P=0.028$) or SH ($r=0.756$; $P=0.007$) performance, but not between MH and SH ($r=0.488$, $P=0.127$).

Responses to exercise *(Table 1)*

SpO_2 decreased from the onset of exercise to near exhaustion in all conditions ($P=0.026$; $\eta^2=0.41$), with lower end-exercise values for each successive altitude (95.5 ± 2.1, 89.4 ± 1.8 and 79.6 ± 3.8% for SL, MH and SH, respectively; $P<0.001$ $\eta^2=0.96$). While $\dot{V}O_2$ didn't differ between conditions at the onset of exercise ($P=0.332$; $\eta^2=0.22$), $\dot{V}O_2$ measured near exhaustion was lower as hypoxia severity increased ($P<0.001$; $\eta^2=0.62$). With the exception of $PetO_2$, none of the cardio-respiratory parameter differed between conditions at exercise onset ($P\geq0.213$; $\eta^2\geq0.22$). All parameters rose from the onset of exercise to near exhaustion ($P\leq0.034$; $\eta^2\leq0.37$) but did not differ between conditions ($P\geq0.213$; $\eta^2\geq0.06$).

Relative to peak values recorded during the pre-experimental $\dot{V}O_{2max}$ assessment (Table 1), similar near maximum percent heart rate (95 ± 5, 93 ± 5 and 93 ± 4%), stroke volume (106 ± 13, 111 ± 12 and 110 ± 18%) and cardiac output (101 ± 11, 104 ± 10 and 103 ± 16%) values were reached near exhaustion during SL, MH and SH exercise, respectively.

RMS activity and NIRS *(Figure 2)*

There was a dose-response relationship between the severity of hypoxia and the increase in VL RMS activity during the intermittent exhaustive cycling exercise ($P=0.020$; $\eta^2=0.22$), with greater increases from exercise onset to near exhaustion occurring in SH ($P=0.004$) compared

to SL but not MH (+13.8 ± 10.9 *vs.* +3.8 ± 8.4 and +6.6 ± 9.2%). A less exaggerated, insignificant response (P=0.277; η^2=0.13) was observed for RF RMS activity (+8.6 ± 12.6 *vs.* −0.9±14.0 and +6.6 ± 9.2%).

Both cerebral (P<0.001; η^2=0.86) and muscle (P=0.003; η^2=0.54) oxygenation decreased throughout the exercise, independent of conditions (P≥0.45; η^2≥0.14). Compared to SL, muscle oxygenation was globally lower in MH and SH (P=0.011; η^2=0.36).

MVC torque *(Figure 3-A)*

After exercise, MVC torque during brief contractions was reduced below baseline in all conditions (pooled values: −9.9 ± 8.3%; P<0.001; η^2=0.48), independent of condition (P=0.844). When maximal contractions were prolonged, exercise-induced decreases in torque were consistent across conditions from the beginning to the end of the 30 s MVC (pooled values: −21.2 ± 11.4%; P<0.001; η^2=0.87). Compared to baseline, mean torque produced ~5 s and ~25 s into the sustained contraction was equally reduced after exercise in all conditions (pooled values: −11.0 ± 10.4 and −16.1 ± 13.9%; P=0.007; η^2=0.42).

Twitches amplitude *(Table 2)*

Compared to baseline, peak twitch amplitude in response to supra-maximal, single (pooled values: −52.8 ± 10.5%; P<0.001; η^2=0.90) and paired (pooled values: −34.4 ± 12.6%; P<0.001; η^2=0.87) electrical stimuli was equally reduced after exercise in all conditions. Estimated resting twitch was similarly reduced below baseline in all conditions (pooled values: −54.2 ± 17.9%; P=0.004; η^2=0.80).

Voluntary activation *(Figure 3-B and C)*

During brief MVCs, VA_{MNS} tended to be reduced below baseline after exercise (P=0.055; η^2=0.25). Compared to baseline, lower post-exercise VA_{MNS} values were obtained during sustained MVCs (all conditions compounded: −0.8 ± 2.0 and −2.9 ± 2.3% after ~5 s and ~25 s, respectively; P=0.030; η^2=0.25), with the magnitude of decrease from the beginning to the end

of the sustained MVC was not different (pooled values: −2.6 ± 2.5%; P=0.196; η^2=0.15) between all conditions.

VA_{TMS} during brief MVC was globally reduced (all conditions compounded: −5.9 ± 5.5%; P=0.034; η^2=0.31) below baseline after exercise. Compared to baseline, mean VA_{TMS} after ~5 s and ~25 s into the sustained 30-s contraction was lower after exercise (SL: −5.4±8.2% *vs.* −27.4±20.2%; MH: −13.4±9.9% *vs.* −24.1±17.2%; SH: −10.9±10.7% *vs.* −27.1±16.2%; P=0.01; η^2= 0.43). VA_{TMS} decreased from the beginning to the end of the 30 s sustained MVC (all conditions compounded: −12.9 ± 7.4%; P=0.002; η^2=0.67), yet with a similar rate of decline between conditions (P=0.143; η^2=0.18).

EMG activity *(Tables 2 and 3)*

Compared to baseline, maximal M-wave amplitude (at rest and during MVCs with the exception of VL-M-wave during the sustained MVC; P≥0.131; η^2≥0.05) and maximal RMS activity of VL and RF muscles (both raw and normalized values; P≥0.142; η^2≥0.07) obtained during MVCs did not change post-exercise in any condition.

MEPs and silent period *(Table 3)*

The silent period duration lengthened both for VL (P<0.001; η^2=0.91) and RF (P<0.001; η^2=0.86) muscles from the beginning to the end of the sustained MVC, while MEP amplitude (raw and normalized values) did not change significantly (P≥0.343; η^2≥0.09). Neither MEP amplitudes (P≥0.343; η^2≥0.09) nor corticospinal silent periods (P≥0.454; η^2≥0.07) measured after exercise differed from baseline.

DISCUSSION

Our novel findings indicate that, with increasing severity of arterial hypoxemia (i.e., higher relative exercise intensity), (i) exercise capacity during exhaustive intermittent cycling is reduced (ii) participants stopped exercising while reaching a similar and near-maximum cardio-

metabolic solicitation, and (iii) end-exercise alterations in quadriceps contractile properties and neural drive didn't differ between conditions.

Exercise performance and responses to exercise

As expected from the hypoxia-induced reduction in maximal aerobic power (Wehrlin and Hallén, 2006), breathing hypoxic gas mixture negatively affected the number of completed intermittent cycling bouts before exhaustion. Indeed, exercise time to exhaustion in MH and SH was only ~70 and ~40% in reference to SL, highlighting differences in the severity of hypoxic conditions. In comparable levels of hypoxia, Jeffries et al. (2019) have reported that exercise time using a fixed RPE protocol was decreased according to a reduction in FiO_2 with also some evidence of smaller inter-individual responses with increasing hypoxia severity. While there were positive correlations between the number of cycle efforts performed in SH and both MH and SH, the smaller range of individual responses in the most severely hypoxic condition may be a side effect of less efforts being completed. During hypoxic cycling, exercise capacity is altered due to a reduction in convective O_2 transport, as evidenced during repeated sprints (Soo et al. 2020), constant-load (Amann et al. 2006) and time trials (Amann et al. 2007). The main mechanism for endurance performance impairment when oxygen availability is compromised primarily relies on a decrease in $\dot{V}O_{2max}$, averaging ~8% per 1,000 m increase in altitude (Wehrlin and Hallén, 2006), ultimately leading to a shift of a given absolute workload to a higher relative intensity. During whole-body exercise, the proposed threshold of arterial hypoxemia where hypoxia-sensitive mechanisms originating in the central nervous system may override other excitatory influence on central motor output is below a SpO_2 of ~75% (Goodall et al. 2012; Goodall et al. 2014a). In our study, SpO_2 values of ~80% and ~90% were observed near exhaustion at simulated altitudes of ~4,200 and ~2,200 m, respectively. It is therefore unlikely that the effects of hypoxia (even in our severe hypoxia condition) on the central nervous system were directly responsible for exercise termination through a 'brain-hypoxic'

effect (SpO_2 <70%), independent of peripheral feedback from the locomotor muscles (Amann et al. 2007).

In our study, increment in RMS EMG activity in both quadriceps muscles was steeper in conditions of reduced O_2 availability, and difference became more apparent toward the end of our exhaustive intermittent cycling exercise in the more severe hypoxic condition. This finding coincides with those from other studies wherein increasing skeletal muscle recruitment to compensate for decreasing muscle contractility in constant-load cycle exercise to exhaustion when participants were exposed to more severe hypoxic conditions (Amann et al. 2007; Goodall et al. 2012). While both cerebral (i.e., early) and muscle (i.e., late) oxygenation levels decreased during exercise, only lower muscle oxygenation readings were observed when participants were exercising in moderate and severe hypoxia conditions in reference to normoxia. Yet, despite clear differences in exercise capacity between all conditions, our data fail to support the existence of a dose-response relationship between hypoxia severity and the magnitude of muscle/cerebral oxygenation levels (Subudhi et al. 2007). This suggests that muscle/cerebral oxygenation levels likely were not the reason for reaching exhaustion during intermittent cycling *per se*. Rates of peripheral fatigue and discharge of group III/IV afferents, originating from the fatiguing locomotor muscles to the central nervous system, are highly sensitive to O_2 availability (Amann et al. 2016) and thereby key determinants of endurance exercise performance. Near exhaustion, cardio-respiratory responses to exercise values did not differ between the three trials and were close to maximal values measured at the end of the incremental test. Although speculative, at end exercise, afferent activity may have been similar due to similar metabolic perturbations (Hogan et al. 1999). Hence, we are confident that participants stopped exercising while reaching a similar and near-maximum cardio-metabolic solicitation.

The psychobiological model of endurance performance postulates that exhaustion is not directly caused by neuromuscular fatigue but rather by a decision-making process based on the maximum effort one is willing to exert in order to succeed in a given task (Smirmaul et al. 2013). Speculatively, hypoxic exposure that increases perception of effort and/or decreases potential motivation likely hampered exercise capacity in our study. Participants stopped exercising prematurely in the most severely hypoxic condition, while the magnitude of neuromuscular fatigue incurred was not different between conditions (see below). In support, exercise-related sensations, rather than neuromuscular function integrity, seem to play a pivotal role in influencing performance of repeated sprints and its recovery (Soo et al. 2020). While similar and near maximal scores for ratings of perceived exertion at exhaustion suggest that hypoxia did not negatively impact motivation, measures of breathing difficulty and/or limb discomfort would have been helpful to determine if *higher than normal* exercise-related sensations may precipitate exercise termination. We further verified that, if intermittent cycling is performed until task failure, all cardiorespiratory variables (except for $\dot{V}O_2$) measured near exhaustion with moderate-to-severe hypoxic exposure do not differ from sea level.

MVC and peripheral fatigue

The MVC torque measured during brief and from the beginning to the end of the sustained contractions decreased below baseline following exercise, (~10-15%), independent of hypoxia severity. This indicates that the current exercise protocol induced a similar global level of neuromuscular fatigue in all conditions, despite less work being performed under hypoxic compared with normoxic conditions. Using cycling as the exercise modality, Amann et al. (2006) were the first to introduce the concept of an individual 'critical' threshold of peripheral fatigue. Since then, mounting evidence using manipulations of systemic (Billaut et al. 2013; Soo et al. 2020), but not localized (Willis et al. 2018), hypoxia indicates that completion of repeated, maximal-intensity cycle bouts coincides with a certain degree of peripheral fatigue.

Recently, it was proposed that exercise-induced reduction in muscle contractility is task-dependent (Thomas et al. 2018). The magnitude of performance fatigability is determined primarily by the active muscle mass engaged in the exercise bout, that in turn also depends on the exercise duration/intensity, and the associated disruption to whole body homeostasis contributing to the perception of fatigue (Rossman et al. 2012; Goodall et al. 2018).

The common observation in the afore-mentioned studies using a closed-loop repeated sprint exercise model is that earlier and larger performance decrement occur in O_2-deprived conditions in order to restrain the total degree of peripheral fatigue (Billaut et al. 2013; Soo et al. 2020). We extent these observations to fixed-intensity, open-loop intermittent cycling exercise performed to the limit of tolerance, while we can only speculate on the time course of neuromuscular adjustments. Unlike previous reports (Goodall et al. 2012; Mira et al. 2020), muscle fatigue assessments were not completed at isotimes (i.e., after a similar number of cycle bouts) in normoxia *versus* moderate and severe hypoxia, which requires further investigation. Finally, whilst the literature has shown mixed results about the effects of cycling exercise in hypoxic conditions on M-wave amplitudes (Perrey and Rupp, 2009), no changes for this parameter could be detected in this study. Observation of well-preserved M-waves would indicate that peripheral fatigue occurred at and/or beyond the sarcolemma as problems in excitation and contraction coupling.

Muscle activation

The novel finding provided by cortical stimulations is that the inability of the motor cortex to maximally drive the knee extensor motor units following exhaustive intermittent cycling was shown using brief and sustained MVCs. There was also a similar time-dependent decrease in sustained torque in all post-exercise conditions in reference to baseline. However, both VA_{MNS} and RMS/M ratios measured at exhaustion remained unchanged compared to baseline. Previously, severe hypoxia ($FiO_2 = 0.10$), but not moderate hypoxia ($FiO_2 = 0.13$), was reported

to accentuate the reduction in VA_{TMS} following exhaustive intermittent isometric quadriceps contractions (Goodall et al. 2010). When cycling at 80% of maximal work rate to the limit of tolerance in hypoxia (FiO_2 = 13%) the decline in VA_{TMS} was more than twofold greater compared to the same duration of exercise in normoxia and likely due to a reduced cerebral oxygen delivery (Goodall et al. 2012; Goodall et al. 2014b). In our study, however, larger hypoxia-mediated supraspinal deficits using brief or prolonged contractions were not observed. As with our study, similar VA_{TMS} reductions occurred after prolonged (three bouts of 80 min separated by 25 min) cycling in hypoxia (FiO_2 = 12%) and normoxia when exercising for the same duration at the same relative sub-maximal power output (Jubeau et al. 2017). Differences in experimental conditions (i.e., delay to install participants on the ergometer before testing and exercise modality, duration and/or intensity) might explain these contrasting results regarding the effects of hypoxia severity on supraspinal fatigue. Although peripheral factors were preponderant, this indicates that fatigue etiology also included a suboptimal drive from the motor cortex. Nonetheless, exercise-induced changes in VA_{TMS} did not differ across the three trials, implying that the magnitude of central fatigue might not be dependent upon hypoxic exposure.

Corticospinal responsiveness

During 30 s maximal voluntary efforts, participants were unable to sustain the same corticospinal output to maximally activate motoneurons. An interesting observation was also that this effect was not larger after exhaustive intermittent cycling or due to hypoxia exposure. If the participants had remained hypoxic, it cannot be ruled out that corticospinal responsiveness would become further impaired with more severe hypoxia. Whereas MEP amplitudes did not change, silent period, as a representation $GABA_B$ receptor-mediated inhibition of corticospinal excitability (Stetkarova and Kofler, 2013), grew longer during the course of the sustained contractions. Despite the development of significant supraspinal fatigue,

the pattern of change in these EMG responses to TMS throughout the sustained MVC was not modulated by the severity of hypoxia or the cycling exercise protocol by itself. Whether corticospinal excitability is altered (Goodall et al. 2012; Goodall et al. 2014b; Peyrard et al. 2019) or not (Goodall et al. 2010; Jubeau et al. 2017) with the addition of hypoxia while exercising is highly controversial. A likely explanation for these discrepant findings is the different time delay at which neuromuscular function was assessed after task failure since neuromuscular function recovery occurs within a few minutes after end of exercise (Mira et al. 2017). In the current study, while neuromuscular function assessment was performed 7 min after task failure, it also took place in different conditions (exercise in hypoxia *vs*. muscle assessment after exercise in normoxia, respectively). Taken as a whole, a partial recovery from the specific effects of hypoxia is likely to have occurred, which may have led to an underestimation of the extent of corticospinal tract excitability adjustments. Previous findings indicate that alterations in indices of corticospinal excitability (MEP/M ratio) and/or intracortical inhibition (silent periods) in O_2 deprived conditions are seen with prolonged hypoxic exposure (several hours; Rupp et al. 2012), while hypoxia exposure on average did not exceed 20 min in either MH or SH. Under the present circumstances, we conclude that our exercise model did not impair the corticospinal responsiveness of motor neurons, at least when evaluated from localized, isometric MVC of the knee extensors.

Additional considerations and limitations

One strength of our study design was to individualize the exercise to the athlete's profile by deriving exercise intensity from the *'anaerobic speed reserve'* concept (i.e., the difference between peak power output during a single, short maximal effort and maximal aerobic power), as opposed to the common practice of using a similar percentage of maximal aerobic power (Buchheit and Laursen, 2013). By considering these two intensity landmarks, a similar physiological demand was imposed on each participant, as they were exercising at a similar

percentage (30%) of their anaerobic speed reserve. Some limitations that require consideration when interpreting our results include the fact that: i) FiO_2 was decreased for inducing hypoxia rather than using a target SpO_2 range (SpO_2 clamp approach), which may increase inter-subject variability, ii) no comparison at isotimes have been implemented between conditions to determine the time course of neuromuscular adjustments, iii) absolute exercise intensities (determined from an incremental test in normoxia) were selected, so that hypoxic trials were likely carried out at a higher relatively intensity than in normoxia due to well-reported deleterious effects of hypoxia on aerobic power, iv) neuromuscular function was not assessed during exercise *per se* nor immediately (within few seconds) at exercise cessation so that our data may not represent the true magnitude and etiology of fatigue.

CONCLUSIONS

This study assessed the effects of hypoxia severity on time to exhaustion during intermittent cycling and the associated cardiorespiratory, tissue oxygenation and neuromuscular fatigue responses characteristics. Our findings showed that the extent and characteristics of neuromuscular fatigue did not differ between conditions despite earlier exercise cessation with increasing hypoxia severity. Our results also display that a similar suboptimal output from the motor cortex was detected after exercise. Quadriceps muscle recruitment and, to a lower extent, muscle/brain oxygenation trends during exercise were exacerbated under increasing levels of hypoxia severity, whereas cardiovascular solicitation neared maximal values at exhaustion in all conditions. The earlier exercise cessation with more severe hypoxic conditions may have been triggered by the higher exercise requirements, while neuromuscular consequences were similar.

CONFLICTS OF INTEREST

The authors have no conflicts of interest, source of funding, or financial ties to disclose and no current or past relationship with companies or manufacturers who could benefit from the results of the present study. The authors report no conflict of interest. At the time of the experiment, Olivier Girard was employed by Aspetar Hospital (Qatar), where the experiment was performed.

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FIGURE LEGENDS

Figure 1 – Number of 'sprints' completed until exhaustion during the intermittent-sprint cycling exercise at sea level (SL), moderate (MH) and severe hypoxia (SH).

Data are mean ± SD for 11 participants.

** significantly different from sea level, † significantly different from moderate hypoxia*.

Figure 2 – Root Mean Square (RMS) surface EMG for *vastus lateralis* (A) and *rectus femoris* (B) muscles, muscle (C) and cerebral (D) tissue saturation index (TSI) data during exhaustive intermittent cycling at sea level (SL), moderate (MH) and severe hypoxia (SH).

Data are mean ± SD for 11 participants.

NIRS and EMG data were time normalized on a scale 1-100% using 20% intervals (1-20, 21-40, 41-60, 61-80 and 81-100% of total exercise duration).

** significantly different from sea level, † significantly different from 1-20%*.

Figure 3. Torque (MVC torque, A), voluntary activation calculated with motor nerve stimulation (VA_{MNS}, B) and transcranial magnetic stimulation (VA_{TMS}, C) during brief (5 s; left panels) and sustained (30 s; right panels) maximal isometric voluntary contractions at baseline and after the exhaustive intermittent cycling test at sea level (SL), moderate (MH) and severe hypoxia (SH).

Data are mean ± SD for 11 participants.

During the 30-s sustained MVC measurements were obtained at the beginning (~5) and at the end (~25 s) of the contraction.

** Significantly different from baseline ($P<0.05$). † SL significantly different from MH and SH. ‡ significantly different between 5 s and 25 s.*