Neuromuscular changes following simulated high-intensity cycling performance in moderate hypoxia

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Abstract

The purpose of this study was to determine whether central activation (CA) is reduced following a simulated 20-km cycling time trial (20TT) under normoxic and hypoxic conditions. It was hypothesized that CA, maximal voluntary contraction (MVC), and peripheral variables would become reduced during the 20TT exercise under both normoxic and hypoxic conditions, but to a greater extent under the hypoxic condition. Eight experienced male cyclists performed two simulated 20TTs in random order in a hypoxic chamber at either 15% or 21% fraction of inspired oxygen (FIO2). Using the interpolated twitch technique during MVC of the quadriceps, measurements were collected before the exercise, and at 1, 2, 3, and 4 minutes postexercise. The CA values at 1, 2, 3, and 4 minutes postexercise were all significantly reduced from the pre-exercise value. Significant decreases were also detected in all four postexercise MVC measurements and in the third and fourth peak twitch force (PTF) measurements. All four postexercise MVC measurements were significantly decreased. These findings suggest that CA, MVC, and PTF values were significantly reduced and remain reduced at 4 minutes following a self-paced, simulated endurance cycling performance. However, the hypoxic condition had no effect on CA, MVC, or peripheral variables when compared with the normoxic condition.

Keywords: Central activation; Fatigue; Hypoxia; Interpolated twitch technique; Maximum voluntary contraction

Introduction

Previous studies have used central activation (CA) to quantify brain’s response to high-intensity exercise. The CA is the ability to voluntarily activate the neuromuscular system in response to a given quantity of exercise. The muscle1 or nerve2 can be electrically stimulated using a procedure called the interpolated twitch technique (ITT)3 to determine CA and assess “central” fatigue. The physiological mechanism(s) of fatigue is dependent on many different factors, including muscle fiber type, contraction type, exercise intensity, and duration.4,5 Thus, because of these factors it is not possible to generalize results from one study to another to determine the precise mechanism of fatigue and task failure.

In general, studies using long-duration, high-intensity, running exercise have found greater changes in CA and peripheral factors than studies using short-duration, low-intensity, low-impact exercise.4–10 In comparison to studies using isometric, isokinetic, constant load cycling, and running-type exercise, few studies have used self-paced [time trial (TT)] cycling exercise.2,9,11–13 Self-paced exercise allows the participant to use a pacing strategy to try to complete the exercise in the fastest possible time to achieve maximal performance.14 Millet et al found only small changes in the maximal voluntary contraction (MVC) and no changes in CA less than 30 minutes following a 140-km road cycling race.9 By contrast, Lepers et al found an 18% decrease in MVC...
and an 8% decrease in CA following 5 hours of constant load cycling at 55% VO\textsubscript{2max}.\textsuperscript{7} In comparison, Sahlin and Seger found a 34% decrease in MVC, but they did not measure CA in response to 40 minutes of cycling at 75% VO\textsubscript{2max}.\textsuperscript{15} Of the cycling studies that used the ITT,\textsuperscript{7} none have used an intensity and duration equivalent of a 20-km cycling TT (20TT) at race pace.\textsuperscript{2,7,9,11,13,16,17} A 20TT was chosen for our study because the bioenergetics of the activity as well as the pacing strategies and mechanisms of fatigue change significantly between long- and short-duration (e.g., 5-km TT) exercise.\textsuperscript{2,5,18} In addition, 20TT performance of competitive cyclists has been found to be a reliable and valid measure of cycling performance.\textsuperscript{19}

A number of factors have been shown to alter central and peripheral fatigue during exercise including hypoxia, hyperthermia, and hypoglycemia.\textsuperscript{20} Of these factors, exercise during acute hypoxia has generated conflicting results.\textsuperscript{2,21–26} The potential reasons for this may be related to the severity of the hypoxic conditions, a variety of exercise paradigms being used, such as high-intensity isometric and isokinetic contractions,\textsuperscript{26} middle distance exercise,\textsuperscript{7} prolonged endurance performance,\textsuperscript{13,17} or possibly how central and peripheral fatigue were quantified (oxygenation, electromyography, MVC, ITT, and transcranial magnetic stimulation).

Several research studies suggested that acute hypoxia may affect CA. However, the results of these studies were unable to show an effect of decreased central drive during acute hypoxia on MVC, indicators of peripheral fatigue, or voluntary activation when compared with normoxia.\textsuperscript{21,25–27} The relatively short exercise duration in these studies is one explanation as to why hypoxia had no effect on the measured variables. By contrast, Goodall et al found that following a constant load cycling, MVC and voluntary activation were reduced to a greater extent in severe hypoxia (3800 m) than in normoxia.\textsuperscript{24} Although interesting, constant load cycling at 3800 m is very different from conditions normally faced by road cyclists in North America. An altitude of 2750 m (Alberta and Colorado) is a more realistic training and racing altitude for cyclists in North America. In addition, Amann et al found impairment in the peripheral measures as well as in MVC force indicating peripheral muscle fatigue when combined with the fact that voluntary activation was similar across conditions.\textsuperscript{21}

Current literature has indicated that an acute hypoxic environment causes alterations in motor cortex excitability as a result of modified motor-cortical ion channel function.\textsuperscript{26} Szubski et al found that acute hypoxia resulted in significant reductions in the resting motor threshold of participants, but this reduction did not result in changes in MVC measurements.\textsuperscript{26} In addition, another hypothesis suggests that under hypoxic conditions the brain experiences reduced output from the motor cortex because of reduced cerebral O\textsubscript{2} delivery.\textsuperscript{24}

To further investigate the influence of hypoxia on both central and peripheral variables associated with fatigue under conditions very similar to those routinely experienced by competitive cyclists, the ITT was used to investigate the effects of a simulated 20TT during normoxia (P\textsubscript{b}: 710 mmHg; 21% FIO\textsubscript{2}) and normobaric hypoxia (P\textsubscript{b}: 710 mmHg; 15% FIO\textsubscript{2}). It was hypothesized that hypoxia would cause significant decreases in CA, MVC, and peripheral measures when compared with normoxia and that CA and MVC would begin to recover within 4 minutes following exercise cessation.\textsuperscript{1} Based on a review of the current literature, to the authors’ knowledge, this is the first study to measure the effects of acute hypoxia during a simulated 20TT on peripheral variables such as time to peak twitch (TPT), peak twitch force (PTF), half relaxation time (HRT), and rate of force development (RFD) in addition to CA and MVC.

**Methods**

**Participants**

This study was approved by the institutional university Ethics Review Board. After a complete description of the study, each participant completed a written informed consent and a Physical Activity Readiness Questionnaire before they participated in the study.

Participants were chosen on a volunteer basis, and data were collected on eight competitive male endurance cyclists between the ages of 18 and 35 (mean: 29.5 ± 4.9 years). However, 10 participants volunteered initially but two were removed from the study because their MVC values obtained during ITT measurements were less than their initial MVC measurement before stimulation.\textsuperscript{28} Patient’s maximal oxygen consumption (mean: 52 ± 7.3 mL/kg/minute) and height (mean: 177.6 ± 6.8 cm) and body mass (mean: 76.4 ± 6.5 kg) were recorded. All data collection occurred during the off-season so as not to interfere with the participant’s competitive season. All cyclists competed in at least one race in the past year, were currently in off-season long slow distance training at the time of data collection, and were accustomed to performing to maximal levels of exertion.

**Procedure**

A familiarization trial was performed for at least 1 week but no more than 2 weeks before the first TT. During the familiarization trial, participants became acquainted to the laboratory environment, completed a VO\textsubscript{2max} test on the Velotron cycle ergometer (Velotron; RaceMate, Seattle, WA, USA) that was used during the 20TT.

The participants performed a 20TT under normoxic conditions (21% FIO\textsubscript{2}; P\textsubscript{b}: 710 mmHg) or during normobaric hypoxic conditions (15% FIO\textsubscript{2}; simulated 2750 m) in a randomized order, separated by 48 hours between trials. An FIO\textsubscript{2} equal to 15% (2750 m) was chosen because 2750 m is equivalent to the highest elevations normally experienced by cyclists in North America. Both trials were completed in a 2.4 × 2.4 × 2.4 m\textsuperscript{3} Hypoxic environmental chamber (Hypoxico, New York, NY, USA). This environmental chamber increases N\textsubscript{2} content in the chamber in order to decrease the O\textsubscript{2} content. Therefore, the FICO\textsubscript{2} remains approximately equal to 0.04%. The trials were completed at the same time of day to account for changes in circadian rhythms. The ITT measurements were performed inside the chamber following
the same protocol in which both trials and participants were blind to the oxygen content in the chamber (generators were running but disconnected from the chamber during the normoxia trial).

Before the 20TT, surface electrodes needed to stimulate the muscle were placed on the patient's quadriceps over the rectus femoris. An athletic therapist was present to locate the rectus femoris to ensure proper placement of the recording electrodes (see description and placement in the following section). The rectus femoris was chosen for analysis because unlike the other muscles in the lower extremities, its activation level is not affected by pedaling rate. This is important when investigating fatigue because as the participant becomes fatigued, pedaling frequency decreases. In addition, the rectus femoris muscle is activated to at least the same degree as the other muscles of the quadriceps during the cycling exercise. Because of this, it should be representative of the fatigue experienced in the leg as a whole. During the ITT, the entire quadriceps was not stimulated to avoid stimulation of opposing muscle groups.

The participant then warmed-up for 5 minutes on the Velotron cycle ergometer inside the environmental chamber. Two minutes subsequent to the warm-up, ITT was performed to determine twitch force characteristics of the quadriceps in a prefatigued condition. During this test the participant was placed in a sitting position on the knee extension chair with the knee and hip positioned at approximately 90° of flexion and strapped down at the hips with their arms crossed at their chest. A second strap secured the ankle to the force transducer, which was fastened with a cable to the back of the chair at the same level as the ankle (i.e., parallel to the floor). Maximal voluntary and twitch forces of the quadriceps were measured during the ITT protocol using a calibrated PT4000 500-lb force transducer (Precision Transducers, Auckland, New Zealand). Dura-Stick II self-adhesive 5-cm square electrodes were used to deliver two 100-µs width square wave pulses (doublets) from a constant current stimulator (Digitimer DS7AH) triggered by a PowerLab 16/30 stimulator panel and separated by 10 ms. The electrodes were placed over the rectus femoris, with the anode placed distally (5 cm above the knee), and the cathode placed proximally over the superior aspect of the rectus femoris (near the superior motor point). To determine the stimulus intensity that each participant required to reach maximum twitch force, the amperage of the stimulus was initially set at 250 mA and pulses were delivered to the resting muscle; the stimulus was increased by 50 mA until a plateau in maximum twitch force was achieved. All eight participants reached a plateau in twitch force.

Once the maximum twitch force was determined, the participant rested for 2 minutes. The participant then performed an MVC of the quadriceps muscle group for 5 seconds. During data analysis, this MVC measurement was compared with the MVC when the participant was expecting a stimulus, thus ensuring that the participant reached their true MVC during the ITT protocol. Two minutes of rest followed this contraction. Following the rest period, three ITT measurements separated by 2 minutes of rest were collected. It should be noted that none of these pre-exercise ITT measurements were significantly different from one another (p > 0.05). The peak value of these three measurements was used for comparison against the postexercise CA measurements. The participant then completed a 20TT on the Velotron cycle ergometer at a self-selected power output in the fastest time possible. The 20-km course was at a constant 2.5% grade and took an average of 51.7 ± 10 minutes during normoxia and 58.6 ± 10.4 minutes during hypoxia. The participant was blind to the time and distance and was instructed to complete the trial in the fastest possible time. The ITT measurements were performed following the 20TT at 1, 2, 3, and 4 minutes postexercise (in the knee extension chair) to observe the changes in CA ratio during recovery after each condition.

The peripheral effects of exercise were measured by calculating PTF, TPT, HRT, and RFD.

The degree of CA was calculated using the following equation:

\[ CA = \left[ 1 - \left( \frac{\text{ITT}_{\text{force}}}{\text{IT}_{\text{force}}} \right) \right] \times 100 \]

where \( \text{ITT}_{\text{force}} \) is the interpolated twitch force and \( \text{IT}_{\text{force}} \) is the control twitch force following ITT.

**Data analysis**

Group mean ± standard deviations were calculated for all dependent variables. SPSS 16 (IBM, North Castle, NY, USA) was used to complete the statistical analysis using a two-way repeated measures analysis of variance (ANOVA) model. This test was chosen to compare preintervention and post-intervention CA values in each of the groups as well as to compare the two environmental conditions (normoxia and hypoxia). The significance level was set at \( p < 0.05 \). Sphericity was tested, and if violated the Greenhouse–Geisser correction was applied. Fisher least significant difference (LSD) test was used as a post hoc analysis to determine where the differences occurred if a significant main effect was found.

**Results**

Central (CA) and peripheral (MVC, HRT, TPT, PTF, and RFD) variables were collected from the eight participants in the hypoxic and normoxic environments. The significance level was \( p = 0.001 \) [\( F(2.08, 14.56) = 12.66; \text{power} = 0.99 \) and \( n^2 = 0.64 \)]. Pairwise comparisons from the LSD test revealed significant differences between pre-exercise MVC values and values obtained at 1, 2, 3, and 4 minutes postexercise, but no differences were found between 1, 2, 3, and 4 minutes postexercise (Table 1 and Fig. 1).

In normoxia, CA was found to be significantly different between the five time intervals (Table 1). The significance level was \( p = 0.028 \) [\( F(4, 24) = 3.29; \text{power} = 0.755 \) and \( n^2 = 0.354 \)]. The post hoc examination of the data revealed significant differences between pre-exercise CA values and values obtained 1, 2, 3, and 4 minutes postexercise, but no
Table 1
Mean and standard deviation of central activation, maximal voluntary contraction, and peak twitch force at pre-exercise and postexercise minutes 1, 2, 3, and 4 under normoxic and hypoxic conditions.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normoxic</th>
<th>Hypoxic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post 1</td>
</tr>
<tr>
<td>CA (%)</td>
<td>93.9</td>
<td>84.7 (13.2)*</td>
</tr>
<tr>
<td>%Δ</td>
<td>—</td>
<td>9.7</td>
</tr>
<tr>
<td>MVC (N)</td>
<td>804.6</td>
<td>699.6 (205.9)*</td>
</tr>
<tr>
<td>%Δ</td>
<td>—</td>
<td>13.0</td>
</tr>
<tr>
<td>PT (N)</td>
<td>536.8</td>
<td>481.0 (106.5)</td>
</tr>
<tr>
<td>%Δ</td>
<td>—</td>
<td>10.4</td>
</tr>
</tbody>
</table>

n = 8 (for normoxic CA, n = 7).
* Significant difference from pre-exercise (p < 0.05).
CA = central activation; MVC = maximal voluntary contraction; PT = peak twitch.

No significant differences were found between normoxic and hypoxic for any of the peripheral variables measured (PTF, TPT, HRT, or RFD). However, the 20TT took a significantly (p < 0.05) longer time to complete in the hypoxic (58.6 ± 10.4 minutes) than in the normoxic (51.7 ± 10 minutes) environment. In addition, average power output was higher under the normoxic condition (237.4 ± 10.2 wattsS\text{AVG}) than the hypoxic condition (198.7 ± 11.5 wattsS\text{AVG}). A significant difference was also found in PTF during the normoxic condition (Table 1 and Fig. 3). In addition, no significant differences were found between the three pre-exercise MVC measurements (p < 0.05), the largest of which was used for the pre-exercise ITT calculation.

Discussion

The purpose of this study was threefold: first, to determine whether central fatigue exists and the extent of central fatigue in experienced cyclists following a 20TT; second, to determine whether mild-to-moderate hypoxia (15% FIO\textsubscript{2}; simulated 2750 m) has an effect on CA and MVC (the rectus femoris muscle) following a 20TT; and third, to establish whether it is necessary to perform ITT measurements immediately postexercise, and what is the effect of short-term recovery (4 minutes) on CA and MVC. These findings are

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**Fig. 1.** Maximal voluntary contraction (MVC) percentage change from pre-exercise to postexercise at minutes 1, 2, 3, and 4 under normoxic and hypoxic conditions. The percentage reduction is the mean percentage reduction ± standard deviation. * Significant difference in values before and after the exercise (p < 0.05).

**Fig. 2.** Central activation (CA) percentage reduction from pre-exercise to postexercise at minutes 1, 2, 3, and 4 under normoxic and hypoxic conditions. The percentage reduction is the mean percentage reduction ± standard deviation. * Significant difference in values before and after the exercise (p < 0.05).
difference in values before and after the exercise (hypoxic conditions. 20TT to postexercise measurements at minutes 1, 2, 3, and 4 under normoxic and hypoxic conditions. Values are the mean peak twitch force ± standard deviation. * Significant difference in values before and after the exercise (p < 0.05).

important to better understand the effects of central and peripheral fatigue during TT cycling exercise in normoxic and hypoxic conditions, and to determine whether ITT data collected within 1–4 minutes postexercise are sufficient to assess CA. In agreement with previous research, CA failure occurred following intense, self-paced endurance cycling exercise (i.e., simulated 20TT) as there was a statistically significant difference between pre-exercise and postexercise values during both normoxia and hypoxia. Although no statistically significant differences were found in CA and MVC values between mild hypoxic (15% FIO2) and normoxic (21% FIO2) environments postexercise, a novel finding in this study was that CA did not recover within 4 minutes of exercise cessation.

One focus in this study was to use a simulated exercise performance (20TT) to assess its effects on CA. Of the ITT cycling papers available in the cited literature, none have looked at CA after a simulated 20TT race at a self-selected power output. We felt that fatigue during and following a 20TT should be investigated as this is a very common distance used in training and evaluating road cyclists. The results of this research indicate that significant decreases in CA and MVC from pre-exercise to postexercise measurements were found at all four postexercise measurements (Figs. 1 and 2).

These results are consistent with other studies of similar duration and intensity. Booth et al showed that MVC was reduced 19% after cycling to exhaustion (72 ± 4 minutes) at 75% of peak oxygen consumption. Lepers et al found a significant 13% decrease in CA of the quadriceps following 30 minutes of cycling exercise at 80% peak oxygen consumption. However, a novel finding of our study was that CA remained significantly decreased from 1 to 4 minutes post-exercise (Fig. 1). No significant differences were detected between the four postexercise CA measurements. The MVC value also remained reduced 4 minutes postexercise (Fig. 2). Each of these postexercise MVC measurements was found to be significantly different from the pre-exercise measurement but not from one another. Limited research is available to show how long a diminished CA or neuromuscular response to intense cycling exercise will last. With the exception of Behm and St-Pierre, all of these studies collected data immediately postexercise but not again until at least 15 minutes postexercise. Behm and St-Pierre did collect data immediately postexercise as well as at 30 seconds, 1, 2, 5, and 10 minutes postexercise. Their results showed a steady increase in MVC force following exercise, indicating neuromuscular recovery. Unfortunately, they did not report CA data to quantify the contribution of central fatigue. As a result of the limited data availability, it is difficult to determine the extent of recovery of the neuromuscular system, and CA in particular, in response to intense cycling exercise. Therefore, indications from our study suggest that it is not necessary to assess CA immediately postexercise to determine central fatigue, but instead to test at a consistent period (e.g., 2 minutes) postexercise.

The trend of postexercise MVC measurements in our study differs from those of Behm and St-Pierre for two possible reasons: first, the rest periods were 60 seconds between MVCs and not long enough between ITT measurements for complete adenosine triphosphate–phosphocreatine phosphorylation following the intense 20TT; second, even though blood lactate levels were not collected in our study, we hypothesize that because our study was of greater intensity and duration, greater amounts of lactic acid were likely produced, causing H+ ion concentrations to continue to increase postexercise. Sahlin and Seger found that following 6–11 minutes of cycle ergometer exercise, blood lactate levels continued to rise during recovery and did not peak until 5–8 minutes post-exercise, and therefore, it is likely that this may have also happened in our study which likely contributed to the reduced MVC measurements during the 4 minutes postexercise. Some researchers have hypothesized that either blood plasma acidosis or high blood lactate may alter muscle afferent feedback, causing an increased perception of effort and decreased motor drive from the central nervous system. Amann et al also suggested that feedback through Group III and Group IV afferents determines force output as well as central motor drive. This afferent feedback may have caused CA values to continue to remain reduced 4 minutes postexercise.

In addition to CA and MVC, peripheral variables were also measured. No significant changes were found in any of the peripheral variables except PTF. The PTF was significantly reduced from pre-exercise to postexercise values at 3 and 4 minutes (Fig. 3). These results are somewhat different from Behm and St-Pierre who found changes in time to PTF, HRT,
and PTF after exercise.4 These phenomena could be explained by the coexistence of twitch potentiation and fatigue as suggested by Rassier and MacIntosh39; however, the reduction in time-to-peak tension and HRT that normally accompanies postactivation potentiation was not present.40 The use of patients with a potentially high percentage of slow twitch muscle reduces the effect of postactivation potentiation.25 Our results suggest that there is limited impairment of the muscle itself during the first 2 minutes of recovery, and only small impairments at 3 and 4 minutes. The results also indicate that the changes in MVC at 1 and 2 minutes postexercise were consistent with central fatigue because no other changes in peripheral variables were significant.

Hypoxia and fatigue

Recent research has investigated that acute hypoxia will alter muscular force generation, but none, to the best of the our knowledge, have used ITT to examine the effects of a simulated 20TT high-intensity cycling exercise in a hypoxic environment.2,21–25 In addition, there is conflicting evidence on whether hypoxia attenuates CA.2,21–26 When we compare the hypoxic and normoxic environments in the present study, large differences in percent change at each of the four time points become apparent in MVC and CA between the two conditions (Figs. 1 and 2). Although these decrements in CA and MVC during hypoxia are large compared with normoxia, they are not statistically significant. Consistent with a number of recent studies, hypoxia does not seem to have an effect on CA or MVC from pre-exercise to postexercise.2,21,23

The results of previous research indicated that acute hypoxia can cause changes in motor cortex excitability but the authors’ found that it had no effect on voluntary activation or MVC.2,25–27 In light of this research, we hypothesized that because of the increased intensity and duration during a 20TT, we would see greater changes in CA% and MVC force under hypoxic conditions similar to Goodall et al.24 However, performance times were significantly (p < 0.05) faster under normoxic conditions compared with hypoxic conditions. These results indicate that the ride was more difficult to complete in the hypoxic condition. However, based on the variables examined, we cannot infer that the patients experienced greater fatigue due to the hypoxic condition. In addition, any changes seen in CA between hypoxia and normoxia (Fig. 4) could be due to the extended exercise time in the hypoxic condition, as opposed to the hypoxic environment itself.

Limitations

Potential limitations to this study are the following: (1) It is possible that some of the participants may not have given a maximal effort during the TT. However, to alleviate this concern, competitive endurance cyclists were used during their “off-season” (VO_{2max} = 52 ± 7 mL/kg/minute) who are accustomed to intense exercise and thus would be capable of exercising until completion of the simulated 20TT. (2) Although food logs were not collected as part of this study, the cyclists were instructed to eat the same foods before both trials. (3) We did not extend our postexercise measurement protocol beyond 4 minutes to see how long CA is diminished following a simulated 20TT exercise performance.

Conclusion

As hypothesised in this study, the 20-km TT did cause reductions in CA of the quadriceps muscle, and these reductions remained for the 4 minutes following exercise cessation. From a research perspective it is important to know that CA and MVC will not significantly recover within 4 minutes following endurance exercise. Traditionally, ITT studies have used the quadriceps muscle during isokinetic (Cybex) exercise, and therefore it was easy to measure MVC and CA immediately postexercise. By performing sequential measurements for 4 minutes postexercise, we have shown that the proximity of the postexercise measurement to exercise is less important provided it is performed at a consistent time. It appears that consistency of measurement time is more important, which has important implications for future research in this area. Furthermore, ITT is an acceptable technique that can be used following an actual performance race to assess both central and peripheral physiology.

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