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Does altitude level of a prior time-trial modify subsequent exercise performance in hypoxia and associated neuromuscular responses?

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Keywords
Altitude, cycling time-trial, hypoxia severity, neuromuscular fatigue, post-exercise recovery.

Abstract
We examined the influence of prior time-trials performed at different altitudes on subsequent exercise in moderate hypoxia and associated cardiometabolic and neuromuscular responses. In normobaric hypoxia (simulated altitude 2000 m; FiO₂: 0.163), 10 healthy males performed (1) an incremental test to exhaustion (VO₂max_2000) and (2) a test to exhaustion at 80% of the power output associated to VO₂max_2000 for a reference time (947 ± 336 sec). Thereafter, two sessions were conducted in a randomized order: a cycle time-trial corresponding to the reference time (TT₁) followed 22 min later (passive rest at 2000 m) by a 6-min cycle time-trial (TT₂). TT₁ was either performed at 2000 or 3500 m (FiO₂: 0.135), while TT₂ was always performed at 2000 m. As expected, during TT₁, the mean power output (247 ± 42 vs. 227 ± 37 W; P < 0.001) was higher at 2000 than 3500 m. During TT₂, the mean power output (256 ± 42 vs. 252 ± 36 W) did not differ between conditions. Before and after TT₁, maximal isometric voluntary contraction torque in knee extensors (pooled conditions: -7.9 ± 8.4%; P < 0.01), voluntary activation (-4.1 ± 3.1%; P < 0.05), and indices of muscle contractility (peak twitch torque: -39.1 ± 11.9%; doublet torques at 100 Hz: -15.4 ± 8.9%; 10/100 Hz ratio: -25.8 ± 7.7%; all P < 0.001) were equally reduced at 2000 m or 3500 m. Irrespective of the altitude of TT₁, neuromuscular function remained similarly depressed after TT₁ both before and after TT₂ at 2000 m. A prior time-trial performed at different altitude influenced to the same extent performance and associated cardiometabolic and neuromuscular responses during a subsequent exercise in moderate hypoxia.

Introduction
Fatigue is a disabling symptom in which physical and cognitive function is limited by interactions between performance fatigability (i.e., the decline in an objective measure of performance such as contractile function and/or muscle activation) and perceived fatigability (i.e., sensations that regulate the integrity of the performer) (Enoka and Duchateau 2016). Under this frame, prior exercise that challenges multiple physiological (i.e., cardiorespiratory, metabolic, neuromuscular) regulatory systems and thereby modifies effort perception and influences performance during the completion of a subsequent exercise bout (Karlsson et al. 1975; Hogan and Welch 1984; Amann 2011). Currently, there is renewed interest in the methodological approach that consists in manipulating the amount/severity/type of pre-established fatigue levels through the induction of localized fatigue of specific muscle groups (e.g., neuromuscular electrical stimulation protocol of the quadriceps; Hureau et al. 2014) or following the completion of an initial whole-body exercise bout (Amann and Dempsey 2008; Girard et al. 2015).

In one study, Amann and Dempsey (2008) demonstrated that the induction of different levels of...
pre-existing locomotor muscle fatigue [following the completion of constant-load cycling of ~10 min at 
~347 W (83% of peak power output) vs. ~276 W (67%)] had a substantial dose-dependent influence (~6%) on 
performance time during a subsequent (i.e., 4 min later) 5-km time-trial (TT). Specifically, the higher the level of 
pre-existing locomotor muscle fatigue, as assessed via pre- 
and post-exercise magnetic femoral nerve stimulation, the lower the central motor drive and power output during 
the subsequent TT. A striking finding, however, was that the end of the subsequent TTs coincided with an almost 
identical level of peripheral fatigue, independent of the 
level of pre-existing fatigue and/or the marked differences in exercise performance. Authors claimed that feedbacks 
from fatiguing muscles play an important role in the 
determination of central motor drive and force output, 
and that the development of peripheral muscle fatigue is 
confined to a certain level (also referred as a “critical” 
threshold), so as not to surpass a sensory tolerance limit.

Increasing the degree of environmental stress such as 
the ambient hypoxia severity [i.e., a reduction in environ-
mental oxygen (O2) availability] is known to exacerbate exercise-induced demands (and thereby recovery require-
ments) in turn leading to excessive fatigue levels (Amann 
2011). With this in mind, we recently manipulated hypoxia severity during an initial set of repeated sprints 
(eight 5-sec sprints; 30 sec rest) under normoxia, moderate, 
or severe hypoxia and examined the effects on perfor-
mance and lower limbs neuromuscular activity during a 
subsequent set (four sprints) performed 6 min later in 
normoxia (Girard et al. 2015). Despite sprint performance 
and neural alterations were largely influenced by the 
hypoxia severity in the initial set, hypoxia had no residual 
effect during the subsequent normoxic set (i.e., similar 
fatigue pattern across conditions), yet the absence of nega-
tive “carry-over” effects would still need to be confirmed 
under hypoxic conditions. In addition, central and 
peripheral mechanisms underpinning neuromuscular fati-
gue, likely influenced by hypoxia severity, were not 
assessed in this latter study.

To date, the majority of studies investigating the inter-
play between central and peripheral mechanisms of fati-
gue through hypoxic perturbations have employed exhaustive, whole-body continuous exercise (Amann et al. 
2006a) and repeated, brief submaximal (Millet et al. 2009, 
2012; Goodall et al. 2010) or maximal (Christian et al. 
2014) isometric contractions. In comparison to exercis-
ing at fixed work rate to fatigue (i.e., open-loop design), self-
paced exercise (i.e., time-trial) of predetermined dura-
tion/distance/work (i.e., closed-loop designs) involves 
pacing strategies for achieving optimal performance. It 
well described that TT performance and associated physi-
ological responses vary between hypoxic and normoxic 
conditions (Amann et al. 2006b; Périard and Racinais 
2016; Saugy et al. 2016). The extent to which the neu-
romuscular consequences also differ between normoxic and 
a range of moderate-to-severe hypoxic conditions is not 
clear.

Our intention was therefore to examine the influence 
of prior TT performed at different altitudes on subse-
quent exercise performance in moderate hypoxia and 
associated cardiometabolic and neuromuscular responses. 
We anticipated that severer hypoxia during a prior TT 
would exaggerate the demands placed on various physio-
logical regulatory systems, in turn resulting in lower 
power output. We were unsure, however, whether specific 
recovery requirements and fatigue-related residual or 
“carry-over” effects would differently influence perfor-
mance and associated cardiometabolic and neuromuscular 
responses during a subsequent exercise performed in same 
moderate hypoxia.

Methods

Participants

Ten healthy men (Mean ± SD: age 34.4 ± 6.8 years; 
height 180.4 ± 7.7 cm; body weight 78.6 ± 10.9 kg; body 
fat 15.0 ± 5.9%) volunteered to participate in the study. 
All participants were born and raised at <1000 m and 
had not traveled to elevations >1000 m in the 3 months 
prior to investigation. They gave their informed, written 
consent preceding the commencement of the experiment. 
Experimental protocol was conducted according to the 
Declaration of Helsinki for use of Human Subjects and 
approved by the Ethics Committee of Valais, Switzerland 
(CECVEM 007/10).

Experimental design

Participants visited the laboratory on two occasions, sepa-
rated by 3–7 days, before the two main experimental tri-
als. During the first preliminary visit, they were initially 
familiarized with neuromuscular testing and performed 
an incremental test in normobaric hypoxia at a simulated 
alitude of 2000 m (FiO2 0.163) for maximal oxygen 
uptake determination (VO2max_2000). During a subsequent 
preliminary visit, again at 2000 m, participants cycled at 
constant workload (80% of the power output associated 
with their VO2max_2000: 245 ± 42 W) to exhaustion 
(Tlim = 947 ± 336 sec). Exercise was terminated when 
pedaling rate dropped below 60 rpm for >5 sec (exhaustion). 
Thereafter, two sessions were conducted in a ran-
donized order: a cycle time-trial (TT1) with Tlim as 
individualized reference duration followed 22 min later 
(passive rest at 2000 m) by a 6-min cycle time-trial
(TT2). TT1 was either performed at 2000 or 3500 m (FiO2 0.135), while TT2 was always performed at 2000 m. After preliminary tests, the duration of 22 min between the two time-trials was chosen as a good trade-off between the times needed to allow significant perceptual recovery from TT1, with the parallel methodological requirement to keep recovery time short enough for a partial recovery of neuromuscular function, both likely to influence subsequent efforts (Minett and Duffield 2014).

Testing procedures

Testing was conducted in a normobaric hypoxic chamber (SL - 400, ATS, Sydney, Australia) of ~30 m³ (2.4 m × 5.0 m × 2.5 m) maintained at a constant temperature of ~25°C and ~40% relative humidity. Prior to each cycling bout, FiO2 (Oximeter Gox 100, Greisinger, Germany) as well as room temperature (°C) and humidity (%) were measured. This chamber allowed modifying the simulated altitude between 3500 and 2000 m in less than 5 min. All tests were performed on a computer-controlled electrically braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands). Participants performed their trials at the same time of the day (±1 h) and wore similar sports gear (cycling shoes, short and jersey). They were instructed to refrain from any strenuous physical activity and maintain their normal diet (i.e., avoiding any nutritional supplements, caffeine, or alcohol consumption) and sleeping habits (≥7 h/night) for the 24 h before each test. They were encouraged 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 at the start of exercise.

Preliminary sessions

During the first preliminary session, participants’ anthropometrical parameters (body height, body weight) and body composition (Bod Pod, Cosmed US Inc., Concord) were first measured. Then, they were requested to perform maximal voluntary contractions (MVC) of the knee extensors until they felt accustomed to the equipment; the coefficient of variation in three successive trials was <5%. Afterwards, the optimal stimulation intensity for one single stimulus was determined by increasing the current gradually from 10 mA until there was no further increase in peak twitch torque and concomitant maximal M-wave amplitudes. This intensity was further increased by 30% (e.g., supramaximal) and subsequently maintained for the entire session. Thereafter, participants performed the complete procedure of neuromuscular tests (see below; total duration ~3 min). Then, they entered the normobaric hypoxic chamber at a simulated altitude of 2000 m, and after ~30 min rest, the incremental test was undertaken. The protocol consisted of cycling for 5-min warm-up at 60 W, then at a starting power output of 90 W and increasing every minute by 30 W until volitional exhaustion, despite strong verbal encouragement. The highest 30 sec average value of VO₂ (see below) was defined as VO₂max_2000.

The second preliminary session was conducted inside the hypoxic chamber at a simulated altitude of 2000 m as follows: (1) rest in a seated position for 30 min; (2) Participants cycled at constant workload (80% of the power output associated with their VO₂max_2000) to exhaustion.

Experimental sessions

Each of the two main experimental session were conducted as follows: (1) rest in a seated position for 30 min inside the chamber, while participants were instrumented; (2) a 10 min cycle warm-up at 60 W (pedaling rate 70–80 rpm); (3) neuromuscular tests before TT1 (pre-TT1); (4) 5-min rest; (5) cycle time-trial for Tim duration (TT1); (6) 22-min rest at 2000 m including neuromuscular tests ~1 min after TT1 (post-TT1) and ~2 min before TT2 (pre-TT2); (7) 6-min cycle time-trial (TT2); (8) neuromuscular tests ~1 min after TT2 (post-TT2).

Cycling time-trials

During each time-trial, participants were asked to maintain the highest sustainable effort, while receiving strong verbal encouragements. The starting work rate was 80% of individual power output at VO₂max_2000 for all exercise trials. Participants were informed of every minute elapsed during time-trials. They were able to continuously self-regulated power output (±10 W). This research was run in double-blinded, controlled manner. Participants were told that the overall goal of the experiment was to test the reproducibility of their cycling time-trial performance in hypoxia, yet without any accurate information about the randomized simulated altitude levels inside the hypoxic room that were set and controlled by an independent research assistant.

Neuromuscular function

The neuromuscular assessment consisted of a 4-sec MVC of the knee extensors with a superimposed 100 Hz doublet (Db100) applied to the peripheral motor nerve when torque had reached a visible plateau. This was followed after 3 sec by (1) one Db10, (2) one Db100, and (3) three single twitches in a relaxed state (all separated by 3 sec). This neuromuscular testing was conducted three times, while ~60 sec of passive rest separated each MVC. Prior to the
pre-TT₁ neuromuscular assessment, participants were warmed up by completing 5 × 4-sec MVC with progressively increasing subjective effort (starting at 50% of subjective maximal effort with increments of 10%) followed by 2 × 4-sec MVC (separated by 1 min of passive rest).

**Measurements**

**Cardiopulmonary and respiratory responses**

Heart rate (HR), monitored via a wireless Polar monitoring system (Polar Electro Oy, Kempele, Finland) and pulse oxygen saturation (SpO₂), estimated noninvasively via pulse oximetry using an earlobe probe (Nonin, Wristwatch, McAllen), were recorded immediately prior to entering the chamber and every 60 sec during exercise. Rating of perceived exertion (RPE) was obtained using the 6-20 Borg scale. The following respiratory variables and pulmonary gas exchange parameters were measured breath-by-breath at rest and throughout all exercises using the portable analyzer Metamax 3b (Cortex Biophysik, Leipzig, Germany). The following parameters were measured: oxygen uptake [VO₂ (mL kg⁻¹ min⁻¹)], minute ventilation [VE (L min⁻¹)], carbon dioxide production [VCO₂ (L min⁻¹)], respiratory exchange ratio (RER), ventilatory equivalent for O₂ (VE/VO₂), and breathing frequency [BF (breath min⁻¹)]. Finally, a capillary blood sample was taken from the fingertip and analyzed for blood lactate concentration with the Lactate Pro (LT-1710, Arkray, Japan) portable analyzer, 2 min before and exactly 2 min after TT₁ and TT₂.

**Force and electromyographic recordings**

During all neuromuscular assessments, participants were seated upright on a custom-built adjustable chair with the hips and knees flexed at 90° (0° corresponding to full knee extension). Restraining straps placed across the chest and hips secured the participants in the chair to prevent extraneous movement, while the dynamometer (Captels, St Mathieu de Treviers, France) was attached 3–5 cm above the tip of the lateral malleoli.

Electromyographic (EMG) signals of the VL and rectus femoris (RF) muscles (cycling and neuromuscular assessment) were recorded via bipolar Ag/AgCl electrodes (Ambu Blue sensor T; Ambu A/S, Denmark) with a diameter of 9 mm and an interelectrode distance of 25 mm. Before electrode placement, the skin was lightly abraded and washed to remove surface layers of dead skin, hair, and oil. The ground electrode was attached to the right wrist. The position of the EMG electrodes was marked with indelible ink to ensure that they were placed in the same location during subsequent trials. To ensure low levels of movement artifact, electrode cables were fastened to participant’ bodies with medical adhesive tape and wrapped in net. The myoelectric signal was amplified (Octal Bioamp, ML138, ADInstruments, Oxfordshire, UK; input impedance = 200 MΩ, common mode rejection ratio >96 dB, gain = 1000), band-pass filtered (bandwidth frequency = 5 to 500 Hz), digitized (sampling frequency = 2,000 Hz), acquired, and later analyzed (LabChart v7.0, ADInstruments Inc, Oxfordshire, UK) with force signal.

**Femoral nerve stimulation**

A high-voltage (maximal voltage 400 V) constant current stimulator (Digitimer DS7AH; Digitimer, Hertfordshire, UK) was used to deliver square-wave stimuli of 1 ms duration. The femoral nerve was stimulated percutaneously via a 10 mm diameter self-adhesive cathode electrode (Skintact, Austria) pressed manually by the investigator onto the skin at the femoral triangle. The anode, a self-adhesive pad (5 × 10 cm, Medicompex; Ecublens, Switzerland) was applied to the gluteal fold.

**Data analysis**

Power output, cardiorespiratory and pulmonary as well as EMG data were averaged over each entire cycling bout to obtain one value for TT₁ (TT₁₂₀₀₀ vs. TT₁₃₅₀₀) and TT₂ (TT₂₂₀₀₀ vs. TT₂₃₅₀₀).

For each neuromuscular test sequence, voluntary force (MVC force) was recorded over the highest 1-sec plateau preceding the superimposed twitch. The peak potentiated twitch force (i.e., the highest value of twitch tension production, Pt) was determined from the mechanical response of the three evoked twitches (and averaged to obtain one Pt value) and one paired doublet at 10 Hz and 100 Hz (Db10 and Db100, respectively). The peak-to-peak amplitude of the concomitant VL and RF M-waves during the three resting twitches was measured and averaged across the three stimulations to obtain one representative M-wave value. Voluntary activation (VA) was assessed using twitch interpolation and defined as follows: VA (%) = \(((1 - \text{(superimposed Db100 doublet/resting potentiated Db100)}) \times 100)\). From the mechanical response induced by paired high-frequency [100 Hz (i.e., 10-ms interstimulus interval)] and low-frequency [10 Hz (i.e., 100 ms interstimulus interval)] supramaximal electrical stimulation, the low- to high-frequency torque ratio was calculated (10/100 Hz) and used as a surrogate of low- and high-frequency tetanic stimulations (Vergès et al. 2009). For all the neuromuscular parameters, the values of three trials were averaged for subsequent analysis. The reliability of measurements of central and peripheral fatigue specific
to the quadriceps both before and after “fatigue” has been reported elsewhere (Place et al. 2007).

Statistics

Values are expressed as mean ± SD. For TT1 and TT2 separately, paired samples t-tests were used to compare cardiorespiratory and pulmonary and EMG data. Two-way analysis of variance (ANOVAs) [Time (pretests and posttests) × Condition (TT1 and TT2)] were also used to compare neuromuscular responses. To assess the assumptions of variance, Mauchly’s test of sphericity was performed using all ANOVA results. A Greenhouse-Geisser correction was performed to adjust the degree of freedom if an assumption was violated, while post hoc pairwise comparisons with Bonferroni-adjusted P values were performed if a significant main effect was observed. For each ANOVA, partial eta-squared was calculated as measures of effect size. Values of .01, .06, and above .14 were considered as small, medium, and large, respectively. All statistical calculations were performed using SPSS statistical software V.21.0 (IBM Corp., Armonk, NY). The significance level was set at P < 0.05.

Results

Preliminary sessions

At a simulated altitude of 2000 m, the maximal oxygen uptake (VO2max_2000) was 54.9 ± 7.3 mL kg⁻¹ min⁻¹ and peak power output was 306 ± 53 W. Tlim performed at 245 ± 42 W was 947 ± 336 sec.

Prior time-trial (TT1)

Mean power output (247 ± 42 vs. 227 ± 37 W; +8.2 ± 3.6%; P < 0.001) and SpO2 (+11.8 ± 1.9%; P < 0.001) were significantly higher during TT1_2000 versus TT1_3500 (Fig. 1). Although VO2 (+8.9 ± 7.8%; P < 0.01) was higher during TT1_2000 in reference to TT1_3500, RER (−6.1 ± 6.7%; P < 0.05), VE/VO2 (−19.9 ± 8.8%; P < 0.001), and BF (−10.8 ± 12.1%; P < 0.05) were lower (Table 1). Averaged RMS activity both for VL and RF muscles did not differ between TT1_2000 and TT1_3500 and remained unchanged post-TT2. For this parameter, no differences were found between conditions either.

Subsequent time-trial (TT2)

Mean power output (256 ± 42 vs. 252 ± 36 W; +0.9 ± 4.1%; P > 0.05) along with accompanying cardiopulmonary and quadriceps muscle activation responses did not differ between TT2_2000 and TT2_3500 (Fig. 1; Table 2). RPE values measured at the completion of TT2 did not differ (P > 0.05) between TT2_2000 (16.9 ± 1.5) and TT2_3500 (16.3 ± 1.7).

Neuromuscular consequences

Maximal voluntary contractions torque (pooled conditions: −7.9 ± 8.4%; P < 0.01), voluntary activation (−4.1 ± 3.1%; P < 0.05), and indices of muscle contractility (peak twitch torque: −39.1 ± 11.9%; doublet torques at 10 Hz and 100 Hz: −38.7 ± 10.2% and −15.4 ± 8.9%; 10/100 Hz ratio: −25.8 ± 7.7%; all P < 0.001) were equally reduced from pre-TT1 to post-TT1, whereas M-wave characteristics of both VL and RF muscles did not differ (Fig. 2; Table 3). Compared to pre-TT1, MVC force and indices of muscle contractility remained similarly depressed at post-TT2 and did not further decrease at post-TT2 time points. Similarly, voluntary activation values returned to baseline levels at pre-TT2 and remained unchanged post-TT2. This parameter was not influenced by hypoxia severity.

Neuromuscular fatigue characteristics post-TT1 were not influenced by hypoxia severity

The development of neuromuscular fatigue (defined as a decrease in MVC force) from pre-TT1 to post-TT1 did
Figure 1. Averaged power output (A), heart rate (B) and pulse oxygen saturation (C, SpO₂) during the first (TT₁) and during the second (TT₂) cycling bouts. The first cycling bout (TT₁) was performed at 2000 m or 3500 m, while the second bout (TT₂) was always performed at 2000 m. Mean ± SD (n = 10). *Denotes a significant difference between 2000 m and 3500 m (P < 0.05).
dropped similarly by ~4%, signifying that muscle activation became suboptimal at the end of both TT1_2000 and TT1_3500. This was further accompanied by near-identical level of peripheral muscle fatigue, whereas M-wave amplitudes of VL and RF muscles were not affected by the time-trial. This observation confirms that the measured changes in twitch amplitude are mainly due to changes occurring within the quadriceps and that neuromuscular propagation failure might be excluded. An increased rate of accumulation of metabolites (e.g., H+, Pi) can directly inhibit the contractile apparatus or disrupt the Ca2+ release and uptake pathways in the sarcoplasmic reticulum (Allen et al. 2008). A valid method to identify excitac–contraction coupling as a major factor of fatigue is the examination of force loss in response to low- (10 Hz) and high- (100 Hz) frequency paired stimuli (Vergès et al. 2009). As confirmed in this study, low-frequency fatigue has already been identified as one of the main fatigue-causing factor for high-intensity exercises, irrespectively of hypoxia severity (Christian et al. 2014).

### No negative “carry-over” effects on subsequent exercise in moderate hypoxia (TT2)

The duration of the recovery period after prior exercise (i.e., SpO2 recovery to initial values) may at least partially dictate performance during completion of a subsequent exercise bout. In this study, hypoxia severity during TT1 had no negative “carry-over” (or residual) effect on performance fatigability and associated cardiorespiratory and EMG variables during TT2 completed after 22 min of rest in moderate hypoxia. In support, we recently observed that despite differing hypoxic severity levels (FiO2 = 0.133, 0.168 and 0.209) during an initial set of eight 5-sec sprints, performance and neuromechanical patterns did not differ during four additional sprints performed, 6 min later (passive rest), in normoxia (Girard et al. 2015). These results somehow contrast with those of Amann et al. (2007) who indicated that after constant load cycling exercise to exhaustion in normoxia (~171 sec), moderate (~278 sec) and severe (~171 sec) hypoxia (FiO2 = 0.209, 0.15, and 0.10, respectively), hyperoxegenation via acute O2 supplementation (FiO2 = 0.30) caused participants to prolong exercise time at task failure in severe hypoxia (+171%), but not in normoxia and moderate hypoxia. Important methodological differences – that is, the range of SpO2 values for the more severe hypoxic conditions (67 vs. 80%), the nature of the exercise (constant load vs. time-trial), the recovery duration between the two subsequent exercises (few seconds vs. 22 min), the O2 condition of the second exercise bout (hypoxia, normoxia vs. hyperoxia) – therefore only

### Table 1. Average responses to exercise during the first cycling bout at 2000 m (TT1_2000) and 3500 m (TT1_3500)

<table>
<thead>
<tr>
<th>Cardiopulmonary variables</th>
<th>TT1_2000</th>
<th>TT1_3500</th>
<th>ANOVA</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (bpm)</td>
<td>160 ± 8</td>
<td>158 ± 10</td>
<td></td>
<td>0.147</td>
</tr>
<tr>
<td>SpO2 (%)</td>
<td>91.0 ± 3.0</td>
<td>80.2 ± 3.1</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VO2 (mL min⁻¹ kg⁻¹)</td>
<td>44.0 ± 7.3</td>
<td>39.9 ± 5.8</td>
<td></td>
<td>0.005</td>
</tr>
<tr>
<td>VE (L min⁻¹)</td>
<td>115 ± 22</td>
<td>124 ± 19</td>
<td></td>
<td>0.079</td>
</tr>
<tr>
<td>RER</td>
<td>0.95 ± 0.04</td>
<td>1.01 ± 0.08</td>
<td></td>
<td>0.024</td>
</tr>
<tr>
<td>VE/VO2</td>
<td>32.3 ± 4.1</td>
<td>38.6 ± 4.0</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BF (breaths min⁻¹)</td>
<td>40 ± 5</td>
<td>44 ± 5</td>
<td></td>
<td>0.014</td>
</tr>
<tr>
<td>[La] (mmol L⁻¹)</td>
<td>10.2 ± 2.5</td>
<td>10.7 ± 3.1</td>
<td></td>
<td>0.597</td>
</tr>
<tr>
<td>RMS VL (mV)</td>
<td>0.165 ± 0.062</td>
<td>0.159 ± 0.058</td>
<td></td>
<td>0.547</td>
</tr>
<tr>
<td>RMS RF (mV)</td>
<td>0.069 ± 0.027</td>
<td>0.066 ± 0.023</td>
<td></td>
<td>0.435</td>
</tr>
</tbody>
</table>

Mean ± SD (n = 10). HR, heart rate; SpO2; arterial oxygen saturation; VO2; oxygen consumption; VE, minute ventilation; RER, respiratory exchange ratio; BF, breathing frequency; [La], blood lactate concentration; RMS VL and RF, root mean square of vastus lateralis and rectus femoris muscles. Bold values indicate the significant of P ≤ 0.05.

### Table 2. Average responses to exercise during the second cycling bout after completing the first time-trial at 2000 m (TT2_2000) and 3500 m (TT2_3500)

<table>
<thead>
<tr>
<th>Cardiopulmonary variables</th>
<th>TT2_2000</th>
<th>TT2_3500</th>
<th>ANOVA</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (bpm)</td>
<td>163 ± 6</td>
<td>160 ± 9</td>
<td></td>
<td>0.101</td>
</tr>
<tr>
<td>SpO2 (%)</td>
<td>93.0 ± 1.9</td>
<td>91.7 ± 4.0</td>
<td></td>
<td>0.188</td>
</tr>
<tr>
<td>VO2 (mL min⁻¹ kg⁻¹)</td>
<td>43.6 ± 7.9</td>
<td>42.7 ± 6.8</td>
<td></td>
<td>0.957</td>
</tr>
<tr>
<td>VE (L min⁻¹)</td>
<td>116 ± 22</td>
<td>116 ± 14</td>
<td></td>
<td>0.751</td>
</tr>
<tr>
<td>RER</td>
<td>0.78 ± 0.02</td>
<td>0.80 ± 0.03</td>
<td></td>
<td>0.361</td>
</tr>
<tr>
<td>VE/VO2</td>
<td>34.3 ± 4.0</td>
<td>33.4 ± 2.3</td>
<td></td>
<td>0.364</td>
</tr>
<tr>
<td>BF (breaths min⁻¹)</td>
<td>45 ± 7</td>
<td>42 ± 5</td>
<td></td>
<td>0.564</td>
</tr>
<tr>
<td>[La] (mmol L⁻¹)</td>
<td>10.4 ± 2.2</td>
<td>10.4 ± 3.4</td>
<td></td>
<td>0.601</td>
</tr>
<tr>
<td>RMS VL (mV)</td>
<td>0.159 ± 0.049</td>
<td>0.168 ± 0.053</td>
<td></td>
<td>0.214</td>
</tr>
<tr>
<td>RMS RF (mV)</td>
<td>0.069 ± 0.029</td>
<td>0.071 ± 0.026</td>
<td></td>
<td>0.684</td>
</tr>
</tbody>
</table>

Mean ± SD (n = 10). HR, heart rate; SpO2; arterial oxygen saturation; VO2; oxygen consumption; VE, minute ventilation; RER, respiratory exchange ratio; BF, breathing frequency; [La], blood lactate concentration; RMS VL and RF, root mean square of vastus lateralis and rectus femoris muscle.
lead to anecdotal comparisons of performance and physiological responses between the aforementioned studies and the present one.

A further interesting observation was that the additional neuromuscular consequences of completing TT₂ were minimal (i.e., no difference between pre-TT₂ and post-TT₂) in both conditions. The short duration (6 min) of the second time-trial might partially explain why we failed to observe the further development of significant decrement in muscle activation (at least as for TT₁), as greater central fatigue has been detected after longer time-trials only (Thomas et al. 2015; Froyd et al. 2016); even though these observations have been obtained near sea level with no prior exercise. In this study, since the level of locomotor muscle fatigue pre-TT₂ versus post-TT₂ was also identical, the individual critical threshold of peripheral fatigue (Amann et al. 2006b) may have already been achieved when TT₂ started. Despite exercise duration was approximately three times shorter, average power output maintained during TT₂ resembles those produced during TT₁, with also similar power values between conditions.

### Prior time-trial (TT₁) performance was lower at severe versus moderate hypoxia

As expected, average power output was lower during TT₁₃₅₀₀ versus TT₁₂₀₀₀. Our results are in line with

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**Figure 2.** Maximal voluntary contraction force (A), voluntary activation (B), and peak twitch force (C) before and after the first and the second cycling bouts. The first cycling bout (TT₁) was performed at 2000 m or 3500 m, while the second bout (TT₂) was always performed at 2000 m. Mean ± SD (n = 10). *Denotes a significant difference from pre-TT₁ (P < 0.05).
Table 3. Muscle function before and after the first (TT1) and the second cycling bout (TT2)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Condition</th>
<th>Time points</th>
<th>ANOVA P value (ES)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pre-TT1</td>
<td>Post-TT1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Db10 (N)</td>
<td>2000 m</td>
<td>305 ± 51</td>
<td>192 ± 49*</td>
</tr>
<tr>
<td></td>
<td>3500 m</td>
<td>319 ± 53</td>
<td>203 ± 50*</td>
</tr>
<tr>
<td>Db100 (N)</td>
<td>2000 m</td>
<td>289 ± 41</td>
<td>242 ± 50*</td>
</tr>
<tr>
<td></td>
<td>3500 m</td>
<td>299 ± 40</td>
<td>256 ± 41*</td>
</tr>
<tr>
<td>Db10,Db100−1</td>
<td>2000 m</td>
<td>1.05 ± 0.08</td>
<td>0.79 ± 0.09*</td>
</tr>
<tr>
<td></td>
<td>3500 m</td>
<td>1.07 ± 0.06</td>
<td>0.79 ± 0.10*</td>
</tr>
<tr>
<td>M-wave VL (mV)</td>
<td>2000 m</td>
<td>20.6 ± 4.5</td>
<td>20.8 ± 4.7</td>
</tr>
<tr>
<td></td>
<td>3500 m</td>
<td>21.7 ± 5.2</td>
<td>22.1 ± 5.0</td>
</tr>
<tr>
<td>M-wave RF (mV)</td>
<td>2000 m</td>
<td>8.6 ± 2.2</td>
<td>8.2 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>3500 m</td>
<td>8.7 ± 3.2</td>
<td>8.5 ± 3.1</td>
</tr>
</tbody>
</table>

Db10, force associated with doublet at 10 Hz; Db100, force associated with doublet at 100 Hz; VL, vastus lateralis; RF, rectus femoris.

*Denotes a significant difference from pre-TT1 (P < 0.05).

previous studies that showed that aerobic performance is altered with altitude: Compared to normoxia, this decrease was reported to be between 10 and 20% at 3000 m (Ventura et al. 2003) in normobaric hypoxia. In this study, we did not measure performance or VO2max in normoxia and therefore cannot compare directly our data. Many studies (Peltonen et al. 2001; Wehrlin and Hallen 2006; Mollard et al. 2007; Périard and Racinais 2016) demonstrated that the reduced VO2max was induced by a decrease in SpO2, leading to aerobic performance impairment. It is possible that the type of hypoxic condition (e.g., normobaric hypoxia) may explain the relatively moderate 8% difference in performance between the two TT1 since we recently showed that the performance is less altered in normobaric than hypobaric hypoxia (Saugy et al. 2016). The main mechanism for endurance performance impairment in hypoxia might be the decrease in VO2max with a mean decrease of 7.7% per 1000 m increase in altitude (−4% at 1000 m (Wehrlin and Hallen 2006; Mollard et al. 2007); −10% (Mollard et al. 2007) to −15% (Peltonen et al. 2001; Wehrlin and Hallen 2006; Périard and Racinais 2016) at 2500–3000 m; or −30% (Mollard et al. 2007) at 4500 m, all in normobaric hypoxia). This reduction in convective O2 transport during hypoxic cycling precipitate a decrement in peak exercise capacity and therefore a shift of a given absolute work load to a higher relative intensity.

Alterations in convective O2 transport to the working muscles are the result of changes in arterial oxygen content and/or limb blood flow (Romer et al. 2006). Obviously, the VO2max was strongly linked to SpO2 levels also tainted by altitude (Chapman 2013). Reportedly, a > 3% reduction in SpO2 from rest has a significant detrimental effect on VO2max (Harms et al. 1997). SpO2 maintenance, and not baseline VO2max levels per se, is a primary limiting factor determining VO2max decline with exposure to acute altitude (Chapman 2013). In this study, 2000 m and 3500 m simulated altitudes corresponded to SpO2 values of ~80% and ~90%, respectively, highlighting differences in the severity of hypoxic conditions. Hyperventilation of heavy sustained exercise (>85% VO2max) causes substantial increases in respiratory muscle work, leading to diaphragm and expiratory muscle fatigue (Dempsey et al. 2006) in turn reducing blood flow, and thus O2 delivery, to the working limb (Harms et al. 2000). To which extent this phenomenon would explain the observed lower performance during TT1_3500 compared to TT1_2000 could not be ascertained here.

In this study, TT1 was a performance test whereby the participants were continuously able to adjust their pace as they attempt to sustain the highest average power output for a predetermined reference time. Under more severe hypoxic conditions (>10% lower SpO2 values during TT1_3500 versus TT1_2000), reductions in power output occurred together with elevated cardiovascular load despite similar skeletal muscle recruitment (EMG). This probably ensured that the rate of peripheral fatigue development was slowed in the more severe hypoxic condition. To date, experimental data are contradictory, with some studies reporting no effect of severe hypoxia on EMG amplitude during dynamic muscle contractions (Donnelly and Green 2013), and other reporting increased activity with a muscle-specific pattern (Fulco et al. 1996; Torres-Peralta et al. 2014). During 5-km cycle time-trials, increased systemic O2 transport from hypoxia to hyperoxia (via wide changes in FiO2) resulted in parallel increases in central motor drive (skeletal muscle recruitment) and power output ultimately leading to improved time-trial performance; yet, the magnitude of peripheral muscle fatigue developed at end-exercise was identical.
(Amann et al. 2006b). In this study, perceived fatigue was not different between conditions, as evidenced by similar RPE values between TT1_2000 and TT1_3500.

Post-exercise recovery of neuromuscular function

Limited research is available to show how long a diminished neuromuscular response to intense cycling exercise will last, notably after hypoxic tasks. In this study, impairment in muscle function persisted at least 22 min into recovery, with remarkably similar post-TT1 and pre-TT2 peak twitch values. Although the extent to which muscle force decreased in response to TT1 varied according to the stimulation frequency considered (1 and 10 Hz vs. points. Contrastingly, VA values returned near baseline at strenuous exercises (Minett and Duffield 2014).

During hypoxic isometric knee extensions, hypocapnia clamping is effective in preventing the development of hypertensivation-induced hypocapnia during intense hypoxic exercise as performed here and what could be the “carry-over” effects on subsequent performance, physiological responses, and neuromuscular consequences need to be researched. Although standard, our neuromuscular assessment based on MVC of an isolated muscle group (knee extensors) pre- versus postcycling might not truly be representative of effective cerebral functioning (and to a lower extent of muscle mechanics) during an actual whole-body dynamic exercise. Rather, future research would benefit from a combination of methodologies (i.e., perfusion, oxygenation, metabolism, neuronal excitability, and electrical activity) offering complementary insights into the brain in hypoxia (Vergès et al. 2012).

An important consideration when measuring stationary cycling performance using normobaric hypoxic gas mixtures in a laboratory setting is that it does not simulate the terrestrial altitude environment where there is a decrease in air density (i.e., drag forces) due to the decrease in barometric pressure (Peronnet et al. 1991). Interestingly, decreases in time-trial performance (~8%) and SpO2 (~2%) values from normoxia to hypoxia were greater in hypobaric versus normobaric hypoxia for a terrestrial/simulated altitude of 3450 m (Saugy et al. 2016), corresponding to the present more severe hypoxic condition of TT1. Our conclusions must therefore remain specific to the context of our study.

Conclusion

During an initial cycling time-trial (approximately 16 min), power output was lower and cardiorespiratory load higher in more severe hypoxia, whereas quadriceps muscle activation (twitch interpolation) trend differed between moderate and severe hypoxia. This resulted, however, in the attainment of similar neuromuscular fatigue characteristics at exercise cessation, thus independent of the hypoxic severity. After 22 min of rest, muscle activation returned near baseline, while peripheral function remained depressed. There was no influence of the altitude of a prior time-trial on performance and associated cardiometabolic responses, with also no additional muscle fatigue development, during a subsequent 6-min time-trial performed in moderate hypoxia. We conclude that prior time-trial performed at higher altitude did not influence further performance and associated cardiometabolic and neuromuscular responses during completion of a subsequent exercise of similar nature in moderate hypoxia.

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Conflict of Interest

The authors have no conflict of interest to disclose.

References


