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Central and peripheral muscle fatigue following repeated-sprint running in moderate and severe hypoxia

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Abstract

We examined the effects of increasing hypoxia severity on repeated-sprint running performance and neuromuscular fatigue. Thirteen active males completed eight sprints of 5 s (recovery = 25 s) on a motorized sprint treadmill in normoxia (sea level, SL; F_{I,O_2} = 0.21), in moderate hypoxia (MH; F_{I,O_2} = 0.17) and in severe hypoxia (SH; F_{I,O_2} = 0.13). After 6 min of passive recovery, in all conditions a second set of four sprints of 5 s was conducted in normoxia. Neuromuscular function of the knee extensors was assessed at baseline (Pre-) and 1 min after set 1 (Post-set 1) and set 2 (Post-set 2). In set 1, the mean distance covered in SL (22.9 ± 1.2 m) was not different to MH (22.7 ± 1.3 m; P = 0.71) but was greater than in SH (22.3 ± 1.3 m; P = 0.04). No significant differences between conditions for mean distance occurred in set 2. There was a decrease in maximal voluntary contraction torque (Δ = −31.4 ± 18.0 N m, P < 0.001) and voluntary activation (%VA; Δ = −7.1 ± 5.1%, P = 0.001) from Pre- to Post-set 1, but there was no effect of hypoxia. No further change from Post-set 1 to Post-set 2 occurred for either maximal voluntary contraction or %VA. The decrease in potentiated twitch torque in SL (Δ = −13.3 ± 5.2 N m) was not different to MH (Δ = −13.3 ± 6.3 N m) but was lower than in SH (Δ = −16.1 ± 4 N m) from Pre- to Post-set 1 (interaction, P < 0.003). Increasing severity of normobaric hypoxia, up to an equivalent elevation of 3600 m, can increase indices of peripheral fatigue but does not impact central fatigue after 'all-out' repeated-sprint running.

KEYWORDS
central fatigue, hypoxia, peripheral muscle fatigue, repeated-sprint running

1 | INTRODUCTION

Team sports represent a complex exercise model. Match durations typically continue for a time span (e.g. 90 min in soccer) that is dominated by oxidative metabolism, whilst a significant anaerobic contribution is required to complete repeated-sprint exercise (RSE), underscoring the development of fatigue throughout a match (Spencer, Bishop, Dawson, & Goodman, 2005). Hypoxia is known to exacerbate performance decline during simulated match play (Sweeting et al., 2017), and during submaximal exercise to exhaustion there is evidence that a shift in central *versus* peripheral fatigue mechanisms occurs as the fraction of inspired oxygen (F_{I,O_2}) decreases from normoxia through 'moderate' ($F_{I,O_2} \approx 0.14$–0.18) to 'severe' ($F_{I,O_2} < 0.14$) hypoxia (Amann, Romer, Subudhi, Pegelow, & Dempsey, 2007; Goodall, Ross, & Romer, 2010; Millet, Muthalib, Jubeau, Laursen, & Nosaka, 2012). Although numerous studies have examined RSE performance in hypo-

xia (Girard, Brocherie, & Millet, 2017), none to date has investigated the balance between central and peripheral fatigue responses to repeated-sprint running in conditions of both moderate and severe hypoxia.

When a single 'all-out' sprint effort (5–6 s duration) is conducted in hypoxia, performance is unaffected compared with normoxic conditions (Billaut & Buchheit, 2013; Brocherie, Millet, Morin, & Girard, 2016). During RSE, however, where the total set duration may last several minutes or more, performance has been shown to decrease for running (Bowtell, Cooke, Turner, Mileva, & Sumners, 2014), cycling (Billaut & Buchheit, 2013; Billaut et al., 2013), and team-sport specific tests (Sweeting et al., 2017). Only two studies have examined the effect of progressively increasing hypoxic magnitude (i.e. F_{I,O_2} decreasing from 0.21 to 0.12) on RSE (Bowtell et al., 2014; Goods, Dawson, Landers, Gore, & Peeling, 2014), both reporting a progressive decline in performance with increasing magnitude of hypoxia. However, neither examined indices of neuromuscular fatigue, and thus no firm conclusions regarding the relative contribution of central *versus* peripheral fatigue were drawn.

Two studies to date have examined the effects of hypoxia on indices of peripheral and central fatigue using neuromuscular electrical stimulation techniques after RSE (Billaut, Rodriguez, Martin, Gore, & Bishop, 2013; Peyrard et al., 2019). In the study by Billaut et al. (2013), participants completed 15 maximal sprints of 5 s on a cycle ergometer in normoxia and hypoxia (F_{I,O_2} = 0.138). The authors reported decreased total work, EMG activity and quadriceps central activation ratio in hypoxia, whereas quadriceps potentiated twitch force did not change significantly. Peyrard et al. (2019), in a study that examined the effect of hypoxia (F_{I,O_2} = 0.13) on elbow neuromuscular function after arm RSE, found an attenuated increase in corticospinal excitability in hypoxia, but no differences in M-wave amplitude or potentiated doublet twitch force. Thus, in both studies, at a magnitude of hypoxia considered to be 'severe' (F_{I,O_2} < 0.14), an increase in central mechanisms of fatigue was evident compared with normoxia. Given that no measurements were made in moderate hypoxia, it is uncertain whether a shift in the relative contribution of central *versus* peripheral fatigue occurred.

In modes of exercise other than RSE, several observations indicate that the central *versus* peripheral contribution to fatigue is shifted with increasing severity of arterial hypoxaemia. Firstly, performance of elbow isometric contractions to exhaustion was diminished in severe hypoxia (F_{I,O_2} = 0.10) compared with moderate hypoxia (F_{I,O_2} = 0.14) despite similar motor-evoked potential amplitudes and cortical silent periods, suggesting that hypoxia induced a direct inhibitory effect on central motor drive (Millet et al., 2012). Secondly, Amann et al. (2007) found markedly lower quadriceps twitch force after constant-load cycling to task failure in severe hypoxia (F_{I,O_2} = 0.10) compared with both moderate hypoxia (F_{I,O_2} = 0.15) and normoxia. Lastly, Goodall, González-Alonso, Ali, Ross, and Romer (2012) observed a greater decline in cortical voluntary activity in severe hypoxia (F_{I,O_2} = 0.13) compared with moderate hypoxia (F_{I,O_2} = 0.16) and normoxia. Collectively, these studies indicate that with increasing severity of arterial hypoxaemia there is a shift from peripheral fatigue dominance towards central mechanisms; however, the exact magnitude of the hypoxic stimulus that induces these changes remains equivocal.

New Findings

- **What is the central question of this study?**
  Increasing severity of arterial hypoxaemia induces a shift towards greater central, relative to peripheral, mechanisms of fatigue during exhaustive exercise. Does a similar pattern exist for 'all-out' repeated-sprint running?
- **What is the main finding and its importance?**
  Severe normobaric hypoxia [fraction of inspired oxygen (F_{I,O_2}) = 0.13] did not induce a greater contribution from central fatigue, but indices of muscle fatigue were elevated compared with normoxia (F_{I,O_2} = 0.21) and moderate hypoxia (F_{I,O_2} = 0.17). This suggests a different fatigue response to repeated-sprint running *versus* other exercise modalities and, consequently, that task specificity might modulate the effect of hypoxia on the central *versus* peripheral contribution to fatigue.

In the present study, we sought to investigate whether the contribution of central *versus* peripheral indices of fatigue obtained after treadmill RSE is modulated by increasing the severity of arterial hypoxaemia. We hypothesized that a progressive decline in RSE performance would occur with increasing severity of the hypoxic stimulus and that lower peripheral fatigue coupled with greater central fatigue would occur in severe hypoxia compared with either normoxia or moderate hypoxia. If true, a reduced magnitude of peripheral fatigue occurring after an initial set of sprints in severe hypoxia would be associated with better performance, compared with either normoxic or moderately hypoxic conditions, in a second bout of RSE occurring only in normoxia.

2 | METHODS

2.1 | Ethical approval

Written informed consent was given by the subjects before the commencement of data collection. The experimental protocol was conducted according to the *Declaration of Helsinki*, except for registration in a database, and approved by Shafallah Medical Genetics Center Ethics Committee, Doha Qatar (institutional review board project no. 2011-011).

2.2 | Participants

Thirteen recreationally trained men (mean ± SD: age, 31.2 ± 4.8 years; height, 178.4 ± 6.6 cm; and weight,74.3 ± 8.2 kg) participated in the study. All participants were active in either team (football, rugby or basketball) or racket (tennis or squash) sports. In the 6 months preceding the study, participants trained on average 4.5 ± 2.5 h week^{-1}, which included sport-specific training (including RSE bouts), aerobic and anaerobic conditioning and basic strength training. All participants were born and raised at <1000 m terrestrial altitude and had not travelled to elevations >1000 m in the 3 months before the investigation.

2.3 | Study design

Approximately one week before testing, participants undertook a familiarization session, in which they first performed a series of seven to ten short (<5 s) sprints at increasing intensities to become accustomed to the instrumented treadmill. Then they performed three maximal sprint efforts lasting 5 s, each separated by 2 min of passive recovery. All habituation trials were conducted whilst wearing a facemask not connected to the hypoxic generator. All participants demonstrated a coefficient of variation <2.2% for distance covered across the three maximal effort trials (Girard, Brocherie, Morin, & Millet, 2016). After 5 min of rest, the complete RSE intervention was completed. Strong verbal encouragement was given during all maximal efforts. Participants were also familiarized with the neuromuscular function assessment protocol (see section 2.7) until they felt accustomed to the equipment (coefficient of variation in three successive knee-extensor trials for maximal torque was <3%).

The experimental design involved three experimental conditions: (i) normoxia (SL; F_{I,O_2} = 0.209, near seal level, ~20 m a.s.l.); (ii) moderate hypoxia (MH; F_{I,O_2} = 0.168, ~1800 m a.s.l.); and (iii) severe hypoxia (SH; F_{I,O_2} = 0.133, ~3600 m a.s.l.). Normobaric hypoxia was obtained using commercially available gas-mixing apparatus (Altitrainer; SMTEC SA, Nyon, Switzerland). Participants inspired the hypoxic gas mixtures through a facemask connected to a two-way Hans Rudolph breathing valve. The order of conditions was randomized, counterbalanced and double-blinded. To facilitate participant 'blinding', each subject breathed through the same facemask, but instead of connecting nitrogen to the Altitrainer as for hypoxic conditions, a cylinder of medical grade, atmospheric air was connected. The efficacy of participant blinding was evaluated after each session via questionnaires.

Participants reported to the climate-controlled (~25°C, 40% relative humidity) laboratory at same time of the day (±1 h), on three occasions separated by a minimum of 3–4 days. Participants were instructed to maintain their normal diet and daily routines throughout the entire experimental period and to avoid high-intensity training for 48 h and consumption of alcohol for 24 h before testing. The consumption of caffeine was restricted on the day of testing. Participants were instructed to drink 4–6 ml of water per kilogram of body mass every 2.5 h on the day before each experimental session to ensure euhydration. They were also permitted to drink *ad libitum* during the warm-up procedure (i.e. before mask attachment).

2.4 | Repeated-sprint exercise test

In a randomized double-blind design, the RSE test involved two sets of 'all-out' maximal efforts lasting 5 s in duration, followed by 25 s of passive recovery. Set 1 consisted of eight sprints of 5 s followed by 25 s of passive recovery completed in the SH, MH or SL conditions. After set 1 there was 6 min of passive recovery in normoxia, followed by a second set of four sprints that was completed in normoxia for all conditions. In all conditions, participants continued to wear the hypoxic facemask until completion of the 25 s recovery period after the last (eighth) sprint effort of set 1. All recovery periods involved standing still on the treadmill.

Before the RSE test, participants completed a standardized warm-up in normoxia consisting of 5 min running at 10 km h^{-1}, then 10 min of sprint-specific warm-up drills (skipping, high knee, butt-kick and high heels), followed by a series of five brief (1–3 s) sprint efforts of gradually increasing intensity ranging from six to nine on a modified Borg 'sense of effort' scale (Christian, Bishop, Billaut, & Girard, 2014). Finally, three maximal 5 s individual sprints, separated by 2 min of passive rest, were completed. These three individual sprint trials were used as the criterion score to assess the coefficient of variation. Subjects then put on the facemask and rested for 5 min. Therefore, in MH and SH, there was a 5 min period of hypoxia 'wash-in' preceding commencement of the experimental trials.

The distance covered for each sprint was taken as the measure of performance, and the sprint decrement score {S_{dec} = [cumulative distance/(largest distance × sprint number) − 1] × 100} was also calculated (Girard, Mendez-Villanueva, & Bishop, 2011).

2.5 | Apparatus for treadmill sprints

All sprinting efforts were performed on a motorized instrumented treadmill (ADAL3D-WR; Medical Development–HEF Tecmachine, Andrézieux-Bouthéon, France). Directly beneath the treadmill belt, there are four piezoelectric force transducers (KI 9077b; Kistler, Winterthur, Switzerland) installed on a custom-engineered concrete slab to ensure maximal rigidity of the supporting structure. The belt drum contains a constant-torque motor, which was set to 160% of the default torque necessary to overcome friction attributable to the body weight. This value was determined based on previous testing and, in combination with a rear wall-mounted horizontal tether (1 cm in diameter, ~2 m in length), enables maximal accelerated running to occur in a comfortable manner whilst maintaining the same position on the treadmill belt (Brocherie et al., 2016; Morin, Samozino, Bonnefoy, Edouard, & Belli, 2010). An additional overhead safety harness with sufficient slack not to impede natural running mechanics

was fastened to the participants to support them in the event of a fall. Participants adopted a standardized start position with their left foot forward and a typical crouched sprint-start forward lean. After a 5 s verbal and visual countdown, the treadmill was released, and the belt began to accelerate upon application of forward propulsive force.

2.6 | Responses to exercise

Heart rate (HR) was measured via a Polar transmitter–receiver (Wearlink T-31; Polar Electro Oy, Kempele, Finland), and arterial oxygen saturation (S_{pO_2}) was estimated using finger-tip pulse oximetry (Palmsat 2500; NONIN Medical Inc., Plymouth, MI, USA). The rating of perceived exertion (RPE) was recorded using the Borg 6–20 scale. Subjects were instructed to reflect on their overall peripheral discomfort during the preceding exercise bout (Christian et al., 2014), and then HR, S_{pO_2} and RPE were recorded exactly 10 s after each sprint. Additionally, S_{pO_2} was recorded 2 min before commencement of both RSE sets 1 and 2. A fingertip capillary blood sample was taken before the warm-up, 2 min after set 1 and 2 min after set 2 and analysed for blood lactate concentration (B[La]) using a portable lactate analyser (Lactate Pro LT-1710; Arkray, Japan).

2.7 | Neuromuscular electrical stimulation

Femoral nerve stimulations (400 V, rectangular pulse of 0.2 ms) were delivered by a high-voltage stimulator (Digitimer DS7AH; Digitimer, Welwyn Garden City, UK). The cathode electrode (diameter of 5 mm) was placed in the inguinal crease, and the anode (5 cm × 10 cm; Medicompex, SA, Ecublens, Switzerland) in the gluteal fold. The intensity of stimulation was determined during the familiarization test session using a passive isometric recruitment curve (Racinais, Maffiuletti, & Girard, 2013). Briefly, the stimulation intensity was increased incrementally by 10 mA until a maximal peak twitch torque was achieved and then increased further by 50% to ensure constant supramaximal stimulation throughout the protocol.

The neuromuscular electrical stimulation (NMES) protocol consisted of a 5 s knee-extension maximal voluntary contraction (MVC) with a superimposed 80 Hz doublet (Db) twitch applied when torque had reached a visible plateau. Three seconds after completion of the MVC, one 80 Hz Db, one 20 Hz Db and three single stimulations were applied in a relaxed state (all separated by 3 s). This sequence was repeated three times, with 30 s of passive recovery in between.

Before the baseline NMES protocol, participants completed a brief warm-up consisting of 5 × 5 s voluntary isometric contractions of increasing subjective effort, separated by 20 s of passive rest. This was followed by two MVCs of 4 s each, separated by 1 min of passive rest. Neuromuscular testing commenced exactly 1 min after completion of both RSE sets.

2.8 | Measurements

2.8.1 | Torque

Knee-extensor torque was measured with participants seated upright on a custom-built adjustable chair, with the hips and knees flexed at 90 deg. Restraining straps placed across the chest and hips secured the participants in the chair to prevent extraneous movement. A strap was placed around the distal tibia 3–5 cm above the tip of the lateral malleoli and attached to the dynamometer (Captels, St Mathieu de Treviers, France). During all contractions, the torque signals were amplified, sent through an A/D board and sampled at 2000 Hz by commercially available hardware and software (MP35 and BSL Pro v.3.6.7; Biopac Systems Inc., Santa Barbara, CA, USA).

2.8.2 | Electromyography

The EMG activity of the vastus lateralis, vastus medialis and rectus femoris muscles was recorded via bipolar Ag/AgCl electrodes (Ambu Blue sensor T; Ambu A/S, Ballerup, Denmark; diameter, 9 mm; inter-electrode distance, 30 mm) fixed longitudinally over the muscle bellies. The reference electrode was attached to the right wrist. Low impedance between the two electrodes was obtained by abrading the skin with emery paper and cleaning with alcohol. The position of the electrodes was marked for consistent placement. The EMG signals were amplified (gain, 1000), bandpass filtered (band-width frequency, 30–500 Hz) and recorded (sampling frequency, 2000 Hz) by commercially available hardware (MP35; Biopac Systems Inc.) and software (Acqknowledge 3.6.7; Biopac Systems Inc.).

2.9 | Data analysis

All analyses were performed using Spike2 Software (Cambridge Electronic Design, Cambridge, UK). For each neuromuscular test sequence, voluntary torque (MVC torque) and associated EMG activity [raw root mean squared (RMS) amplitude] were recorded over the highest 1 s plateau preceding the superimposed Db. The peak-to-peak amplitude of superimposed maximum compound action potential (M-wave) responses was measured for each agonist muscle, and raw RMS amplitude was divided by the M-wave to give the RMS to M-wave ratio ($RMS.M^{-1}$). The percentage voluntary activation (%VA) was assessed using twitch interpolation and defined as follows: %VA = [1 × (superimposed 80 Hz Db/resting potentiated 80 Hz Db)] × 100. The average of all three NMES test sequences was used for data analysis.

Variables determined from electrically evoked resting single twitches are as follows: peak twitch torque (Pt; the highest value of twitch torque produced, in newton metres), time-to-peak torque (TPT; time from stimulation to Pt, in milliseconds), one half-relaxation time (HRT; time from Pt to 50% Pt, in milliseconds), maximal rate of torque development (MRTD; the maximal value of the first derivative of the torque signal, in newton metres per millisecond) and maximal rate of torque relaxation (MRTR; the lowest value of the first derivative of the

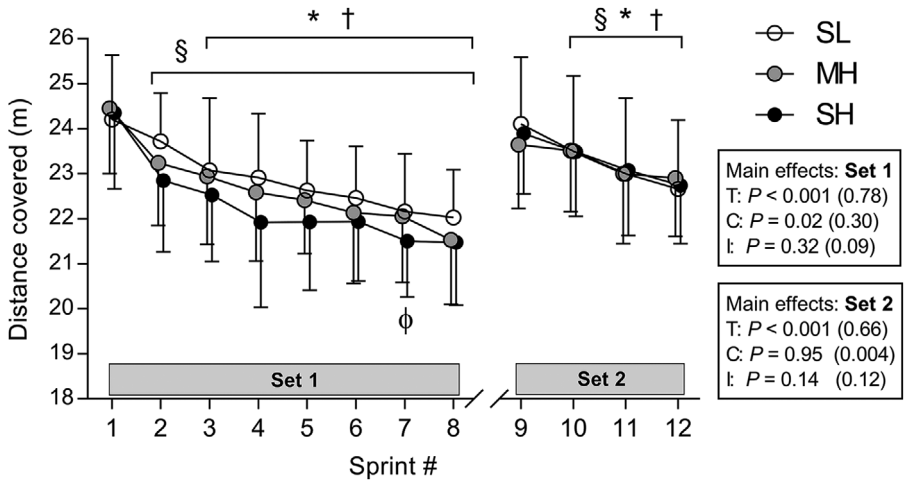

FIGURE 1 Performance (distance covered) during repeated-sprint exercise conducted for set 1 (sprints 1–8) in severe hypoxia [SH; fraction of inspired oxygen (F_{I,O_2}) = 0.13], moderate hypoxia (MH; F_{I,O_2} = 0.17) and at sea level (SL; F_{I,O_2} = 0.21) and for set 2 (sprints 9–12) in normoxia. All values are presented as the mean ± SD (n = 13). T, C and I refer to two-way ANOVA main effects of time, condition and interaction, respectively, with the P-value and η_p^2 in parentheses. The time point indicated is different ($P < 0.05$) from sprint 1 (baseline in panel d) for *SL, †MH and §SH

torque signal, in newton metres per millisecond). Additionally, peak torque after each of the three Db at both 20 and 80 Hz was determined and averaged to obtain a single value (in newton metres) for Db20 and Db80, respectively. Lastly, the low- to high-frequency doublet torque ratio was calculated ($Db20.Db80^{-1}$).

2.10 | Statistical analysis

Values are expressed as means ± SD. A two-way repeated-measures ANOVA [time (sprint number) × condition (SL *versus* MH *versus* SH)] was used to examine performance and selected physiological responses, and a [time (Pre- *versus* Post-set 1 *versus* Post-set 2) × condition (SH *versus* MH *versus* SL)] model was used to compare blood lactate and neuromuscular responses. All dependent variables were tested using Mauchly's procedure for sphericity. When the assumption of sphericity was violated, P-values and degrees of freedom were adjusted using a Greenhouse–Geisser correction. When significant effects were found, *post hoc* analysis was conducted using a Bonferroni adjustment for multiple comparisons. Effect sizes are expressed as partial eta-squared (η_p^2) for main effects, and Hedges' g for pairwise *post hoc* comparisons. All statistical calculations were performed using SPSS statistical software v.21.0 (IBM Corp., Armonk, NY, USA). The significance level was set at $P < 0.05$.

3 | RESULTS

3.1 | Repeated-sprint exercise performance

3.1.1 | Set 1 (Figure 1, sprints 1–8)

There was a significant main effect of condition ($P = 0.02$; $\eta_p^2 = 0.30$) on mean sprint distance in set 1. Compared with the mean distance in SL (22.9 ± 1.2 m), *post hoc* analysis revealed no change in MH (22.7 ± 1.3 m; $P = 0.71$; $g = 0.13$), but a decrease in SH (22.3 ± 1.3 m; $P = 0.04$; $g = 0.19$). Compared with sprint 1, a significant decrease in performance first occurred at sprint 3 in SL (24.2 ± 1.4 *versus* 23.1 ± 1.6 m; $P = 0.003$; $g = 0.72$) and MH (24.5 ± 1.5 *versus* 22.9 ± 1.5 m; $P = 0.003$; $g = 0.97$), whereas a significant decrease in performance occurred at sprint 2 for SH (24.4 ± 1.7 *versus* 22.9 ± 1.6 m; $P = 0.03$; $g = 0.89$). There was no main effect of condition ($P = 0.08$; $\eta_p^2 = 0.21$) on S_{dec} (SL, −5.7 ± 1.5%; MH, −7.5 ± 3.4%; SH, −8.3 ± 3.8%).

3.1.2 | Set 2 (Figure 1, sprints 9–12)

No main effect of condition was found for mean sprint distance ($P = 0.95$; $\eta_p^2 = 0.004$). Compared with sprint 9, a significant decrease in performance first occurred at sprint 11 in SL (24.1 ± 1.5 *versus* 23.0 ± 1.7 m; $P = 0.006$; $g = 0.66$), sprint 12 in MH (23.5 ± 1.4 *versus* 22.9 ± 1.3 m; $P = 0.003$; $g = 0.97$) and sprint 11 in SH (23.9 ± 1.3 *versus* 23.1 ± 1.4 m; $P = 0.004$; $g = 0.57$). There was a main effect of condition for S_{dec} ($P = 0.006$; $\eta_p^2 = 0.35$), with *post hoc* analysis revealing SL (−3.6 ± 1.9%) to be lower than MH (−2.1 ± 0.9%; $P = 0.04$; $g = 0.98$), but not different from SH (−3.3 ± 1.7%; $P = 1.00$; $g = 0.16$). Furthermore, S_{dec} in MH was greater than in SH ($P = 0.049$; $g = 0.85$).

3.1.3 | Set 1 *versus* set 2

For distance covered in sprint 1 compared with sprint 9, there was a significant main effect of time (pooled mean, 24.3 ± 1.5 *versus* 23.9 ± 1.4 m; $P = 0.003$; $\eta_p^2 = 0.54$), but no effect of condition ($P = 0.70$; $\eta_p^2 = 0.03$) and no interaction effect ($P = 0.21$; $\eta_p^2 = 0.12$).

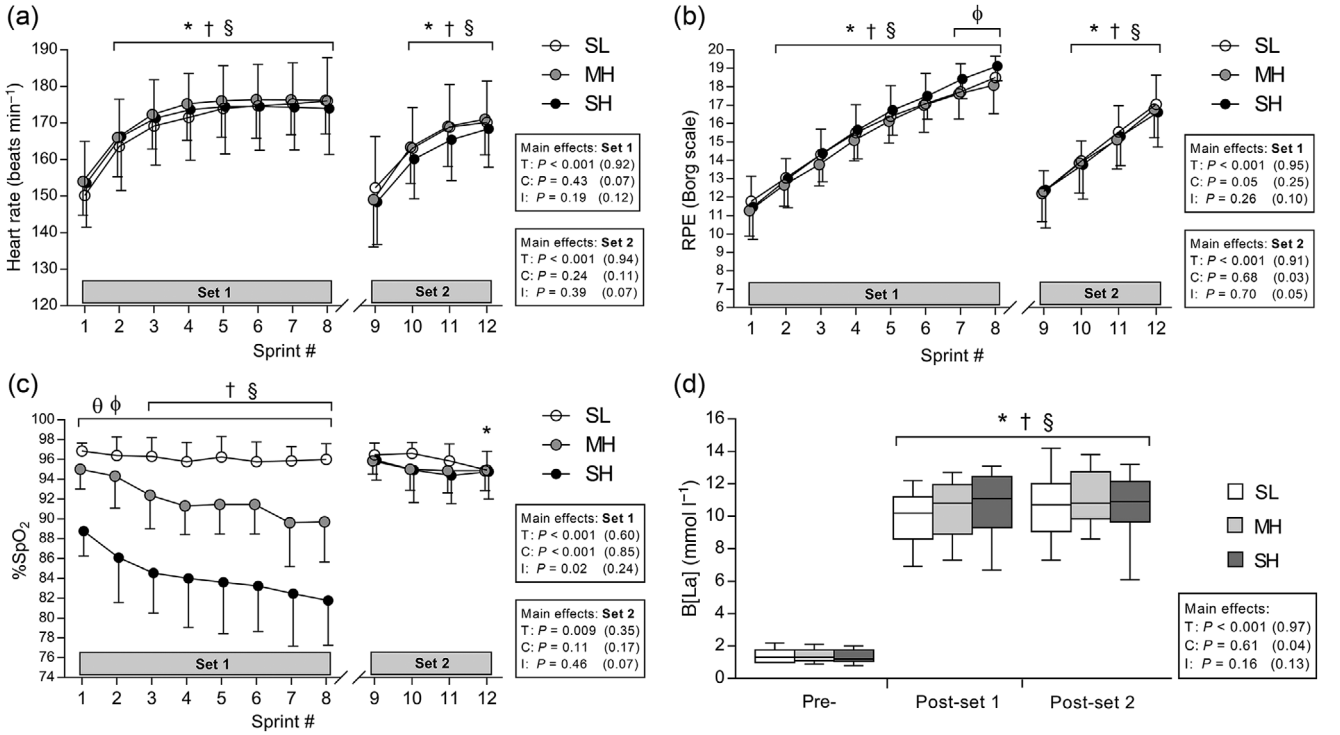

FIGURE 2 Physiological responses to repeated-sprint exercise conducted for set 1 (sprints 1–8) in severe hypoxia [SH; fraction of inspired oxygen (F_{I,O_2}) = 0.13], moderate hypoxia (MH; F_{I,O_2} = 0.17) and at sea level (SL; F_{I,O_2} = 0.21) and for set 2 (sprints 9–12) in normoxia. (a) Heart rate (in beats per minute). (b) Rating of perceived exertion (RPE; Borg 6–20 scale). (c) Percentage oxygen saturation (%S_{pO_2}). (d) Blood lactate concentration (B[la], in millimoles per litre). Values in panels a–c are presented as the mean ± SD (n = 13) and those in panel d as a box and whisker plot (mean, 25–75% percentiles, range) (n = 13). T, C and I refer to two-way ANOVA main effects of time, condition and interaction, respectively, with the P-value and partial η_p^2 in parentheses. The time point indicated is different ($P < 0.05$) from sprint 1 (baseline in panel d) for *SL, †MH and §SH. ⊖MH is different from SL at the sprint number indicated ($P < 0.05$). ϕSH is different from SL at the sprint number indicated ($P < 0.05$)

3.2 | Physiological responses

3.2.1 | Set 1

Heart rate increased rapidly and then plateaued in all three conditions (Figure 2a), but there was no main effect of condition (P = 0.43; η_p^2 = 0.07). Likewise, RPE also rose progressively, but there was no main effect of condition (Figure 2b; P = 0.05; η_p^2 = 0.25). As expected, a significant main effect of condition on S_{pO_2} did occur (Figure 2c; P = 0.001; η_p^2 = 0.85). *Post hoc* analysis demonstrated mean S_{pO_2} during set 1 in SL (96.0 ± 1.7%) to be higher than in MH (91.5 ± 2.6%; $P < 0.001$; g = 2.06) and in SH (83.7 ± 4.3%; $P < 0.001$; g = 3.85). Mean S_{pO_2} was also significantly higher in MH than in SH ($P < 0.001$; g = 1.66).

3.2.2 | Set 2

There was no main effect of condition on HR (P = 0.24; η_p^2 = 0.11), S_{pO_2} (P = 0.11; η_p^2 = 0.17) or RPE (P = 0.68; η_p^2 = 0.03) during set 2.

3.2.3 | Set 1 and set 2

There was no main effect of condition on B[La] (Figure 2d; P = 0.61; η_p^2 = 0.04), whereas a main effect of time did occur ($P < 0.001$; η_p^2 = 0.97). *Post hoc* analysis revealed a significant increase from Pre- to Post-set 1 (pooled mean, 1.4 ± 0.4 *versus* 10.3 ± 1.7 mmol l^{-1}; $P < 0.001$; g = 7.3) and a further small, but significant increase from Post-set 1 to Post-set 2 (10.3 ± 1.7 *versus* 10.9 ± 1.9 mmol l^{-1}; $P < 0.001$; g = 0.1).

3.3 | Neuromuscular electrical stimulation

Changes in the key variables of interest MVC, %VA and Pt are shown in Figure 3, and detailed neuromuscular function test results are presented in Table 1. There was no main effect of condition on MVC torque (P = 0.22; η_p^2 = 0.12), whereas a significant main effect of time was observed ($P < 0.001$; η_p^2 = 0.77). *Post hoc* analysis indicated a significant decrease in MVC torque from Pre- (pooled mean, 270 ± 42 N m) to Post-set 1 (239 ± 38 N m; $P < 0.001$; g = 0.76), but with no further change at Post-set 2 (234 ± 43 N m; P = 0.52; g = 0.12). Likewise, for %VA there was no main effect of condition (P = 0.51;

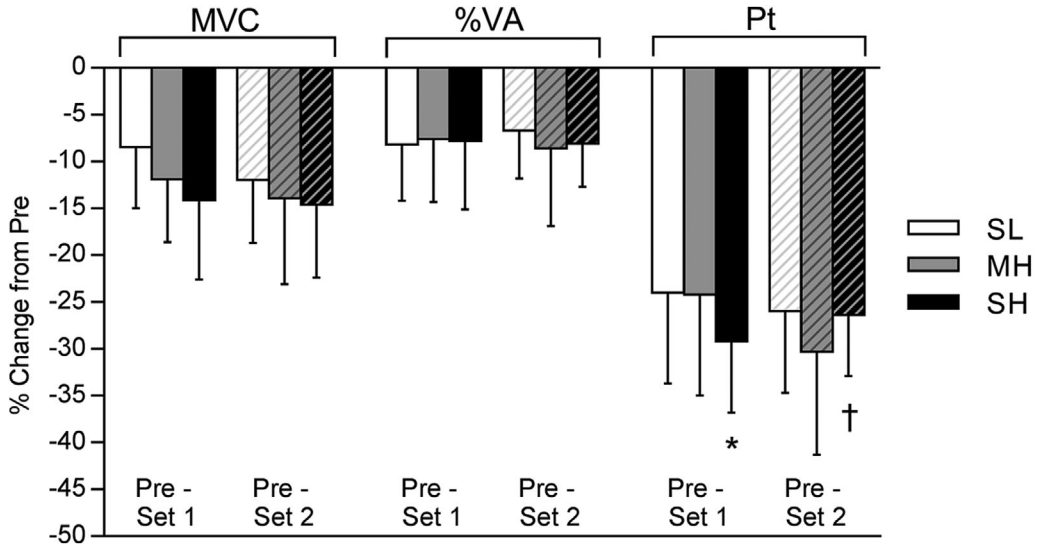

FIGURE 3 Selected neuromuscular fatigue responses to repeated-sprint exercise ($n = 13$). Results show the percentage change from baseline to post-set 1 and from baseline to post-set 2. Abbreviations: MH, moderate hypoxia; MVC, maximum voluntary contraction (isometric leg extension); Pt, resting potentiated quadriceps twitch torque; SH, severe hypoxia; SL, sea level; and %VA, percentage voluntary activation. * SH is different from SL and MH at the time point indicated ($P < 0.05$). † SH is different from MH at the time point indicated ($P < 0.05$)

$\eta_p^2 = 0.05$), but there was a main effect of time ($P < 0.001$; $\eta_p^2 = 0.66$), whereby a reduction in %VA occurred from Pre- (pooled mean, 91.2 ± 5.4%) to Post-set 1 (84.1 ± 8.4%; $P = 0.001$; $g = 1.0$) without any further change at Post-set 2 (84.1 ± 8.0%; $P = 1.0$; $g < 0.01$). There were no interaction effects for either MVC or %VA, and no other variables recorded during the MVC showed a main effect of condition (Table 1).

Of the three muscles where EMG was recorded, a significant main effect of time was observed only for the RMS.M^{-1} ratio of rectus femoris ($P < 0.001$; $\eta_p^2 = 0.58$). *Post hoc* analysis revealed a decrease in RMS.M^{-1} of rectus femoris from Pre- (pooled mean, 0.038 ± 0.018 a.u.) to Post-set 1 (0.032 ± 0.014 a.u.; $P = 0.006$; $g = 0.82$), but no additional change at Post-set 2 (0.031 ± 0.015 a.u.; $P = 0.61$; $g = 0.07$). No significant differences between conditions in peak-to-peak M-wave amplitude were observed, and there were no interaction effects.

Measures of muscle contractility in the potentiated rested state are presented in Table 2. No main effect of condition was observed for Pt ($P = 0.12$; $\eta_p^2 = 0.16$), but there was a significant main effect of time ($P < 0.001$; $\eta_p^2 = 0.92$). *Post hoc* analysis revealed a significant decrease from Pre- (pooled mean, 55.7 ± 6.8 N m) to Post-set 1 (41.4 ± 7.8 N m; $P < 0.001$; $g = 1.11$), but no further change at Post-set 2 (40.5 ± 8.0 N m; $P = 0.56$; $g = 0.11$). A significant interaction was also observed ($P = 0.003$; $\eta_p^2 = 0.28$), whereby *post hoc* analysis demonstrated greater Pt at Post-set 1 in SL (42.7 ± 8.1 N m) compared with SH (39.8 ± 7.5 N m; $P = 0.02$; $g = 0.34$). No significant differences between conditions for Pt, from Post-set 1 to Post-set 2, were observed.

For Db20, no main effect of condition was found ($P = 0.38$; $\eta_p^2 = 0.08$), whereas there was a main effect of time ($P < 0.001$; $\eta_p^2 = 0.79$), but not a significant interaction ($P = 0.08$; $\eta_p^2 = 0.20$). Db20 decreased from Pre- (pooled mean, 87.7 ± 19.3 N m) to Post-set 1 (70.3 ± 4.5 N m; $P < 0.001$; $g = 0.9$), and no further change at Post-set 2 (68.9 ± 4.6 N m; $P = 0.81$; $g = 0.07$) was evident.

Likewise, for Db80, no main effect of condition was observed ($P = 0.15$; $\eta_p^2 = 0.15$), and there was a main effect of time ($P < 0.001$; $\eta_p^2 = 0.77$). Db80 decreased from Pre- (pooled mean, 92.9 ± 15.0 N m) to Post-set 1 (77.9 ± 14.0 N m; $P < 0.001$; $g = 0.95$), with no further change at Post-set 2 (77.9 ± 14.0 *versus* 76.7 ± 14.8 N m; $P = 1.0$; $g = 0.08$). A significant interaction occurred ($P = 0.006$; $\eta_p^2 = 0.25$), whereby Db80 was greater at Post-set 1 in SL (79.4 ± 14.0 N m) compared with SH (74.5 ± 15.2 N m; $P = 0.03$; $g = 0.32$), but not compared with MH (79.5 ± 14.1 N m; $P = 1.0$; $g = 0.01$).

A significant main effect of time occurred for both MRTD ($P < 0.001$; $\eta_p^2 = 0.88$) and MRTR ($P < 0.001$; $\eta_p^2 = 0.78$), in addition to a time × condition interaction for MRTD ($P = 0.03$; $\eta_p^2 = 0.20$) and MRTR ($P = 0.02$; $\eta_p^2 = 0.21$). At Post-set 1, MRTD was higher in SL (1235 ± 295 N m ms^{-1}) than in SH (1142 ± 285 N m ms^{-1}; $P = 0.007$; $g = 0.12$), and MRTR was also higher in SL (543 ± 148 N m ms^{-1}) than in SH (475 ± 112 N m ms^{-1}; $P = 0.04$; $g = 0.49$).

4 | DISCUSSION

The main finding of the present study was that larger decreases in muscle contractility parameters (Pt and Db80) occurred in Post-set 1 in SH compared with SL, whereas there was no difference between MH and SL. In addition, we did not observe significant differences between conditions, or interactions for Db20, Db20.Db80^{-1}, MVC, %VA, M-wave or RMS.M^{-1} in Post-set 1. These results contradict our hypothesis of a shift towards increased central fatigue in SH

TABLE 1 Muscle function during brief MVC before (Pre-) and after the first (Post-set 1) and second (Post-set 2) repeated-sprint sets

	Time point			ANOVA *P*-value (effect size)		
Variable	Pre-	Post-set 1	Post-set 2	Condition	Time	Interaction
MVC torque (N m)						
SL	271 ± 46	247 ± 35*	239 ± 46*	0.216	<0.001	0.148
MH	269 ± 42	238 ± 39*	232 ± 43*	(0.12)	(0.77)	(0.14)
SH	270 ± 42	232 ± 41*	232 ± 43*			
VA (%)						
SL	92.0 ± 5.5	85.5 ± 7.7*	85.6 ± 5.1*	0.510	<0.001	0.747
MH	91.4 ± 5.6	84.8 ± 8.7*	83.5 ± 9.9*	(0.05)	(0.66)	(0.03)
SH	91.0 ± 5.8	84.3 ± 9.7*	84.5 ± 8.7*			
RMS.M^{-1} VL (a.u.)						
SL	0.055 ± 0.026	0.052 ± 0.025	0.052 ± 0.025	0.582	0.100	0.627
MH	0.053 ± 0.025	0.050 ± 0.029	0.048 ± 0.021	(0.04)	(0.19)	(0.05)
SH	0.057 ± 0.030	0.050 ± 0.021	0.049 ± 0.021			
RMS.M^{-1} VM (a.u.)						
SL	0.049 ± 0.018	0.044 ± 0.018	0.046 ± 0.023	0.999	0.154	0.461
MH	0.047 ± 0.023	0.047 ± 0.027	0.046 ± 0.029	(0.00)	(0.14)	(0.07)
SH	0.048 ± 0.019	0.047 ± 0.023	0.045 ± 0.018			
RMS.M^{-1} RF (a.u.)						
SL	0.040 ± 0.019	0.034 ± 0.016*	0.032 ± 0.016*	0.322	0.001	0.592
MH	0.041 ± 0.021	0.033 ± 0.014*	0.035 ± 0.015*	(0.09)	(0.58)	(0.06)
SH	0.039 ± 0.017	0.032 ± 0.015*	0.031 ± 0.014*			
M-wave VL (mV)						
SL	11.3 ± 4.5	11.3 ± 4.5	11.3 ± 4.5	0.824	0.377	0.295
MH	11.8 ± 4.5	11.2 ± 4.4	11.0 ± 4.2	(0.02)	(0.08)	(0.10)
SH	11.3 ± 4.5	11.6 ± 4.8	11.2 ± 4.7			
M-wave VM (mV)						
SL	10.3 ± 4.0	10.8 ± 4.4	10.1 ± 4.4	0.922	0.103	0.557
MH	10.6 ± 3.1	10.1 ± 3.5	9.9 ± 2.9	(0.01)	(0.17)	(0.05)
SH	10.7 ± 3.9	10.3 ± 4.2	10.1 ± 3.8			
M-wave RF (mV)						
SL	12.2 ± 2.0	12.6 ± 2.0	12.8 ± 2.2	0.219	0.570	0.160
MH	12.5 ± 3.3	12.3 ± 1.5	11.6 ± 1.4	(0.12)	(0.04)	(0.13)
SH	12.3 ± 2.1	12.7 ± 1.8	12.6 ± 2.2			

Values are the mean ± SD ($n = 13$). Abbreviations: MVC, maximal voluntary contraction; RMS.M^{-1}, M-wave-normalized root mean square activity; RF, rectus femoris; VA, voluntary activation; VL, vastus lateralis; and VM, vastus medialis. For set 1, all eight sprints were performed at sea level (SL), moderate (MH) or severe hypoxia (SH), whereas for set 2, all sprints were conducted in normoxia. *Significant difference from Pre- ($P < 0.05$).

compared with either MH or SL and indicate that greater peripheral muscle fatigue occurred in SH. This suggests that greater central fatigue with increasing severity of hypoxia, as reported elsewhere (Billaut et al., 2013; Peyrard et al., 2019), did not occur during the present experimental protocol. Instead, given that the maximum obtainable quadriceps peripheral muscle fatigue was elevated in SH, this challenges the notion of a tightly regulated 'peripheral muscle fatigue threshold' (Hureau, Romer, & Amann, 2016b) when RSE running is conducted in severe hypoxia ($F_{I,O_2} \approx 0.13$). A caveat to these findings, however, was that no differences in performance between conditions occurred during set 2. Furthermore, we did not observe significant differences between conditions in the B[la], HR or RPE responses to RSE in set 1 or set 2 even though S_{pO_2} was markedly lower in set 1 in both MH and SH conditions compared with SL. Thus, despite a slightly greater magnitude of peripheral fatigue during Post-set 1 in SH, the difference was insufficient to have a negative impact on performance during set 2 or to alter whole-body metabolic and perceived exertion responses to RSE.

TABLE 2 Muscle function at rest before (Pre-) and after the first (Post-set 1) and second (Post-set 2) repeated-sprint sets

	Time point			ANOVA *P*-value (effect size)		
Variable	Pre-	Post-set 1	Post-set 2	Condition	Time	Interaction
Paired stimulations						
Db20 (N m)						
SL	85.9 ± 22.4	74.3 ± 18.7*	71.4 ± 17.2*	0.375	<0.001	0.080
MH	86.8 ± 18.1	70.0 ± 14.6*	67.5 ± 19.1*†	(0.08)	(0.79)	(0.20)
SH	90.3 ± 19.6	66.4 ± 16.6*†‡	67.7 ± 18.2*†			
Db80 (N m)						
SL	92.8 ± 17.2	79.4 ± 14.0*	79.6 ± 15.9*	0.147	<0.001	0.006
MH	91.9 ± 13.3	79.5 ± 14.1*	73.3 ± 15.6*	(0.15)	(0.77)	(0.25)
SH	93.9 ± 16.5	74.7 ± 15.2*†	77.2 ± 13.1*			
$Db20.Db80^{-1}$						
SL	0.92 ± 0.13	0.92 ± 0.09	0.90 ± 0.12	0.869	0.016	0.225
MH	0.94 ± 0.12	0.88 ± 0.11	0.91 ± 0.12	(0.01)	(0.29)	(0.12)
SH	0.96 ± 0.15	0.89 ± 0.14	0.87 ± 0.16			
Single stimulation						
Pt (Nm)						
SL	56.0 ± 7.6	42.7 ± 8.3*	41.7 ± 8.5*	0.119	<0.001	0.003
MH	55.1 ± 6.7	41.8 ± 8.0*	38.5 ± 8.2*	(0.16)	(0.92)	(0.28)
SH	56.0 ± 6.5	39.8 ± 7.5*†‡	41.4 ± 7.4*‡			
TPT (ms)						
SL	76.4 ± 11.6	71.8 ± 11.1*	73.0 ± 15.6*	<0.001	0.002	0.277
MH	85.6 ± 24.2	76.2 ± 14.4*	72.1 ± 10.0*	(0.83)	(0.41)	(0.10)
SH	78.4 ± 20.6†‡	69.2 ± 13.7*†‡	57.9 ± 8.3*†‡			
HRT (ms)						
SL	69.7 ± 16.3	57.9 ± 7.1*	58.8 ± 8.7*	0.878	0.002	0.452
MH	74.4 ± 15.9	56.1 ± 9.0*	57.5 ± 7.6*	(0.01)	(0.55)	(0.06)
SH	72.9 ± 16.9	58.5 ± 8.7*	57.9 ± 8.3*			
MRTD ($N\ m\ ms^{-1}$)						
SL	1622 ± 341	1235 ± 295*	1193 ± 324*	0.163	<0.001	0.031
MH	1629 ± 299	1172 ± 272*	1089 ± 310*	(0.14)	(0.88)	(0.20)
SH	1661 ± 315	1142 ± 285*†	1181 ± 290*			
MRTR ($N\ m\ ms^{-1}$)						
SL	809 ± 237	543 ± 148*	532 ± 137*	0.389	<0.001	0.020
MH	769 ± 183	535 ± 120*	476 ± 113*	(0.07)	(0.78)	(0.21)
SH	814 ± 187	475 ± 112*†‡	517 ± 117*			

Values are the mean ± SD ($n = 13$). Abbreviations: Db20, torque associated with doublet at 20 Hz; Db80, torque associated with doublet at 80 Hz; HRT, half-relaxation time; MRTD, maximal rate of torque development; MRTR, maximal rate of torque relaxation; Pt, peak twitch torque; and TPT, time-to-peak torque. For repeat sprint set 1, all eight sprints were performed at sea level (SL), moderate (MH) or severe hypoxia (SH), whereas for set 2 all sprints were conducted in normoxia. *Significant difference from Pre- ($P < 0.05$). †Significant difference from SL ($P < 0.05$). ‡Significant difference from MH ($P < 0.05$).

4.1 | Repeated-sprint performance in hypoxia

During set 1, there was no difference in performance on sprint 1 across conditions and, as expected, the distance covered in each individual sprint decreased progressively in all conditions. The mean distance for sprints 1–8 was lower in MH and SH compared with SL, but the difference was significant only for SH. The finding that performance of a single, brief 'all-out' effort was not affected by hypoxia aligns with studies demonstrating that short-duration (<60 s), high-intensity exercise, characterized by a large anaerobic energetic contribution, remains unaffected by hypoxia (Calbet et al., 2003; Weyand et al., 1999). During RSE tasks, the aerobic contribution is increased relative

to a single sprint effort, and performance is therefore likely to be impacted by diminished oxygen delivery (Billaut, Gore, & Aughey, 2012). Bowtell et al. (2014) observed similar peak running velocity in moderate hypoxia (F_{I,O_2} = 0.15) compared with normoxia, but reduced performance in severe hypoxia (F_{I,O_2} = 0.12). Additionally, Goods et al. (2014) found similar performance (expressed as mean power output) at $F_{I,O_2} \approx 0.16$ compared with normoxia, during the first three of nine repeated sprints. Hence, these findings are in accordance with our data, but contrast with studies also conducted in moderate hypoxia ($F_{I,O_2} \approx$ 0.15–0.17), which generally report a decrease in sustained high-intensity exercise performance (Amann et al., 2007; Clark et al., 2007; Goodall et al., 2010; Shearman, Dwyer, Skiba, & Townsend, 2016; Townsend, Nichols, Skiba, Racinais, & Périard, 2017). Likewise, studies investigating the impact of hypoxia on aerobic function, including maximal oxygen uptake ($\dot{V}_{O_2}$) and critical power, report decreased performance and/or aerobic capacity in moderate hypoxia (Shearman et al., 2016; Squires & Buskirk, 1982; Wehrlin, Marti, & Hallén, 2016). Therefore, it appears that a greater magnitude of hypoxia may be necessary to induce performance decrement in RSE compared with either incremental or continuous severe-intensity exercise. This could be attributable to the very brief 'all-out' nature of each sprint effort, which necessitates a high anaerobic contribution (Girard et al., 2011).

Repeated-sprint performance declined more rapidly in SH compared with MH and SL. In SH, we observed a significant decrease in performance at sprint 2, whereas for MH and SL a significant decline only occurred at sprint 3. This pattern of earlier performance decline in set 1 of the SH conditions is similar to results of previous investigations of RSE during running (Goods et al., 2014) and cycling (Billaut & Buchheit, 2013). Slowed $\dot{V}_{O_2}$ kinetics are a hallmark feature of exercise in hypoxia (Hughson, Xing, Butler, & Northey, 1990). This characteristic response has been associated with an increase in the accumulated O_2 deficit during a Wingate test conducted in severe hypoxia (F_{I,O_2} = 0.10), whilst performance was unaffected (Calbet et al., 2003). Moreover, in trained soccer players completing RSE in normoxia, a strong correlation was observed between the $\dot{V}_{O_2}$ primary component and the decline in sprint performance (Dupont, Millet, Guinhouya, & Berthoin, 2005). Given that performance on the initial sprint effort was unaffected by hypoxia, but there was a larger decrease in performance on sprint 2 in SH compared with either SL or MH, our results might be explained by a similar mechanism, whereby the increased anaerobic energy contribution during brief 'all-out' exercise compensates for slower $\dot{V}_{O_2}$ kinetics. It is well established that anaerobic metabolism contributes to the mechanisms of peripheral muscle fatigue, including an increase in inorganic phosphate and decreased muscle pH (Allen, Lamb, & Westerblad, 2008; Kent-Braun, Fitts, & Christie, 2012). Furthermore, these changes in the intracellular milieu within working muscles are proportional to elevations in group III/IV muscle afferent stimulation during high-intensity exercise (Blain et al., 2016). Therefore, if an increase in anaerobic energy contribution occurred in SH during the early phase of set 1, this would be expected to exacerbate the rate of fatigue development, as observed in our study.

Interestingly, the rate of performance decline from sprint 2 onwards was not different between conditions. Given that RSE is characterized by a maximal effort on each individual sprint bout, this finding suggests that the rate of fatigue development was not markedly different across conditions from sprint 3 until completion of set 1. This observation is in accordance with numerous other studies (Billaut & Buchheit, 2013; Billaut et al., 2013; Girard et al., 2017; Goods et al., 2014) and aligns with the notion that fatigue is not an 'all-or-none' phenomenon, but instead occurs progressively (Grassi, Rossiter, & Zoladz, 2015). Thus, although an initial maximal effort remains unhindered by hypoxia, any increase in muscle fatigue occurring early within a set of RSE might behave akin to a feedback mechanism limiting maximal performance, in turn reducing the subsequent rate of fatigue development thereafter. This would explain why there were no differences between conditions in the rate of performance decline from sprint 3 to sprint 8 of set 1 (Figure 2c).

4.2 | Relative contribution of central *versus* peripheral fatigue

In the present study, we observed similar changes in indices of central fatigue (%VA and RMS.M^{-1}) from Pre- to Post-set 1 across all conditions. Furthermore, this was coupled with larger decreases in Pt and Db80 in SH compared with SL, which suggests that a greater magnitude of peripheral fatigue was obtained in SH. These results contradict our hypothesis that a shift towards greater central fatigue, coupled with a diminished magnitude of peripheral fatigue, would occur with increasing severity of hypoxia. This hypothesis was partly based on evidence revealing that severe hypoxia exacerbates the magnitude of central fatigue experienced during exhaustive exercise, leading to a reciprocal decrease in peripheral fatigue (Amann et al., 2007; Millet et al., 2012). In a study by Billaut et al. (2013), participants completed 15 cycling sprint intervals (6 s work, 25 s recovery) in hypoxia (F_{I,O_2} = 0.14) and normoxia. The authors reported a greater decrease in RMS.M^{-1} and central activation ratio in the hypoxic conditions. In another study, reduced corticospinal excitability was found following elbow flexion sprint intervals (10 s work, 20 s recovery) to exhaustion in hypoxia (F_{I,O_2} = 0.13) compared with normoxia (Peyrard et al., 2019). The discrepancy in findings might be attributable to differences in muscle mass involvement, which appears to modulate the magnitude of peripheral fatigue achievable (Rossman, Venturelli, McDaniel, Amann, & Richardson, 2012). In the present study, participants completed 'all-out' sprint running as opposed to cycling or elbow flexion. Larger muscle mass engagement (double *versus* single leg extension) was shown to limit the magnitude of peripheral fatigue obtained during exhaustive exercise in normoxia (Rossman, Garten, Venturelli, Amann, & Richardson, 2014), which suggests that the magnitude of muscle group-specific peripheral fatigue was not maximal during whole-body exercise in normoxia. This leaves open the potential for hypoxia to allow for an increase in peripheral fatigue, as observed in the present study; however, whether this finding is related to active muscle mass requires further investigation.

No previous studies have examined the relative contribution of central *versus* peripheral indices of fatigue after RSE conducted in graded hypoxia; therefore, our hypothesis was also based on investigations that investigated other modes of fatiguing exercise (Amann et al., 2007; Goodall et al., 2010; Millet et al., 2012). After cycling at 80% peak power until exhaustion, Amann et al. (2007) observed a reduction in the potentiated quadriceps twitch force in severe hypoxia (F_{I,O_2} = 0.10), whereas no differences were found between moderate hypoxia (F_{I,O_2} = 0.15) and normoxia. Upon subsequent hyperoxygenation (F_{I,O_2} = 0.30) in a second bout of exercise, the time to exhaustion was prolonged in the severe hypoxic conditions, and the magnitude of peripheral fatigue rose to meet that obtained in moderate hypoxic and normoxic conditions. The authors interpreted this finding as supplementary evidence that increased central fatigue in severe hypoxia led to attenuated peripheral fatigue. Likewise, we expected to find better performance on set 2 in the SH conditions, because this would corroborate our hypothesis that a centrally mediated limitation of peripheral fatigue would occur after set 1. Therefore, although our results differ from those of Amann et al. (2007), it is noteworthy that we observed similar performance in all conditions during set 2, in combination with a consistent magnitude of central fatigue after set 1. In another study, intermittent isometric quadriceps contractions at 60% MVC were performed until task failure in normoxia (F_{I,O_2} = 0.21), mild hypoxia (F_{I,O_2} = 0.16), moderate hypoxia (F_{I,O_2} = 0.13) and severe hypoxia (F_{I,O_2} = 0.10) (Goodall et al., 2010). Cortical voluntary activation declined in all conditions, but was greatest in severe hypoxia and was coupled with an attenuated decline in potentiated quadriceps twitch force (Goodall et al., 2010). In both of these studies, the authors concluded that reduced cerebral oxygenation probably inhibited descending motor drive, thereafter reducing peripheral muscle fatigue. In the present study, the lack of differences between conditions and from Post-set 1 to Post-set 2 for both %VA and MVC lends support to the theory that central fatigue modulates the force-producing capacity at the limit of tolerance (Hureau et al., 2016b).

In the present study, we did not observe any difference in %VA or RMS.M^{-1} between conditions; therefore, we cannot conclude that hypoxia inhibited descending motor drive independently of afferent feedback. However, the magnitude of severe hypoxia that induced elevated central fatigue (F_{I,O_2} = 0.09–0.10) was greater in the abovementioned studies (Amann et al., 2007; Goodall et al., 2010) than for SH in the present study. Results from these three studies also demonstrate a greater magnitude of hypoxia at which an increase in central fatigue occurred compared with the data of Billaut et al. (2013) and Peyrard et al. (2019). Therefore, it is likely that interaction of multiple factors, including the magnitude of hypoxia, active muscle mass and task specificity ('all-out' RSE *versus* submaximal contractions to exhaustion), might influence the overall magnitude of central fatigue experienced.

Despite the finding that indices of central fatigue were unaffected by hypoxia in the present study, we still observed larger decreases in Pt and Db80 in SH compared with SL Post-set 1, although this was not true for Db20 or Db20.Db80^{-1}. Conversely, there was no effect of condition or a time × condition interaction on M-wave responses. The M-wave response is reflective of muscle membrane properties (Millet, Martin, Martin, & Vergès, 2011); hence, our results confirm those of other studies (Amann et al., 2007; Millet et al., 2012; Peyrard et al., 2019). This indicates that the underlying mechanism of peripheral fatigue after exhaustive exercise primarily occurs distal to the sarcolemma and is unaffected by hypoxia *per se*. Peak twitch torque and Db20 are affected by attenuated sarcoplasmic Ca^{2+} release, and reduced sensitivity of the contractile apparatus to Ca^{2+} and changes in Db80 are attributed to an accumulation of extracellular K^+, both of which lead to impaired excitation–contraction coupling (Millet et al., 2011). Changes in Db20.Db80^{-1} are considered to reflect low-frequency fatigue, which is also characterized by excitation–contraction uncoupling (Millet et al., 2011). Therefore, given that decreases were observed for Pt and P80 postexercise, but Db80, Db20.Db80^{-1} and the M-wave remained unchanged, this suggests that reduced muscle contractile function, rather than impaired excitation–contraction coupling, was the likely mechanism underlying the enhanced peripheral fatigue found in SH. Moreover, our data also indicate that these changes can occur in the absence of significant differences in MVC, %VA and RMS.M^{-1}. This would appear to challenge the concept of a 'critical peripheral fatigue threshold' (Hureau et al., 2016b), but it should be noted that this concept has been challenged on grounds of task specificity (Neyroud, Kayser, & Place, 2016). Regardless of differences in experimental protocol and integrated neuromuscular fatigue response observed across studies, a common feature emerges when exhaustive exercise is conducted in hypoxia in the range F_{I,O_2} = 0.13–0.17. Within this range, numerous studies, including ours, have consistently observed decreases in MVC that are similar amongst conditions (Amann et al., 2007; Goodall et al., 2012; Peyrard et al., 2019). These findings lend support to the notion that a global level of fatigue, i.e. loss of MVC force, might be the variable that is regulated rather than peripheral muscle fatigue *per se* (Neyroud et al., 2016).

A limitation of the present study is that we did not measure central and peripheral indices of fatigue immediately after a single sprint effort; therefore, we cannot know the relative contribution of central *versus* peripheral fatigue mechanisms at each time point. Only one study (Hureau, Ducrocq, & Blain, 2016a), conducted in normoxia, has examined muscle contractility changes after one, four, six, eight or 10 sprint repetitions within a single set (10 s effort with 30 s recovery). That experimental protocol was achieved by conducting each RSE test session on different days. The authors reported progressive decreases in quadriceps twitch torque, low- and high-frequency doublet torque from one to six sprint efforts, followed by no further change between six and 10 sprint efforts. This indicated that to achieve maximum obtainable decreases in muscle contractility, approximately six sprint efforts were required. This leaves open the potential for hypoxia to induce a more rapid onset of central and/or peripheral fatigue within the first one to three sprint efforts, as suggested by our results. Further research is required to explore fully the theory that a more rapid development of peripheral fatigue occurs in severe hypoxia, and hence underlies the faster decline in performance observed in this study and

others (Billaut & Buchheit, 2013; Billaut et al., 2013; Goods et al., 2014). Another limitation, which is a common critique of experimental protocols involving NMES after whole-body exercise, is that fatigue was not assessed instantly after the bout of exercise, but after a short recovery interval (which we standardized to 1 min). It is plausible that severe hypoxia could have induced significant differences in central fatigue but that we were unable to detect these using the present protocol.

4.3 | Summary and conclusions

This study is the first to examine the effect of graded hypoxia on indices of central and peripheral fatigue after 'all-out' repeated-sprint running. Contrary to previous studies that examined either RSE of a smaller muscle mass (Billaut et al., 2013; Peyrard et al., 2019) or sub-maximal exercise to task failure (Amann et al., 2007; Goodall et al., 2010; Millet et al., 2012), we did not observe a shift towards greater central fatigue with increasing severity of hypoxia, but instead an increased magnitude of peripheral fatigue was obtained in the most severely hypoxic conditions (F_{I,O_2} = 0.13). This suggests that the threshold level of hypoxia required to induce a direct impact on central motor drive is likely to be modulated by both active muscle mass and task specificity. Therefore, we conclude that a reciprocal relationship between indices of central and peripheral fatigue is not always evident during exhaustive exercise in hypoxia. Additionally, the magnitude of peripheral fatigue obtained within working muscle is not necessarily associated with changes in large-muscle mass, whole-body exercise performance.

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COMPETING INTERESTS

None declared.

AUTHOR CONTRIBUTIONS

This project was completed within the Athlete Health and Performance Research Center, Aspetar Orthopaedic and Sports Medicine Hospital, Doha, Qatar. F.B., G.P.M. and O.G. were involved in conception and design of the study. All authors were involved in data acquisition, analysis and interpretation of results and in drafting and revision of the manuscript for relevant content. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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