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Original Article

Neuromuscular and Muscle Tissue Hemodynamic Responses When Exposed to Normobaric Hypoxia during Lower-Body Fatiguing Muscle Actions

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Abstract

Objectives: This study examined effects of acute hypoxia on the neuromuscular responses (electromyographic (EMG) amplitude and EMG frequency) and localized muscle tissue oxygenated hemoglobin (oxygenated hemoglobin (Oxy_{Hb}), deoxygenated hemoglobin ($Deoxy_{Hb}$), total hemoglobin ($Total_{Hb}$), and muscle tissue oxygenation saturation (StO_2) during the process of fatigue. **Methods**: Fifteen male participants (21.4±2.8yr) performed leg extension repetitions to failure at 70% 1-repetition maximum until volitional exhaustion under Normoxic (FiO_2:21%) and Hypoxic (FiO_2:12.9%) conditions. Electromyographic amplitude, EMG frequency, Oxy_{Hb}, $Deoxy_{Hb}$, $Total_{Hb}$, and StO_2 were measured from the vastus lateralis at Initial, 20, 40, 60, 80, and 100% of the repetitions to failure. **Results**: There was no significant difference in the patterns of responses for EMG amplitude, Oxy_{Hb}, or $Deoxy_{Hb}$ between Normoxia and Hypoxia. For EMG frequency, Hypoxia was greater than Normoxia and decreased with fatigue. $Total_{Hb}$ and StO_2 were greater under Normoxia compared to Hypoxia. The patterns of responses for EMG amplitude, $Deoxy_{Hb}$, and $Total_{Hb}$ increased throughout the repetitions to failure. Oxy_{Hb} and StO_2 exhibited decreases throughout the repetitions to failure for Normoxic and Hypoxic conditions. **Conclusion**: The EMG and oxygenation measurements non-invasively suggest a sympathoexcitatory response (indicated by EMG frequency) and provided complimentary information regarding the process of fatigue in normoxic and hypoxic states.

Keywords: EMG, Hypoxemia, NIRS, StO_2, Sympathoexcitatory

Introduction

Hypoxemia, a below-normal level of blood oxygen content, commonly occurs during mountaineering, aviation, and military activities[1,2]. It is well documented that aerobic performance is negatively impacted under hypoxia, however, the effects of hypoxia during high-intensity, strength-based movements are conflicting[3–6]. Numerous studies[3,5,7] have reported differing neuromuscular responses between normoxic and hypoxic conditions, however, other studies[6,8,9] have reported no differences in strength, performance, or neuromuscular responses. For example, Scott et al. 2017 reported greater electromyographic (EMG) amplitude under moderate hypoxic conditions (FiO_2: 16%) compared to normoxia from the vastus lateralis during 3 sets of fatiguing back squats at 70% one repetition maximum (1-RM)[5]. In contrast, Scott et al. 2018 also reported no differences in EMG amplitude under normoxic, moderate hypoxia (FiO_2: 16%), or severe hypoxia (FiO_2: 13%) from the vastus lateralis during 5 sets of fatiguing back squats or deadlifts at 80% 1-RM[6]. These conflicting neuromuscular responses under similar conditions suggest that hypoxic-related differences in neuromuscular responses may be mediated by additional factors such as the metabolic status of the muscle.

Many studies that examine the effects of hypoxia on neuromuscular physiology primarily utilize EMG amplitude[3,5,6,8,10]. Electromyographic amplitude represents the level of muscle excitation during muscular contraction and is influenced by many factors including intensity,

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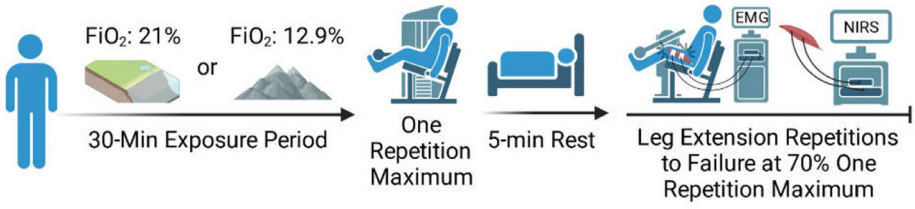

Figure 1. Depiction of the testing visits study flow.

fatigue, motor unit recruitment, and synchronization[11–13]. Thus, muscle excitation may be a useful tool for examining the effects of hypoxia on neuromuscular physiology as a reduction in FiO_2 may result in a relative increase in intensity, which would manifest as an increase in EMG amplitude. However, EMG amplitude is only one domain of the EMG signal resulting in the frequency component of signal often being overlooked. The frequency component of the EMG signal reflects the motor unit action potential conduction velocity characteristics of the activated muscle fibers and is highly influenced by the buildup of metabolic byproducts, excitation-contraction coupling failure, and the extra-cellular environment[14]. Hypoxemia has been shown to increase the rate that metabolic byproducts accumulate, negatively impacting the extra-cellular environment which would potentially affect the neuromuscular physiological properties that are reflected by EMG frequency[15,16]. The EMG signal is well documented to be impacted by the buildup of metabolic byproducts, damage to the neuro-contractile properties of the muscle tissue, and reduced neural drive from the central nervous system[14,15,17–21]. For example, recent work utilizing decomposition methods has shown a fatigue induced reduction in the frequency component of the EMG signal which was attributed to a derecruitment of low threshold, higher firing frequency motor units and an increase in higher threshold, lower firing frequency motor units[21]. Furthermore, foundational work has shown the buildup of metabolic byproducts such as Mg^{++}, ADP, Pi, Lactate, H^+, or NH_3 slows the ability for the muscle to efficiently conduct action potentials along its sarcolemma[14,17,18]. For example, Hargreaves, 2005 found that fatigue resulted in a buildup of metabolic byproducts that inhibited the sarcoplasmic reticulum and myofilament's function, ultimately slowing motor unit action potential conduction velocities and negatively impacting performance[18]. Thus, the addition of the EMG frequency component in hypoxic-related neuromuscular physiological research may provide valuable information regarding the muscles motor unit action potential conduction velocity characteristics and fatigue status.

Muscular near-infrared spectroscopy (NIRS) examines the concentration and oxygenation status of the hemoglobin and myoglobin within the skeletal muscle and reflect the muscle tissue oxygenation status (StO_2), total hemoglobin + myoglobin content ($Total_{Hb}$), oxygenated hemoglobin + myoglobin content (Oxy_{Hb}), and deoxygenated hemoglobin + myoglobin content ($Deoxy_{Hb}$)[22]. Measuring StO_2 allows for the quantification of oxygen status at the muscle tissue which more closely reflects the metabolic status than arterial oxygenation alone when examining fatigue and neuromuscular performance[1,22]. In addition, the simultaneous assessment of Oxy_{Hb} and $Deoxy_{Hb}$ provide useful information regarding the concentration of bound and unbound oxygen to the hemoglobin and myoglobin content at the muscle which may change under some hypoxic and fatiguing conditions[3,22]. Furthermore, $Total_{Hb}$ measured during an acute bout of exercise has been suggested to reflect blood flow to the active muscle as an increase in blood flow to the muscle would result in greater hemoglobin and myoglobin concentration[23–25]. Therefore, the examination of muscle oxygenation using NIRS allows for the quantification of Oxy_{Hb}, $Deoxy_{Hb}$, $Total_{Hb}$, and StO_2 which provide an assessment of the muscles metabolic, blood flow, and oxygen status in the active muscle. These NIRS measures can be combined with EMG amplitude and frequency to provide complementary information to better understand the neuromuscular physiological processes during fatigue under hypoxic conditions. Thus, the aim of this study was to examine the effects of acute hypoxic exposure on the EMG amplitude, EMG frequency, Oxy_{Hb}, $Deoxy_{Hb}$, $Total_{Hb}$, and StO_2 during the fatiguing process of leg extensions to failure at 70% 1-RM.

Materials and Methods

Subjects

Fifteen male participants volunteered in the study (age: 21.4±2.8 yr., height: 174.9±13.1 cm, weight: 85.6±14.3 kg). All participants lived at ≤1,066 m without any travel (greater than 24-hr) to altitudes greater than 1,524 m. within the past 6-mo. In addition, all participants were resistance trained (minimum 3 days per week for 1-hr a day consistently for at least 6-mo), U.S. Army Reserve Officer Training Corp members, and free from any musculoskeletal injuries or neuromuscular disorders. Participants were non-smokers, free from any asthma, or history of acute mountain sickness, and asked to refrain from consuming any caffeine or alcohol within 24-hr of their scheduled testing visit.

The study consisted of three visits to the laboratory and all participant visits occurred at the same time of day ±1-

hr. The first visit was designed as a familiarization visit for the participants to allow them to become familiar with all testing procedures including acute hypoxic exposure, NIRS on the vastus lateralis, peripheral capillary oxygen saturation (SpO_2), and EMG on the vastus lateralis. After subjects were familiarized with the testing procedures, all follow-up visits were scheduled. Visits two and three consisted of a 30-min exposure period, 1-RM leg extension protocol, 5-min of rest under exposure, and then leg extensions to failure at 70% 1-RM (Figure 1).

Normobaric Hypoxia

Hypoxia was induced using a Hypoxico HYP123 altitude generator (Hypoxico, New York, NY USA) with an in-line 300L Douglas bag with a pressure relief valve connected to a Hans-Rudolph 7450 full-face metabolic mask (Shawnee, KS USA). A calibrated in-line MySignO sensor was utilized to monitor the oxygen percent being delivered to the subjects (FiO_2) under normobaric hypoxia conditions (Envitec Wismar, Germany). During all testing, humidity and temperature were controlled for each visit at 20°C and 35% relative humidity. Therefore, the current study utilized normobaric hypoxia for all hypoxic testing visits. Subjects were blinded to their hypoxic exposures on all testing visits. During the Normoxic condition, an FiO_2 of 21% was delivered through the system to the participants. During the Hypoxic condition, a FiO_2 of 12.9% was delivered to the participants. Prior to the leg extension repetitions to failure, all participants were exposed to either Normoxic or Hypoxic conditions for 30 mins. Furthermore, peripheral capillary oxygenation saturation (SpO_2) was taken from the non-dominant index finger at rest prior to placing the hypoxic generator mask on the participant (Pre-Exposure) as well as after 30-min of wearing the hypoxic generator mask (Post-Exposure).

Leg Extensions

The 1-RM and repetition to failure leg extensions were performed on a commercial Cybex leg extension machine with an attached weight stack (Life Fitness, Rosemont, IL USA). All leg extensions were unilaterally using the dominant limb based on kicking preference. The range of motion was controlled and monitored throughout testing. A leg extension chair, lever arm, and pad position were adjusted so each participant's leg was resting at a 90° angle. Full extension was considered the range of motion that the participant could maximally extend their leg in the leg extension machine without any weight placed on the arm. A contact point on the lever arm of the leg extension machine was utilized to verify the range of motion for all participants and visits (contact being full extension). Failure was determined if subjects were unable to perform a full range of motion during the 1-RM or repetitions to failure. All 1-RM procedures were performed by Certified Strength and Conditioning Specialists and in accordance with the National Strength and Conditioning Associations testing procedures[27]. Specifically, the subjects performed a warm-up set of 5 to 10 repetitions at approximately 50% of an estimated 1-RM, then 3 to 5 repetitions at approximately 75% of their estimated 1-RM, a 1-min rest was given between 1-RM trials, and then subjects performed a series of single repetitions to determine their 1-RM leg extension within 2.27 kg. A 1-RM was performed before each testing visit to assure similar work and conditions were across all testing visits, however, the calculated 70% 1-RM weight for all repetitions to failure was based on the 1-RM performed during the Normoxia familiarization visit. A 5-min rest was provided immediately following the 1-RM attempt to assure all EMG and NIRS data return to a resting state prior to beginning the leg extension repetitions to failure. During the repetitions to failure, subjects were instructed to perform as many repetitions as they can until either they cannot lift the weight, or they feel that they can no longer perform any additional leg extensions. Failure was also determined if subjects paused for greater than 1-s between repetitions.

Electromyography

A bipolar electrode arrangement (Ag/AgCl, AccuSensor, Lynn Medical, Wixom, MI, USA) was placed on the vastus lateralis of the dominant leg (based on kicking preference) 66% of the distance between the anterior superior iliac spine and the lateral border of the patella and orientated at a 20° angle to approximate the pennation angle of the muscle fibers with an interelectrode distance of 30 mm[28]. A reference electrode was placed over the anterior superior iliac spine. The subjects' skin was dry shaved, abraded, and cleaned with isopropyl alcohol prior to the placement of electrodes. All EMG signals were collected at a sampling frequency of 2,000 Hz (BioPac Systems Inc., MP150 EMG100C, Goleta, CA). The EMG signals were notch-filtered (59-61 Hz), zero-meaned, and bandpass filtered (fourth-order Butterworth) at 10-500 Hz prior to analysis. The concentric phase was examined using the range of motion analysis derived from an accelerometer placed on the lever arm. The EMG signals corresponding to the repetitions to the Initial, 20%, 40%, 60%, 80%, and 100% of repetitions to failure were used for analysis. If a repetition selection was between repetitions, the greater repetition was selected for analysis (i.e. 40% was calculated as repetition 5.8, repetition 6 was selected). During each repetition, the root mean square (RMS) and mean power frequency (MPF) were analyzed for the entire concentric phase of the leg extension. All EMG values were normalized to their Pre-Exposure values which allows for the examination of what occurs due to the 30-min exposure period independent of the fatiguing protocol. All EMG analyses were performed utilizing a custom written NI LabView program (NI Austin, TX).

Near-Infrared Spectroscopy

A NIRS device was placed between the bipolar EMG electrode arrangement of the dominant legs vastus

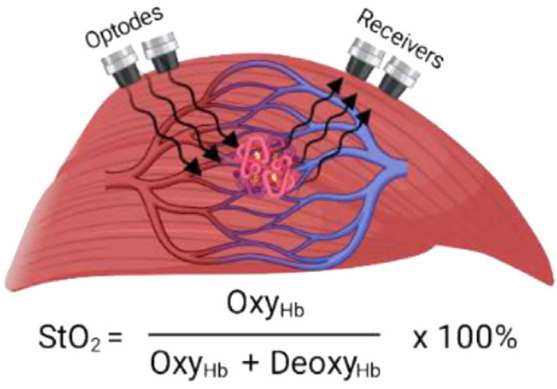

Figure 2. Muscle tissue oxygenation saturation (StO_2) calculation and the methodological approach for placement and obtaining near-infrared spectroscopy data from a dual optode and dual receiver model. Oxygenated hemoglobin (Oxy_{Hb}); Deoxygenated hemoglobin ($Deoxy_{Hb}$).

lateralis (Oxymon MkII, Artinis Medical Systems Einsteinwig, Nethlerlands). A single channel, dual optode setup was utilized with an optode to receiver distance of 45 mm. Each optode consisted of a dual-wavelength of 848 and 762 nm. A differential path-length factor (DPF) of 4.0 was utilized for all measurements. The signal was sampled at 10 Hz for all participants. In addition, the signal amplification and power were determined for each subject during the familiarization visit and were held constant for each subsequent visit. This allowed for repeatability of the measurement and improved the sensitivity of the NIRS. During the repetitions to failure, only the repetitions corresponding to Initial, 20%, 40%, 60%, 80%, and 100% of repetitions to failure were used for analysis. Using percentages of the repetitions to failure was utilized to allow subjects' data to be compared as a function of fatigue since the number of repetitions was not controlled. The mean Oxy_{Hb}, $Deoxy_{Hb}$, $Total_{Hb}$, and StO_2 were measured during each repetition utilizing a custom written LabView program and normalized to their Pre-Exposure value obtained before undergoing to the 30-min exposure period. This allowed for the quantification of change associated with the 30-min of exposure prior to the fatiguing task.

Theory

Near-Infrared Spectroscopy is based on the light absorption properties of oxygenated and deoxygenated hemoglobin which are between 700-900 nm. Using a dual-wavelength of 848 and 762 nm we can monitor the composite hemoglobin and myoglobin saturations through a non-invasive spectroscopy device. The NIRS device utilized optode transmitters and receivers whose distance impact the measurement depth (Figure 2). An increase in the distance between optode and receiver results in a reduced pickup depth, but greater width. A balance between the pickup depth and width must be achieved based on specific location being measured. In the current study, a distance of 45 mm allowed for optimal depth and width based on signal quality and sensitivity based on previous works[9,29]. The combination of spectroscopy measures with non-invasive neuromuscular metrics compliments one another improving the physiological interpretation and clinical applications. To achieve this, we aim to improve the independent understanding of each metric and then fuse these data in a clinically meaningful way that improves the interpretation through simplifying these metrics. This study aims to build upon the foundation combining EMG and NIRS data during fatiguing muscle contractions presented by Keller et al. 2021 with the goal of improving field-ready, non-invasive NIRS and EMG measures that can be fused to improve human monitoring[30].

Statistical Analyses

Six separate 2 (Condition: Normoxia vs Hypoxia) x 6 (Time: 0, 20, 40, 60, 80, and 100% repetitions to failure) repeated measures ANOVAs were performed for EMG RMS, EMG MPF, Oxy_{Hb}, $Deoxy_{Hb}$, $Total_{Hb}$, and StO_2. In addition, a 2 (Condition: Normoxia vs Hypoxia) x 2 (Time: Pre-Exposure vs Post-Exposure) repeated measures ANOVA was performed for SpO_2. Follow-up one-way ANOVAs as well as post-hoc paired sampled t-tests with Tukey-LSD were performed when appropriate. In addition, two separate t-tests were performed to examine repetitions performed and 1-RM between each condition. If sphericity was violated, the Greenhouse-Geisser correction was used. An alpha of $p \leq 0.05$ was considered statistically significant for all statistical analyses (IBM SPSS Version 25.0, Armonk, NY).

Results

Neuromuscular Responses

For EMG RMS, there was no significant two-way interaction for Condition x Time ($p=0.081$; $\eta_p^2=0.128$) or main effect for Condition ($p=0.641$; $\eta_p^2=0.061$). There was a significant main

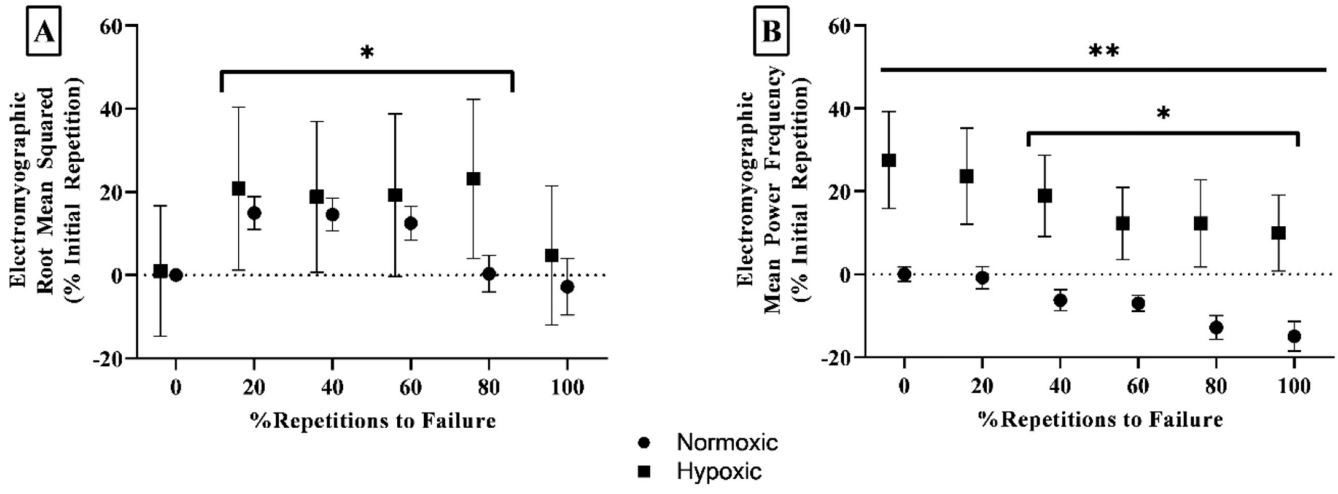

Figure 3. Electromyographic root mean squared (A) and mean power frequency (B) results under Normoxic (FiO_2: 21%) and Hypoxic (FiO_2: 12.9%) conditions during repeated leg extensions to failure at 70% one repetition maximum. (* Indicates significantly different than Initial. ** Indicates Hypoxia is greater than Normoxia).

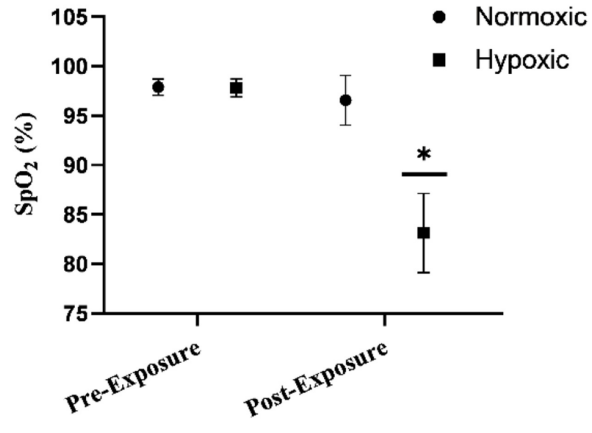

Figure 4. Normoxic and Hypoxia peripheral oxygen saturation (SpO_2) immediately before (Pre-Exposure) and after (Post-Exposure) 30-min of exposure. (* indicates significantly less than Pre-Exposure and Normoxia).

effect for Time (p=0.010; η_p^2 =0.276) which indicated that Initial < 20% (p<0.001), 40% (p<0.001), 60% (p<0.001), and 80% (p=0.020); Initial = 100% (p=0.945); 20% >100% (p=0.010); 40% >100% (p<0.001); 60% >100% (p=0.013); and 80% >100% (p<0.001) (Figure 3A).

For EMG MPF, there was no significant two-way interaction for Condition x Time (p=0.380; η_p^2 =0.072). There was a significant main effect for Condition (p=0.031; η_p^2 =0.290) which indicated that Normoxic < Hypoxic condition (p=0.031) (Figure 3B). There was a significant main effect for Time (p<0.010; η_p^2=0.512) which indicated that Initial >40%, 60%, 80%, and 100% (p<0.010 for all comparisons); 20%= Initial; 20% >60%, 80%, and 100%; and 60% >80% and 100% (Figure 3B).

Peripheral Oxygenation Saturation

For SpO_2, there was a significant two-way interaction for Condition x Time (p<0.001; η_p^2 =0.783). The follow-up paired t-tests across Time for the Normoxic condition was not significant (p=0.098; η_p^2 =0.155). The follow-up paired t-tests across Time for the Hypoxic condition was significant (p<0.001; η_p^2 =0.783) which indicated that Pre-Exposure > Post-Exposure (p<0.01) (Figure 4). The paired t-tests were across Condition for Time indicated no significant difference in SpO_2 at Pre-Exposure (p=0.751), however, there was a significant difference in SpO_2 Post-Exposure which indicated that Normoxic was significantly greater than the Hypoxic condition (p<0.01) (Figure 4).

Near-Infrared Spectroscopy

For Oxy_{Hb}, there was no significant two-way interaction for Condition x Time (p=0.070; η_p^2 =0.133) or main effect for Condition (p=0.122; η_p^2 =0.009). There was a significant main effect for Time (p<0.001; η_p^2 =0.472) which indicated that Initial >20%, 40%, 60%, 80%, and 100% (p<0.001 for all comparisons); 20% > 40%, 60%, 80%, and 100% (p<0.010 for all comparisons); 40% >60%, 80%, and 100% (p<0.010 for all comparisons); 60% >80% (p=0.040) and 100% (p=0.015) (Figure 5C).

For $Deoxy_{Hb}$, there was no significant two-way interaction

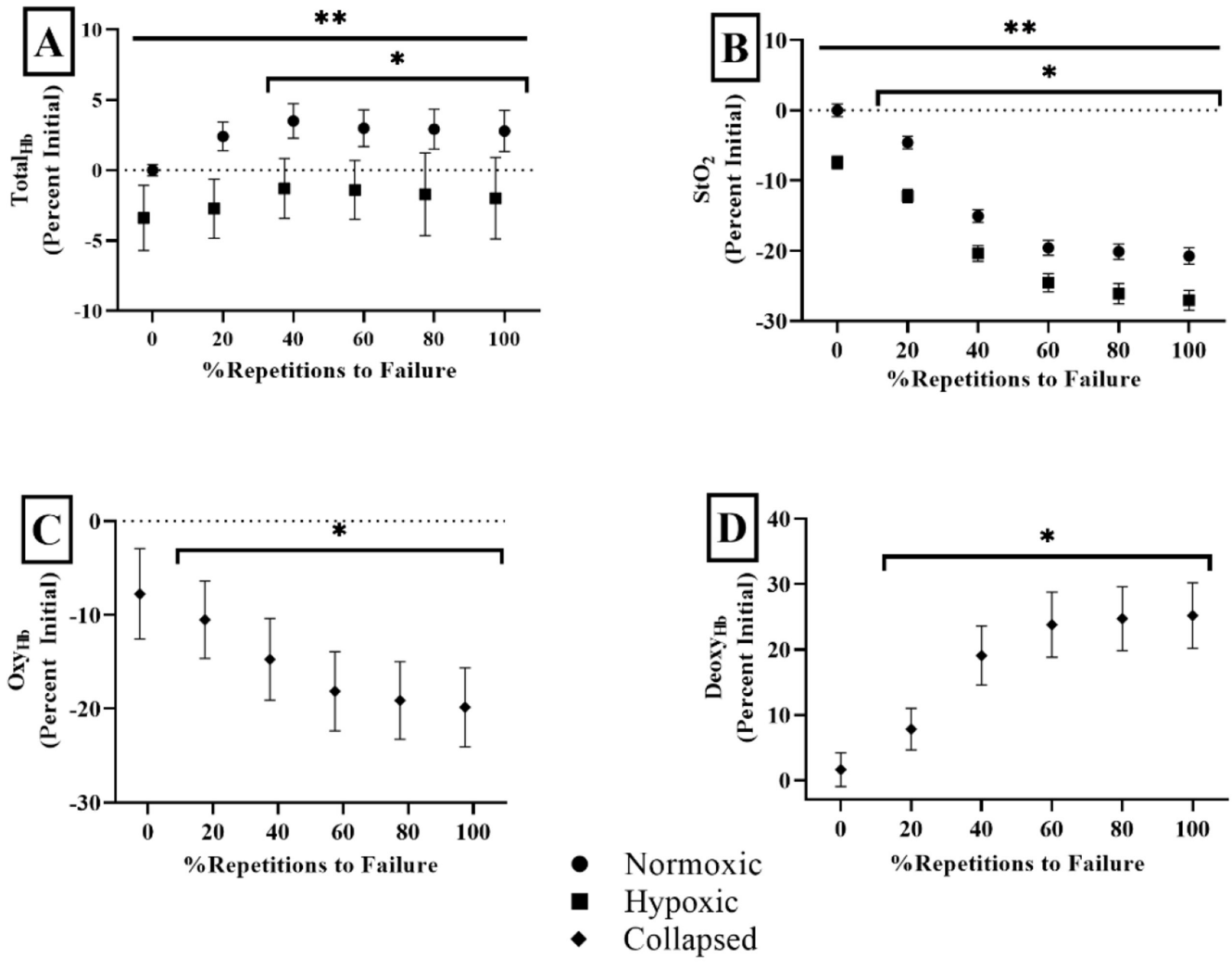

Figure 5. Muscle tissue oxygenation measures determined from muscle near-infrared spectroscopy during repeated leg extension to failure at 70% one repetition maximum under • Normoxic (FiO_2: 21%), ■ Hypoxic (FiO_2: 12.9%) conditions, or the ◆ collapsed data when no significant difference between conditions was observed. Total hemoglobin ($Total_{Hb}$); Muscle tissue oxygenation saturation (StO_2); Oxygenated hemoglobin (Oxy_{Hb}); Deoxygenated hemoglobin ($Deoxy_{Hb}$). (* Indicates significantly different than Initial. ** Indicates a significant difference between Normoxia and Hypoxia).

for Condition x Time (p=0.098; η_p^2 =0.159) or main effect for Condition (p=0.820; η_p^2=0.009). There was a significant main effect for Time (p<0.001; η_p^2 =0.758) which indicated that Initial <20%, 40%, 60%, 80%, and 100% (p<0.001 for all comparisons); 20% <40%, 60%, 80%, and 100% (p<0.001 for all comparisons); 40% <60%, 80%, and 100% (p<0.001 for all comparisons); 60% <80% (p=0.011) and 100% (p<0.001) (Figure 5D).

For $Total_{Hb}$, there was no significant two-way interaction for Condition x Time (p=0.071; η_p^2=0.129). There was a main effect for Condition (p=0.021; η_p^2 =0.683) which indicated that the Normoxic > Hypoxic condition (p=0.020) (Figure 5A). There was, however, a significant main effect for Time (p=0.004; η_p^2 =0.425) which indicated that Initial <40%, 60%, 80%, and 100% (p<0.010 for all comparisons); 20% <40% (p=0.012) (Figure 5A).

For StO_2, there was no significant two-way interaction for Condition x Time (p=0.561; η_p^2 =0.034), however, there was a main effect for Condition (p=0.048; η_p^2=0.193) which indicated that Normoxic condition was greater than Hypoxic condition (p=0.042). In addition, for StO_2 there was a significant main effect for Time (p=0.010; η_p^2=0.603) which indicated that Initial >20%, 40%, 60%, 80%, and 100%; 20% >40%, 50%, 80%, and 100%; 40% >60%, 80%, and 100%; 60% > 80% and 100%; 80% >100% (p<0.010 for all comparisons) (Figure 5B).

Fatiguing Task

There was no significant difference between the repetitions performed between the Normoxic (13.5±3.4 repetitions)

and Hypoxic conditions (12.9±2.4 repetitions) (p=0.603). In addition, there was no significant difference between the 1-RM performed between the Normoxic (88.3±16.1 kg) and Hypoxic conditions (87.5±21.7 kg) (p=0.431).

Discussion

Neuromuscular Responses to Fatigue

The neuromuscular responses in the current study indicated that there was no effect of hypoxia on EMG RMS, but there was greater EMG MPF throughout the Hypoxic condition compared to the Normoxic condition (Figure 3A, B). The level of muscle excitation, as indicated by EMG RMS, was not influenced by hypoxia, but did increase due to the fatiguing task from the Initial repetition from 20 to 80% of the repetitions to failure under the Normoxic and Hypoxic conditions. These findings were similar to those of Osawa et al. 2011 who reported no differences in muscle excitation from the vastus lateralis under normoxic or hypoxic conditions during a ramp cycling exercise[8]. The similar patterns of responses and levels of EMG RMS during the Normoxic and Hypoxic condition suggest that similar levels of muscle excitation are required to complete the 70% 1-RM leg extensions to failure. In addition, the increase in EMG RMS from 20 to 80% of the repetitions to failure indicated that greater muscle excitation is required to maintain adequate force production through the recruitment of higher threshold motor units[13,31] (Figure 3B). The plateau and reduction in EMG RMS that occurred from 80 to 100% of the repetitions to failure is atypical compared to most literature that examined the process of fatigue, but may be attributed to the a derecruitment of motor units or an initial overshoot of the required force to move the resistance of the leg extension throughout its full range of motion that was reduced to the minimum amount of muscle excitation required by 80 to 100% of the repetitions to failure[21,32].

There was no hypoxic-related difference in the time course of responses for EMG MPF which decreased from the Initial at 40 to 100% of the repetitions to failure during the Hypoxic and Normoxic conditions (Figure 3B). The similar fatigue-induced decreases in EMG MPF suggested that the rate of metabolic byproduct accumulation (Mg^{++}, ADP, Pi, Lactate, H^+, or NH_3) and slowing of motor unit action potential conduction velocity was similar between conditions[14,17]. For example, Smith et al. 2017 reported a fatigue-induced decrease in EMG MPF following repeated leg extensions to failure at 70% 1-RM which reflected a fatigue-induced buildup of metabolic byproducts resulting in a slowing in motor unit action potential conduction velocity[33]. Hargreaves, 2005 reported the accumulation of Mg^{++}, ADP, and Pi during the process of fatigue in skeletal muscle which inhibits the Ca^{++} release from the sarcoplasmic reticulum, slowing of the myofilament Ca^{++} uptake, and a reduction in energy optimization ultimately slowing motor unit action potential conduction velocity and decreasing performance[14,18]. Furthermore, similar accumulation of metabolic byproducts and fatigue status have been shown to result in similar pattern of responses for EMG MPF during leg extension to failure at 70 and 80% 1-RM[33,34]. Therefore, the decreases in EMG MPF indicated a similar buildup of metabolic byproducts throughout the process of fatigue during Hypoxia and Normoxia despite the mechanistic differences between conditions (Figure 3B).

It can be hypothesized that greater EMG MPF during hypoxia could be attributed to a greater sympathoexcitatory responses or recruitment of higher-threshold motor units[19–21]. The EMG signal has been reported to reflect the localized summation of motor unit action potential conduction velocity and is influenced by the recruitment of motor units, pH, ion concentrations, and motor unit firing rate[15,17,33]. During severe hypoxic conditions, greater increases in sympathetic outflow, muscle sympathetic nerve activation, neurotransmitter release (epinephrine and norepinephrine), and motor unit excitability occur compared to identical conditions in normoxia[19,20,35]. This greater physiological response would result in a global increase in motor unit action potential conduction velocity, ultimately reflecting in a greater EMG MPF as reported in the present study (Figure 3B). In addition, the reduced oxygen availability increases the recruitment of higher-threshold, less oxidative, motor units which may result in greater EMG MPF compared to Normoxic condition. Therefore, the greater EMG MPF during hypoxia, reflecting differences in motor unit recruitment and sympathoexcitatory physiological responses under Normoxic and Hypoxic conditions while performing the identical 70% 1-RM fatiguing protocol.

Hypoxia-Induced Sympathoexcitatory Response

The combined responses of the SpO_2, StO_2, $Total_{Hb}$, and EMG MPF responses support the occurrence of a hypoxic induced sympathoexcitatory response during lower body exercise to failure under extreme hypoxic condition[15,19,20,36] (Figure 3B, 4, 5A, 5b). Hanada et al. 2003 and Joyner and Casey, 2013 have reported that the stimulation of carotid and aortic chemoreceptors under hypoxic conditions triggers a greater sympathetic response from the central nervous system[19]. In addition, the lower StO_2 in the Hypoxic condition throughout the study indicated a decrease in oxygenation status of the Hb and myoglobin of the vastus lateralis (Figure 5B). The localized, reduced oxygenation during hypoxia has been shown to augment the sympathetic outflow from the central and peripheral nervous system[19,20]. Thus, the neuromuscular and muscle tissue oxygenation physiological responses in the current study indicate the presence of systemic and localized hypoxia which likely resulted in increased efferent and afferent sympathetic activation.

Total Hemoglobin

The $Total_{Hb}$ in the current study did not respond as expected, however, literature examining NIRS responses under hypoxic conditions during high-intensity, large muscle mass movements are limited[37]. That is, we expected

to observe an increase in $Total_{Hb}$ due to hypoxic exposure which has been suggested to reflect blood flow due to the sympathoexcitatory response increasing cardiac output and compensatory vasodilation[19,20,38]. However, $Total_{Hb}$ was lower during the severe Hypoxic condition compared to the Normoxic condition which may reflect competing compensatory mechanisms or motor unit recruitment differences between conditions[16,19,33]. Under hypoxic conditions, there are competing physiological vasodilation or vasoconstriction responses, which under hypoxic conditions, typically result in a net vasodilation in the working skeletal muscle[15,19,20,36,38]. The increased sympathetic response during large muscle mass activities has been suggested to match perfusion and metabolic demand to the active muscle, ultimately vasoconstricting non-working muscles and vasodilating the working muscles to provide adequate oxygenation under hypoxic conditions[35,39]. Under extreme hypoxic conditions, however, some authors have suggested a local vasoconstriction of microcirculation to active muscles which may reduce overall blood flow in favor of greater hyperoxemia[19,38]. For example, Joyner and Casey 2014 suggested that the release of norepinephrine from sympathetic nerves may cause vasoconstriction in active skeletal muscle despite the compensatory vasodilation response, ultimately reducing blood flow to the working skeletal muscle[20]. The methodological limitations of the current study, however, cannot definitively determine if either of these mechanisms occurred, however, the SpO_2, StO_2, and EMG MPF provide strong evidence for a sympathoexcitatory response under severe hypoxic conditions, which may not fully account for the lower $Total_{Hb}$ during severe hypoxia (Figure 3B, 4, 5B). It is plausible, however, that the lower $Total_{Hb}$ may, in part, be due to the high-intensity exercise being performed restricting blood flow through mechanical force[16]. With high-intensity skeletal muscle contractions result in mechanical compression of the veins and arteries, which may be more influential to the $Total_{Hb}$ compared to the compensatory vasodilation under hypoxic conditions than normoxic conditions[16]. That is, the combined effect of mechanical compression and hypoxia may be more influential to $Total_{Hb}$ than the same mechanical forces under normoxic conditions. For example, Hoelting et al. 2001 suggested that increased leg extension force and contraction frequency reduces femoral blood flow to the activate skeletal muscle due to consistent mechanical compression force[40]. A greater buildup of metabolic byproducts during the hypoxic conditions would result in higher threshold motor unit being recruited, ultimately increasing compressive forces. Thus, in the current study, the constant contractions result in greater time under tension during high-intensity leg extension likely resulted in mechanical compression of the vasculature in the leg.

Oxy and Deoxy Hemoglobin Responses to Fatigue

The Oxy_{Hb} and $Deoxy_{Hb}$ exhibited fatigue-related responses beginning at 20% and continuing until 100% of the repetitions to failure during the process of fatigue (Figure 5C, 5D). Specifically, there was a decrease in Oxy_{Hb} with a corresponding increase in $Deoxy_{Hb}$ similarly during the Normoxic and Hypoxic conditions. These findings were similar to Amann et al. 2007 who reported a 12% decrease in Oxy_{Hb} and 14% increase in $Deoxy_{Hb}$ from the vastus lateralis during cyclical, fatiguing exercise[41]. DeLorey et al. 2004 also reported a similar inverse relationship with a decrease in Oxy_{Hb} and increase in $Deoxy_{Hb}$ from the vastus lateralis during step-wise changes in lower body work rate[42]. This inverse Oxy_{Hb} and $Deoxy_{Hb}$ relationship that did not differ in severity under hypoxia which suggested that the fatiguing leg extension muscle actions in the current study required a similar demand of oxygen delivery to the skeletal muscle throughout the process of fatigue. The similarities between the Normoxia and Hypoxia conditions may be attributed to a lack of power to detect changes or that the severity of the exposure (duration and FiO_2), and that the combined differences between the Oxy_{Hb} and $Deoxy_{Hb}$ were detectable when calculating the StO_2. Future research should examine perfusion pressures, duration of exposure, and hypoxemic levels under various fatigue protocols.

Conclusion

In conclusion, there were similar increases in EMG RMS during the Normoxic and Hypoxic conditions which indicated greater muscle excitation was required to maintain adequate force production during the process of fatigue. In addition, the similar patterns of responses as reflected from the decreases in EMG MPF during fatigue indicated a similar buildup of metabolic byproducts under Hypoxia and Normoxia despite the mechanistic differences between conditions. The Hypoxic condition, however, exhibited greater EMG MPF, likely reflecting greater sympathoexcitatory physiological responses under the Hypoxic condition while performing the identical 70% 1-RM fatiguing protocol. The physiological responses in the current study indicated the presence of systemic (SpO_2) and localized hypoxia (StO_2) which likely resulted in increased efferent and afferent sympathetic activation. Due to the methodological limitations, we cannot quantify or determine the differences in sympathetic activation, however, the SpO_2, StO_2, and EMG MPF responses provide strong evidence for a sympathoexcitatory response under severe hypoxia. The pattern of responses for $Total_{Hb}$, Oxy_{Hb} and $Deoxy_{Hb}$ were similar across the Normoxic and Hypoxic conditions. The differences in $Total_{Hb}$ did not respond as expected with the Hypoxic Condition having lower $Total_{Hb}$ than the Normoxic condition. The difference in $Total_{Hb}$ between conditions may be attributed to greater mechanical compression due to the recruitment of higher threshold motor units and/or local vasoconstriction of microcirculation to the active muscles which may reduce overall blood flow in favor of greater hyperoxemia. Future

studies should further examine blood flow, biomarkers, and the metabolic costs associated with fatiguing, high-intensity muscle actions during acute hypoxic exposure.

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Ethics approval

The study was approved by the University's Institutional Ethical Review Board and aligned with the principles outlined within the Declaration of Helsinki (approval no. 1482628-1)[26].

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