The effects of priming exercise on the VO2 slow component and the time-course of muscle fatigue during very-heavy intensity exercise in humans.
Original research

Title
The effects of priming exercise on the VO₂ slow component and the time-course of muscle fatigue during very heavy intensity exercise in humans.

Running title

VO₂ slow component and time-course of muscle fatigue during exercise

Authors:
Paulo Cesar do Nascimento Salvador¹, Kristopher Mendes de Souza¹, Ricardo Dantas De Lucas¹, Luiz Guilherme Antonacci Guglielmo¹, Benedito Sérgio Denadai².

¹Physical effort Laboratory, Sports Center, Federal University of Santa Catarina, Florianópolis, Brazil
²Human Performance Laboratory, São Paulo State University, Rio Claro, Brazil

Corresponding author:
Paulo Cesar do Nascimento Salvador
ORCID iD 0000-0001-8228-5115
Adresse: Rua Silvio Possobon, 70, apartamento 1009, Abraão, CEP: 88085-190, Florianópolis, Santa Catarina, Brasil.
Phone: +55 48 9949-6762, Fax: +55 48 3721-6248
Email: nascimentoopc84@hotmail.com

Kristopher Mendes de Souza - kristophersouza@yahoo.com.br
Ricardo Dantas De Lucas - tridantas@hotmail.com
Luiz Guilherme Antonacci Guglielmo - luiz.guilherme@ufsc.br
Benedito Sérgio Denadai - bdenadai@hotmail.com

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Abstract

We hypothesized that prior exercise would attenuate the muscle fatigue accompanied by the VO$_{2\text{SC}}$ behavior during a subsequent very heavy (VH) intensity cycling exercise. Thirteen healthy male subjects performed tests to determine the critical power (CP) and W' and six square-wave bouts until 3 or 8 min, each at a work rate set to deplete 70% W' in 8 min, with an maximal isokinetic effort (MIE) before and after the conditions without (VH$_{\text{CON}}$) and with prior exercise (VH$_{\text{EXP}}$), to measure the cycling peak torque decrement. The VO$_{2\text{SC}}$ magnitude at 3 min (VH$_{\text{CON}}$ = 0.280 ± 0.234, VH$_{\text{EXP}}$ = 0.116 ± 0.109 L.min$^{-1}$; p=0.04) and the VO$_{2\text{SC}}$ trajectory were significantly lower for VH$_{\text{EXP}}$ (VH$_{\text{CON}}$ = 0.108 ± 0.042, VH$_{\text{EXP}}$ = 0.063 ± 0.031 L.min$^{-2}$; p<0.01) leading to a VO$_{2\text{SC}}$ magnitude at 8 min significantly lower than VH$_{\text{CON}}$ (VH$_{\text{CON}}$ = 0.626 ± 0.296 L.min$^{-1}$, VH$_{\text{EXP}}$ = 0.337 ± 0.179; p<0.01). Conversely, peak torque progressively decreased from pre-exercise to 3 min (∆Torque = 21.5 ± 7.7 vs. 19.6 ± 9.2 Nm) and to 8 min (∆Torque = 29.4 ± 15.8 vs. 27.5 ± 12.0 Nm) at VH$_{\text{CON}}$ and VH$_{\text{EXP}}$ respectively, without significant differences between conditions. Regardless of the condition, there was a significant relationship between ∆torque and the VO$_{2\text{SC}}$ ($R^2$ VH$_{\text{CON}}$ = 0.23, VH$_{\text{EXP}}$ = 0.25; p=0.01). Considering that “priming” effects on the VO$_{2\text{SC}}$ were not accompanied by the muscle force behavior, these findings do not support the hypothesis of a “causal” relationship between the time-course of muscle fatigue and VO$_{2\text{SC}}$.

Key words priming exercise; slow component of oxygen uptake; VO$_2$ kinetics, muscle force production; high-intensity exercise
Introduction

During exercise performed above the gas exchange threshold (GET), the fundamental oxygen uptake (VO$_2$) kinetics is supplemented by a delayed-onset VO$_2$ slow component (VO$_{2SC}$). The VO$_{2SC}$ delays the attainment of a steady state during heavy-intensity exercise [below the critical power (CP)] or drives the VO$_2$ toward to its maximum (VO$_{2peak}$) during VH-intensity exercise (above the CP) (Jones et al. 2011, Rossiter 2011). Although the development of the VO$_{2SC}$ indicates a progressive loss of muscle efficiency as supra-GET exercise proceeds (Jones et al. 2010, Grassi et al. 2015, Poole et al. 2016), the putative mechanisms determining the VO$_{2SC}$ are still controversial and are not entirely understood (Jones et al. 2011, Grassi et al. 2015). The majority of the VO$_{2SC}$ (>85%) originates in the locomotor muscles (Poole et al. 1991, Rossiter et al. 2002a). Indeed, previous studies using different techniques such as electromyography (Borrani et al. 2001, Burnley et al. 2002a, Lanzi et al. 2012), magnetic resonance imaging (Saunders et al. 2000, 2003, Endo et al. 2007), glycogen depletion (Carter et al. 2004, Krustrup et al. 2004a), muscle biopsy (Krustrup et al. 2004b) and neural blockade of type I muscle fibers (Krustrup et al. 2008), confirm that the dominant portion of the VO$_{2SC}$ derives from intramuscular sites (Jones et al. 2011). Thus, the VO$_{2SC}$ might be caused by an additional recruitment of less-efficient type II muscle fibers in order to compensate for progressive fatigue in the early recruited fibers and/or by an increased metabolic demand within those already recruited fibers (Jones et al. 2011).

In this context, the VO$_{2SC}$ is related to muscle fatigue (defined as an exercise-induced reduction in maximal muscle force, torque or power-generating capacity) during supra-GET exercise (Cannon et al. 2011, Grassi et al. 2015, Keir et al. 2016). Cannon et al. (2011), analyzed three different work rates (below the GET, between GET and CP, and above the CP) and three different pedal cadences (60, 90 and 120 rpm) in the cycling exercise, and demonstrated a significant correlation between VO$_{2SC}$ and muscle fatigue (quantified by reductions in peak torque and power during an all-out isokinetic sprint
However, the authors observed that the time course of muscle fatigue was unrelated to the VO\textsubscript{2SC} progression, because the reductions in peak torque and power were unchanged between 3 and 8 min of heavy- and VH-intensity exercise (Cannon et al. 2011). This finding suggests that loss of muscle efficiency during supra-GET exercise might occur without progressive fatigue development. Moreover, the development of the VO\textsubscript{2SC} seems to be independent of an additional recruitment of less-efficient type II muscle fibers (Jones et al. 2011, Cannon et al. 2011, Grassi et al. 2015).

On the other hand, Keir et al. (2016) reported that the time course of peripheral muscle fatigue (as determined by pre- vs. post-exercise differences in electrically stimulated quadriceps muscle torque) was associated with the development of the VO\textsubscript{2SC} during 18 min of a VH-intensity cycling exercise. This quantitative and temporal relationship between VO\textsubscript{2SC} and peripheral muscle fatigue suggests, therefore, that both phenomena share common mechanisms. Indeed, above the CP, the trajectory of the VO\textsubscript{2SC} mirrors those of depletion of substrates (e.g., [PCr] and glycogen) and the accumulation of fatigue-related metabolites (e.g., [ADP], [P\textsuperscript{i}], [H\textsuperscript{+}] and [K\textsuperscript{+}]) (Carter et al. 2004, Jones et al. 2010, 2011, Burnley and Jones 2016, Poole et al. 2016, Black et al. 2017). In addition, the development of the VO\textsubscript{2SC} is limited by the attainment of the VO\textsubscript{2peak}, and the level of metabolic muscle perturbation is also restricted to the attainment of some critical threshold (Burnley et al. 2010, Burnley and Jones 2016, Vanhatalo et al. 2016, Black et al. 2017). This is consistent with the 'critical threshold of peripheral muscle fatigue' proposed for high-intensity exercise (Amann et al. 2011).

Although the relationship between VO\textsubscript{2SC} and muscle fatigue seems to be a ‘cause-effect relationship’, there is not enough evidence regarding this issue. There are no studies that have investigated the influence of an intervention changing the VO\textsubscript{2SC} in this relationship. It has been demonstrated that the performance of a prior heavy- or VH-intensity exercise results in a reduction of the VO\textsubscript{2SC} during a subsequent VH-intensity exercise (Koppo and Bouckaert 2002, Burnley et al. 2002b, 2006, Jones et al. 2004, Sahlin et al. 2005, Bailey et al. 2009, do Nascimento et al. 2015). The overall effects of
prior high-intensity exercise on the VO$_{2SC}$ of a subsequent supra-CP exercise are attributed to an increase in the muscle blood flow, O$_2$ extraction, and mitochondrial enzyme activity, as well as reductions in additional motor unit recruitment (Burnley et al. 2002a, Jones et al. 2011). Moreover, decreasing the substrate-level phosphorylation and an accumulation of metabolites by reducing the muscle fatigue during supra-CP exercise, would therefore be expected to result in an attenuated VO$_{2SC}$ magnitude (Rossiter et al. 2001, 2002b).

Thus, the aim of this study was to verify whether a prior VH-intensity exercise attenuates the muscle fatigue accompanying the reductions on the VO$_{2SC}$ dynamic in a subsequent VH-intensity exercise. In the same way as previous studies (Cannon et al. 2011, de Souza et al. 2016), we used instantaneous switching from constant work rate to isokinetic (constant pedal cadence) cycling to quantify reductions in peak torque at specific timings (pre-exercise, 3 and 8 min) during a VH-intensity exercise without (control condition - VH$_{CON}$) and with (experimental condition - VH$_{EXP}$) 'priming' influence. We hypothesized that reductions in VO$_{2SC}$ amplitude would be accompanied by attenuation of falls in peak torque during VH$_{EXP}$. In addition, we hypothesized that the magnitude and time course of muscle fatigue would be related to the development of the VO$_{2SC}$ in both conditions (i.e., VH$_{CON}$ and VH$_{EXP}$).

**Methods**

**Ethical approval**

The experimental protocol was approved by the Research Ethics Committee of the Federal University of Santa Catarina and was conducted in accordance with the Declaration of Helsinki. After being fully informed of the risks and stresses associated with the study, the subjects gave their written informed consent to participate.

**Participants**

Sample size was calculated *a priori* based on the effect size (ES) = 0.5 (moderate effect) and $p = 0.05$, set at a minimum power of 80% of the statistical analysis.
Based on these parameters, a minimum of 12 participants was required. Thus, thirteen healthy male subjects (age 28 ± 7 years; mass 82.6 ± 12.7 kg; height 180.8 ± 6.9 cm; \( VO_{2\text{peak}} 51.5 ± 7.4 \text{ ml.kg.min}^{-1} \)) volunteered to participate in the study. They participated in any exercise at a recreational level (3-4 sessions per week; 150-300 min per week), but were not highly trained, and were familiar with laboratory exercise testing procedures.

**Overview of study design**

Subjects were required to visit the laboratory on 10-12 occasions. Breath-by-breath pulmonary gas exchange and heart rate (HR) data were measured continuously during all tests. On the first visit, each subject performed a maximal incremental ramp test for the determination of the GET, \( VO_{2\text{peak}} \) and maximal power output (\( P_{\text{max}} \)). On subsequent visits, subjects performed 3-5 maximal constant work rate prediction tests for the determination of the CP and \( W^* \) (fixed amount of work above CP). After that, a series of six VH-intensity exercises without (\( \text{VH}_{\text{CON}} \)) and with (\( \text{VH}_{\text{EXP}} \)) ‘priming’ effect was completed to verify the relationship between \( VO_{2\text{SC}} \) and muscle fatigue above the CP (Figure 1). Subjects were instructed to avoid any intake of caffeine for 3 h, or alcohol and strenuous exercise in the 24 h preceding the test sessions and to arrive at the laboratory in a rested and fully hydrated state, at least 2 h postprandial. All tests were performed at the same time of day in a controlled environmental laboratory condition (19-22°C; 50-60%RH) to minimize the effects of diurnal biological variation on the results. Subjects performed only one test on any given day, and each test was separated by 24-72 h but completed within a period of five weeks.

“Figure 1 here”

**Equipment**

All tests were performed on an electromagnetically braked cycle ergometer (Excalibur Sport PFM, Lode BV, Groningen, Netherlands). Respiratory and pulmonary gas exchange variables were measured using a breath-by-breath analyzer (Quark PFTergo,
Cosmed, Rome, Italy). Before each test, the O\textsubscript{2} and CO\textsubscript{2} analysis systems were calibrated using ambient air (20.94% O\textsubscript{2} and 0.03% CO\textsubscript{2}) and a gas of a known O\textsubscript{2} and CO\textsubscript{2} concentration (16.00% O\textsubscript{2} and 5.00% CO\textsubscript{2}) according to the manufacturer’s instructions. Likewise, the turbine flow meter was calibrated before each test using a 3 L syringe (Quark PFTergo, Cosmed, Rome, Italy). A monitor coupled to the gas analyzer was used to measure the HR. Capillary blood samples (25 µl) were obtained from the earlobe of each subject and the blood lactate concentration ([La]) was measured using an electrochemical analyzer (YSL 2700 STAT, Yellow Springs, Ohio, USA). The cycle ergometer, the breath-by-breath analyzer and the electrochemical analyzer were calibrated in accordance with specific manufacturer’s recommended procedures.

**Determination of GET and VO\textsubscript{2peak}**

On the first laboratory visit, 15 min after the isokinetic sprint familiarization, subjects performed an incremental ramp test for the determination of the GET, VO\textsubscript{2peak} and P\textsubscript{max}. After a 4-min period of cycling at 20 W (baseline), an incremental ramp test to exhaustion was undertaken with power output increasing by a rate of 30 W.min\textsuperscript{-1}. Subjects were instructed to maintain their preferred cadence (83 ± 6 rpm) throughout the test. The preferred cadence along with saddle and handle bar height and configuration was recorded and reproduced in subsequent tests. Each subject was verbally encouraged to undertake maximal effort. The test was terminated when the cadence fell by more than 10 rpm below the preferred cadence for more than 5 s despite strong verbal encouragement (Black et al. 2015). The VO\textsubscript{2} values were averaged over 15-s periods. The VO\textsubscript{2peak} was defined as the highest value obtained in a 15-s interval, or if a VO\textsubscript{2} plateau observed, the VO\textsubscript{2peak} was considered as the average of the final minute of exercise (Day et al. 2003). The attainment of VO\textsubscript{2peak} was defined using the criteria proposed by Bassett and Howley (2000). The P\textsubscript{max} was considered as the highest power output attained during the test.

The GET was determined using a cluster of measurements as the V-slope method and the ventilatory equivalent method (Beaver et al. 1986). The data from the
A ramp test was used to calculate the work rate corresponding to 70% ∆ (i.e., GET plus 70% of the difference between the work rate at the GET and VO$_{2peak}$), with the lag in VO$_2$ during incremental exercise taken into account by a deduction of two-thirds of the ramp rate from the work rate at the GET (Burnley et al. 2011). The work rate at 70% ∆ was calculated by interpolation of the linear regression of VO$_2$ with respect to work rate during the incremental test. This excluded the first few minutes (2 – 3 min), as well as the time following the attainment of any plateau in VO$_2$.

**Determination of CP and $W'$**

The participants performed a series of 3-5 constant work rate prediction trials in a randomized order on separate days for the determination of the CP and $W'$. The work rates were chosen to elicit exhaustion between ~2 and ~15 min (e.g., 75% to 105% of $P_{\text{max}}$), which is the range recommended for the determination of the CP and $W'$ (Jones et al. 2010, Burnley et al. 2011). After the performance of the first two trials, a linear regression was used to estimate the subsequent trials. The work rates were estimated using CP and $W'$ parameters derived from equation 1. Each test started with a 5 min warm-up at GET followed by 5 min of passive rest. Furthermore, after 3 min of cycling at 20 W, the work rate was adjusted to one of the previously established work rates and subjects were instructed to perform until exhaustion. Subjects were instructed to remain seated and to maintain their preferred cadence for as long as possible with the test being terminated when the cadence fell by more than 10 rpm below the preferred cadence for more than 5 s despite strong verbal encouragement (Black et al. 2015). The time to exhaustion ($T_{\text{lim}}$) was recorded to the nearest second. For each subject, the following three equivalents of the 2-parameter model were used to fit the data and to estimate CP and $W'$:

The linear power output ($P$) versus 1/time to exhaustion:

$$P = \left(\frac{W'}{T_{\text{lim}}}\right) + CP$$

(1)
The linear work \((W)\) versus time to exhaustion:

\[ W = (CP \times T_{lim}) + W' \quad (2) \]

Non-linear power output \((P)\) versus time to exhaustion:

\[ T_{lim} = \left( \frac{W'}{P - CP} \right) \quad (3) \]

As a quality control measure of the mathematical modeling of power-time parameters, a priori criteria were set for the standard errors of estimate (SEE) associated with the CP and \(W'\), such that if the SEE exceeded 1% and 5%, respectively, after 3 predictions trials had been performed, a fourth prediction trial was then completed. If required a fifth prediction trial would be performed and inserted in the models to bring the SEE of the CP and \(W'\) below the criteria. The SEE values from the equations 1-3 were compared in order to select the CP and \(W'\) estimates from the best fit (Vanhatalo et al. 2010, de Souza et al. 2016).

**Maximal isokinetic effort**

The muscle fatigue was assessed for each subject before and after square-wave bouts \((VH_{CON}\) and \(VH_{EXP}\)) by a 5-s maximal isokinetic effort (MIE) at 120 rpm, in order to quantify peak torque and power. This protocol was similar to the previously used by Cannon et al. (2011) and de Souza et al. (2016). In the pre-exercise muscle function assessment subjects performed a 5 min warm-up at 80 % GET immediately followed by the MIE. After this, the subjects performed 5 min of active recovery at 80 % GET and a period of 12 min rest before the square-wave bouts. In post-exercise muscle fatigue assessment, the MIE was performed immediately after the \(VH_{CON}\) and \(VH_{EXP}\) (see figure 1 for more details). Subjects were given an auditory cue to begin the all-out effort in the seated position and strong verbal encouragement was given throughout the 10 crank revolutions (5 s at 120 rpm). The cycle ergometer was instrumented with pedal force measurement (Excalibur Sport PFM, Lode BV, Groningen, Netherlands). For the muscle fatigue measurements, the torque and power data were recorded continuously during all
the MIE. As described by Altenburg et al. (2007), the peak torque and power in each crank arm were determined by visual inspection as the average of the four consecutive highest torque and power values (2 s) excluding any occasional overshoot. Thus, the peak torque and power during the MIE were then considered as the average of the peak values of both left and right crank arms.

**Square-wave bouts**

In the main part of this investigation, six square-wave bouts set to deplete 70 % $W'$ in 8 min were performed in a randomized order until 3 min or 8 min (twice), three with ($VH_{\text{EXP}}$) and three without ($VH_{\text{CON}}$) prior VH exercise. In $VH_{\text{CON}}$, 17 min after the pre-exercise measurements of MIE the exercise protocol started with 3-min of baseline (cycling at 20 W), immediately followed by an abrupt transition to the target work rates. Considering that the muscle fatigue is related to the total amount of work that can be performed above the CP (i.e., $W'$) (Murgatroyd et al. 2011, Vanhatalo et al. 2016), the work rates were calculated to induce a depletion 70 % $W'$ at 8 min (70 % $W'$) according to equation 1.

In $VH_{\text{EXP}}$, in the same way to control, 17 min after the pre-exercise MIE and 3 min of baseline cycling, an abrupt transition to a 70 % $\Delta$ during 6 min was set. After 7 min of passive recovery and 3 min of baseline an abrupt transition to a 70 % $W'$ work rate until 3 min or 8 min were set. This intensity and this duration of time to recovery were shown by Bailey et al. (2009) as an efficient prior exercise strategy that attenuates the VO$_{2\text{SC}}$ and improves $T_{\text{im}}$. Capillary blood samples were collected from the ear lobe 30 s before the beginning, as well as 30 s before the 6th min at prior exercise bouts, 3rd min or 8th min of exercise during the square-wave bouts. The $\Delta$ [La] was defined as the difference between the [La] at the end, and the [La] at the beginning of each exercise bout. To ensure that the pre-exercise muscle assessments performed before VH priming would be reflective of those that could be measured after priming, all participants performed a constant work
rate exercise bout to exhaustion at a VH intensity (~70 % ∆) with the MIE performed before (as in the same way that the pre-measurements) and 1 min after the intolerance.

Data analysis

Breath-by-breath data for each test were initially examined to exclude outlier values caused by sighs, swallowing and coughs (Lamarra et al. 1987). For each exercise transition, the breath-by-breath data was linearly interpolated to 1-s intervals. Each condition was then time-aligned to the start of exercise, and ensemble-averaged (three trials until 3 min and two trials until 8 min for each condition) to yield a single profile for each subject. The single profile data was reduced to a 5-s stationary average to decrease the influence of “signal error” and improve parameter estimation (Whipp and Rossiter 2005). The first 20-s of data after the onset of exercise (i.e., the “cardiodynamic” phase) were not included in the analysis (Rossiter et al. 2005). Non-linear regression techniques were used to fit the data after the onset of a fundamental phase with an exponential function (OriginPro 8; OriginLab). An iterative process ensured that the sum of squared errors was minimized. The mathematical model consisted of an exponential term (equation 4) as described previously (Barstow et al. 1996). The fundamental VO$_2$ kinetics (phase II) was isolated following the iterative method to identify the exponential region (Murgatroyd et al., 2011; Rossiter et al., 2002; Rossiter et al., 2001). Based on previous study (Barstow et al. 1996), the model was constrained in VO$_2$baseline to aid in the identification of the key parameters according to the following equation:

$$VO_2(t) = VO_{2\,\text{baseline}} + A \times [1 - e^{\frac{(t-TD)}{\tau}}]$$

(4)

where: VO$_2$(t) represents the value of VO$_2$ at a given time (t); VO$_2$baseline is the average value over the last minute of baseline cycling; A is the asymptotic amplitude for the exponential term describing changes in VO$_2$ from baseline to its asymptote; τ is the time constant; and the TD is the time delay.
The identification of VO\textsubscript{2SC} during the VH intensity exercise, in which the exponential region varies in duration among the subjects due to the variably delayed appearance of the VO\textsubscript{2SC} (Murgatroyd et al. 2011) was performed individually. The identification at the end of fundamental phase (i.e., TD\textsubscript{s}) was performed by fitting a window from the start of the fundamental phase (i.e., after 20 s cardio-dynamic phase) initially set at 60 s. The window was lengthened iteratively until the exponential model fit demonstrated a discernible and consistent departure from the measured VO\textsubscript{2} values by considering the following criteria: 1) the narrowest confidence interval for $\tau$; 2) a breakpoint and systematic increase in both $\tau$ and $A$, with a decrease in TD; 3) a breakpoint and systematic rise in the $x^2$ for the fitted model; and 4) a departure from an even distribution of residuals around zero (as judged from the visual inspection of a plot of the residuals of the fit) (Murgatroyd et al., 2011; Rossiter et al., 2002; Rossiter et al., 2001). Thus, the fitting window was constrained to this time point and a single-exponential fitting was performed only on the fundamental phase to identify the kinetics parameters.

The VO\textsubscript{2SC} was calculated according to the following equation:

$$\text{VO}_{2\text{SC}} = \text{VO}_{2\text{END}} - (\text{VO}_{2\text{baseline}} + A)$$

(5)

Where: VO\textsubscript{2END} is the average VO\textsubscript{2} value over the last 15 s at 3 or 8 min of exercise. The functional gain of the fundamental phase with respect to work rate (in ml.W\textsuperscript{-1}.min\textsuperscript{-1}) was calculated according to equation 6:

$$\text{Gain} = \frac{A}{W - \text{unloaded baseline}}$$

(6)

To analyze the rate of increase in VO\textsubscript{2} during the second phase (i.e., the slow component trajectory - VO\textsubscript{2SC} trajectory), an index of efficiency that does not depend on the value of the primary amplitude or the VO\textsubscript{2END} and demonstrates the VO\textsubscript{2SC} behavior without the influence of the other parameters (Burnley et al. 2011). The VO\textsubscript{2SC} trajectory was calculated using the following equation (Burnley et al. 2011):
\[ VO_{2SC} \text{trajectory} = \frac{VO_{2SC}}{(t - TDs)} \] (7)

**Statistical analysis**

Descriptive statistics are expressed as mean ± standard deviation. The Shapiro-Wilk test was applied to ensure a Gaussian distribution of the data (n < 50). The \( VO_2 \) kinetics parameters obtained from the square-wave bouts were compared between the conditions (\( VH_{CON} \) vs. \( VH_{EXP} \)) using the paired “t” test. Relationships between variables were assessed using Pearson’s product-moment correlation coefficients. A two-way ANOVA with repeated measures was used to analyze the interaction over time and conditions for torque and power output parameters, \([La]\) and as well for \( VO_{2SC} \) at 3 or 8 min. Assumptions of sphericity were assessed using the Mauchly test, and any violations were corrected using the Greenhouse-Geisser correction factor. The Shapiro-Wilk test was used to verify the normality of residuals. When significant effects were observed the Bonferroni post hoc test was used for comparisons. Analyzes were performed using the Statistical Package for Social Sciences Windows (SPSS Inc. version 17.0; Chicago, IL, USA) and Graph-pad Prism software package for Windows (version 5.0; Graph-Pad Prism Software Inc., San Diego, California, USA). The level of significance adopted was set at p < 0.05.

**Results**

*Incremental ramp test and predictive tests of CP and W'*

The results of selected peak variables obtained during incremental tests were presented in Table 1. The GET occurred at 25.8 ± 5.5 ml.kg.min\(^{-1}\) (50 ± 5 % \( VO_{2peak} \)) and at a power of 131 ± 25 W. The \([La]\) at the beginning and the end of the ramp tests were 1.4 ± 0.3 and 10.6 ± 2.3 mmol.L\(^{-1}\), respectively. The CP and W’ were 249 ± 31 W (54 ± 10 % \( \Delta \)) and 21.9 ± 7.6 kJ, respectively. The SEE associated with the estimated CP and W’ were 0.6 % and 2.6 % with 95 % confidence intervals between 0.6 to 2.8 W and 0.3 to 1.0
kJ for CP and W’, respectively. The CP and W’ were not correlated with $\tau$ or $\text{VO}_{2\text{SC}}$, respectively. The prior exercise work rate corresponding to 70 % $\Delta$ was 284 ± 31 W (75 ± 3 % $P_{\text{max}}$; 115 ± 9 % CP) and according to equation 1, depleted ~62 % W’ in 6 min. The square-wave bouts set to deplete 70 % W’ (15.3 ± 5.3 kJ) in 8 min were performed at 281 ± 34 W (74 ± 3 % $P_{\text{max}}$; 69 ± 8 % $\Delta$).

**Square-wave bouts and VO$_2$ kinetics responses**

The $\tau$ ($p = 0.19$), TD for the slow component phase (i.e., $\text{TD}_s$; $p = 0.26$) and the $\text{VO}_{2\text{END}}$ (i.e., VO$_2$ at 8 min; $p = 0.51$) were not significantly different between the conditions (Table 2). $\text{VO}_{2\text{END}}$ in both conditions was not significantly different from $\text{VO}_{2\text{peak}}$ in the ramp test ($F = 0.39$, $p = 0.64$). Conversely, in the $\text{VH}_{\text{EXP}}$, higher total amplitude of phase II was observed ($A_{\text{TOTAL}}$; i.e., the VO$_2$$_{\text{baseline}}$ + $A_2$; $p < 0.01$). In this condition $\text{VO}_{2\text{SC}}$ magnitude at 3 min was significantly lower ($p = 0.04$). Despite the significant increase in both conditions, the $\text{VO}_{2\text{SC}}$ trajectory was significantly lower for $\text{VH}_{\text{EXP}}$ ($p < 0.01$) leading to a $\text{VO}_{2\text{SC}}$ magnitude at 8 min significantly lower than $\text{VH}_{\text{CON}}$ ($p < 0.01$) with a decrease of 37 ± 34 % (table 2 and figure 2).

“Figure 2 here”

The [La] increased from rest to 3 min and to 8 min in $\text{VH}_{\text{CON}}$, whereas in $\text{VH}_{\text{EXP}}$ [La] at rest was markedly augmented ($p < 0.05$), and it did not increase further after the first 3 min but increased from 3 min to 8 min. There were observed differences ($p < 0.05$) between conditions for the baseline values, at 3 min, but no differences were found between the conditions at 8 min of exercise (Figure 3). There was no significant correlation ($p > 0.05$) between the $\text{VO}_{2\text{SC}}$ and [La] at any time in both conditions. The HR was slightly higher ($p < 0.05$) during $\text{VH}_{\text{EXP}}$ protocol throughout most of the test, but that difference gradually decreased and at the end of the exercise no differences were observed between the two conditions ($p = 0.19$). The HR at the end of $\text{VH}_{\text{CON}}$ showed a
significant difference in relation to the $HR_{\text{max}}$ obtained during the ramp test ($F = 7.06; p < 0.01$) but HR at the end of VH$_{\text{EXP}}$ and $HR_{\text{max}}$ were similar ($p = 0.34$).

“Figure 3 here”

“Figure 4 here”

“Figure 5 here”

Peak torque and peak power output during MIE

No differences were observed for both peak torque (intraclass correlation coefficient = 0.99; typical error = 3.7 %) and peak power output (intraclass correlation coefficient = 0.99; typical error = 4.2 %) obtained during the pre-exercise assessments. The average of the three pre-exercise assessments was taken for each condition. Peak torque and power output progressively decreased from pre-exercise to 3 min and to 8 min ($p < 0.05$) in both conditions. At all assessment times, no differences between VH$_{\text{CON}}$ and VH$_{\text{EXP}}$ were observed ($p > 0.05$). Thus, prior exercise had no effect on muscle force production (Table 3 and figure 4). The peak torque and power output decreases were recovered 1 min after the intolerance (Table 4). Regardless of the condition, there was a significant relationship between muscle force behavior and the changes in the VO$_{2SC}$.

When the peak torque was analyzed in each separate condition, a significant temporal relationship between VO$_{2SC}$ magnitude and $\Delta$ torque at 3rd and 8th min (VH$_{\text{CON}}$: $R^2 = 0.23$, $p = 0.01$ and VH$_{\text{EXP}}$: $R^2 = 0.25$, $p = 0.01$; figure 5) was found. The $\Delta$ power was significantly associated with the VO$_{2SC}$ magnitude in a similar way (VH$_{\text{CON}}$: $R^2 = 0.24$, $p = 0.01$ and VH$_{\text{EXP}}$: $R^2 = 0.34$, $p < 0.01$; figure 5, B and D). There was no significant association between the decrease in muscle performance indices and $\tau$ of VO$_2$ kinetics ($p = 0.40$).

Discussion

This is the first study to investigate the effects of prior exercise on the relationship between muscle fatigue and VO$_{2SC}$. Some recent papers suggested a direct association
between muscle fatigue and the VO$_{2SC}$ (Cannon et al. 2011, Keir et al. 2016). The present work aimed to test whether the time course of muscle fatigue follows a “cause-effect” relationship by manipulating the VO$_{2SC}$ with “priming” exercise. Compared to a non-priming control (VH$_{CON}$), the VO$_{2SC}$ during VH exercise was attenuated with priming (VH$_{EXP}$ protocol) but there were no between-condition differences in the magnitude of the reduction (from pre-exercise) of maximal isokinetic force and power at the third and eighth minutes of VH exercise. Therefore, the within-condition results confirm a relationship between the magnitude (Cannon et al. 2011) and time-course (Keir et al. 2016) of muscle fatigue and the VO$_{2SC}$ during VH exercise but the between-condition findings refute a “cause-effect” relationship between VO$_{2SC}$ and muscle fatigue.”

Relation between muscle fatigue and VO$_{2SC}$

Cannon et al. (2011) observed that when the exercise was performed at heavy domain the force production decreased significantly within 3 min of exercise with no additional reduction in peak torque between 3 and 8 min of exercise. Even more, at VH exercise which VO$_{2SC}$ magnitude is higher than other intensities the muscle fatigue was similar between 3 and 8 min of exercise. This data suggests that additional recruitment of motor units was not obligatory for VO$_{2SC}$ appearance, and besides that a reduction in mechanical efficiency in fatigued fibers was implicated and precedes VO$_{2SC}$. The authors affirmed that an increased ATP and/or O$_2$ cost of power production in fatigued fibers, that is a precedent fatigue, would be responsible for the VO$_{2SC}$. In the present study, it was observed that a significant decrease in torque production from pre-exercise to 3 min (VH$_{CON}$ = 16.4 ± 6.4 %; VH$_{EXP}$ = 14.9 ± 7.1 %), and that a continued to decrease significantly from 3rd to 8th min for both conditions (VH$_{CON}$ = 5.8 ± 7.6 %; VH$_{EXP}$ = 6.2 ± 8.3 %). Likewise, the beginning of VO$_{2SC}$ on the 3rd min of exercise (VH$_{CON}$ = 7.8 ± 6.4 %; VH$_{EXP}$ = 3.2 ± 3.4 % A$_{TOTAL}$) significant increases to the 8th min (VH$_{CON}$ = 17.8 ± 8.3 %; VH$_{EXP}$ = 9.0 ± 4.9 % A$_{TOTAL}$) with similar values of TD$_s$ (VH$_{CON}$ ~140 s and VH$_{EXP}$ ~160 s; Table 2). Contrary to the findings of Cannon et al. (2011), this study found a significant
temporal relationship between VO$_{2SC}$ magnitude and the time-course of force production for both conditions (figure 5). However, it is important to note that the bigger reductions in torque in both conditions occurred within 3 min of exercise (~15 % vs. ~6 %) agreeing with Cannon et al. (2011) showing a muscle fatigue that precedes the emergence of the VO$_{2sc}$. However, Keir et al. (2016) showed a mirror image between the temporal pattern of VO$_{2SC}$ and the decrements in muscle torque production as well as significant correlation between the VO$_{2SC}$ with the peripheral muscle fatigue (p<0.01; r$^2$ = 0.69) in a constant power-output cycling exercise (i.e., 60 % ∆) during 18 min. The authors observed that VO$_{2sc}$ increases 25, 59, 71, 77 % of VO$_{2sc}$ reserve (VO$_{2peak}$ – extrapolated A) in the 3, 8, 13 and 18 min during exercise, respectively. In the same way, the percentages of decreases in the maximal voluntary contraction (MVC) were 7, 9, 19 and 22 % in the cited time intervals. The present findings confirmed the study cited above to an exercise performed above the CP at a higher intensity (69.4 % ∆ with the attainment of VO$_{2peak}$ vs. 60 % ∆ no attainment of VO$_{2peak}$) and a protocol to measure the force production immediately in a specific mode of exercise.

However, the likely ‘causal’ relationship between muscle fatigue and VO$_{2sc}$ is still under debate in the literature. To test the hypothesis of a cause-effect relation between muscle fatigue and VO$_{2sc}$, this relationship should be influenced by an intervention changing one of the factors. The study of de Souza et al. (2016) verified that two different conditions set to deplete 70 % W’ within 3 or 10 min triggered different VO$_{2sc}$ magnitudes (0.12 vs. 0.44 L.min$^{-1}$, respectively), although the peak torque (assessed with the same technique in the present study) at the end of exercise was the same. Deley et al. (2006) verified a greater muscle fatigue of type II fibers and lower VO$_{2sc}$ during cycling exercise after a fatiguing electromyostimulation protocol. It is interesting that in this case, the VO$_{2sc}$ showed an inverse behavior compared to the muscle fatigue and the muscle fatigue after voluntary contractions did not alter the VO$_{2sc}$. Furthermore, Thistlethwaite et al. (2008) showed that a 2-fold increase in the activation of the additional motor units (~38 % vs. ~21 % MVC) had a similar VO$_{2sc}$ during a subsequent heavy-intensity cycling exercise in
relation to exercise bout after a priming condition at the same intensity, indicating that muscle fatigue is not a primary determinant of the VO$_{2ac}$. Moreover, Hopker et al. (2016) argued that progressive muscle fatigue per se, may not be associated with the development of the VO$_{2ac}$ and suggested that the correlations presented by Cannon et al (2011) across exercise intensities may be misbegotten. Therefore, our results corroborate with these studies (Deley et al. 2006, Thistlethwaite et al. 2008, de Souza et al. 2016, Hopker et al. 2016) showing that an acute intervention changing the VO$_{2ac}$ dynamic does not change the post force production behavior at least during cycling exercise. The relation between VO$_{2ac}$ and muscle fatigue could not be causal.

*Physiological Mechanisms that could explain the attenuation of VO$_{2ac}$*

When the prior exercise is performed above the CP the W' depletion could attain critical values or a total depletion in the some cases (Ferguson et al. 2010). This triggers a metabolic disturbance such as impairment on Ca$^+$ regulation, an increase in H$^+$ concentrations, ADP and Pi inside the sarcoplasm, and a critical decrease in PCr concentrations. In the present study, according to equation 1 the W' depletion at prior bout during the VH$_{EXP}$ was 62 ± 17 % with a 10 min recovery interval. After a prior VH exercise that depleted 100 % W' in 6 min, Ferguson et al. (2010) found a W' restoration of 85 % with a 15 min recovery interval. Thus, it is possible that W' had not totally recovered at the beginning of second bout of exercise and influenced the metabolic efficiency. Consequently, the recovery between the prior and subsequent exercises need to be enough to recover totally W' (Bailey et al. 2009).

Furthermore, our results showed a higher primary gain in VH$_{EXP}$, more specifically a higher cost of O$_2$ for the same work rate. Bailey et al. (2009) observed that longer recovery intervals could be needed (e.g., 20 min) to associate a reduced VO$_{2ac}$ with a better metabolic efficiency. The results of the present study showed a significant attenuation of the VO$_{2ac}$ ~40 % in VH$_{EXP}$ (with a 10 min recovery interval), with the same force production in relation to VH$_{CON}$ and with higher HR values for the same power.
output. These results confirm that accelerating the overall VO$_2$ kinetics, per se, is not necessarily ergogenic and indicates a dissociation between changes in HR kinetics (a possible enhancement of muscle O$_2$ supply), and VO$_2$ kinetics (Bailey et al. 2009). Additionally, if the primary amplitude is increased and the VO$_{2\text{END}}$ does not change, the VO$_{2\text{SC}}$ amplitude must decrease, even without mechanistic significance (Burnley et al. 2011). Nonetheless, the VO$_{2\text{SC}}$ trajectory was lower after priming and could be more meaningful than the VO$_{2\text{SC}}$ amplitude.

Grassi et al. (2015) suggest that muscle inefficiency may be a consequence of higher ATP cost to determine force production, specifically a progressive increase in ATP turnover instead of a decrease in ATP production (Grassi et al., 2015; Jones et al., 2011; Rossiter et al., 2002). Rossiter et al. (2001) showed that prior high-intensity exercise decreases the VO$_{2\text{sc}}$, the “slow component” of PCr and the phase II $\tau$ (lower O$_2$ deficit). Considering the significant attenuation of VO$_{2\text{sc}}$ during VH$_{\text{EXP}}$, as well as an increase of $A_{\text{TOTAL}}$, it is possible to consider that these putative mechanisms happened in this work. Thus, a better equilibrium on ATP turnover in the first minutes of exercise would be more evident after VH$_{\text{EXP}}$. On the other hand, the [La] was markedly augmented (~7 mmol.L$^{-1}$) at the start of VH$_{\text{EXP}}$ main bout and remained stable during 3 min ($\Delta$ [La] 0.4 ± 0.9 mmol.L$^{-1}$) in spite of a significant increase ~3 mmol.L$^{-1}$ in VH$_{\text{CON}}$. This difference at the start of the exercise could be indicating a higher oxidative production of ATP to maintain the work rate since that glycolytic production could be limited to “critical levels”. The VO$_{2\text{sc}}$ could be caused by inhibition of ATP production by anaerobic glycolysis by progressive cytosol acidification (Korzeniewski and Zoladz 2015, Zoladz et al. 2016) and, this scenario slows down further progress on the VO$_{2\text{sc}}$ (Zoladz et al. 2016). Considering that $\Delta$ [La] 3min at VH$_{\text{EXP}}$ was very close to zero because the higher acidosis caused by prior exercise, it is reasonable to assume these reasons in light of the present results.

Lastly, Hopker et al. (2016) observed significant muscle fatigue assessed by 6 s MIE (90 rev.min$^{-1}$) after a muscle damage protocol but, the metabolic acidosis and VO$_{2\text{SC}}$ (464 ± 302 mL.min$^{-1}$ in fatigue situation vs. 556 ± 223 mL.min$^{-1}$ in control) were not
significantly different during the cycling exercise. These authors concluded that a locomotor muscle fatigue without inducing metabolic stress could be dissociated from the VO$_{2sc}$ and, an additional recruitment of motor units to compensate for muscle fatigue might not be the main driver of the VO$_{2sc}$ during high-intensity cycling exercise. Unfortunately, in the present work it is not possible to dissociate a possible decrease within muscle contractile capacity from metabolic acidosis induced by VH$_{EXP}$. Nevertheless, agreeing to Hopker et al. (2016) it is noteworthy that changes caused by priming on the VO$_{2sc}$ does not mean a “mirror effect” on the muscle force production behavior. Thus, these results, do not confirm the cause-effect relationship between muscle fatigue and VO$_{2sc}$.

Limitations

The present study used a 5-s MIE to quantify muscle fatigue during VH intensity cycling. With this technique, the fatigue measured is an “overall” picture of fatigue and cannot discriminate its central and peripheral components. Thus, it is not possible to ascertain whether the proportional contributions of central and peripheral factors to the reductions in maximal isokinetic torque were consistent across time or between conditions. For example, a significant reduction in central drive may have influenced the MIE differently in VH$_{EXP}$ relative to VH$_{CON}$. In addition, the pre-exercise MIE in VH$_{EXP}$ was performed before the bout of VH priming (rather than after) and therefore it was assumed that the pre-exercise muscle torque and power values were the same before versus after the bout of VH priming in VH$_{EXP}$. In support of this assumption, our pilot data showed that torque production returned to pre-exercise values as early as 1 minute after a bout of exhaustive priming (Table 4). Therefore, it was assumed that the pre-exercise values in VH$_{EXP}$ would have been the same regardless of whether they had been measured before or after priming.

Conclusion
This study observed a decrease on the VO$_{2SC}$ after a priming VH intensity exercise that was accompanied by higher metabolic acidosis and cardiovascular responses. Besides, the time-course of muscle force production was the same in the control and prior exercise conditions. It was observed that there was a temporal relationship between muscle fatigue and VO$_{2SC}$ regardless of the condition. Therefore, it seems that the mechanisms that explain muscle fatigue in part could explain the VO$_{2SC}$. Considering that VO$_{2SC}$ attenuation was not accompanied by the muscle force production behavior, these findings do not convincingly support the hypothesis of a causal relationship between time-course of muscle fatigue and VO$_{2SC}$ during VH intensity exercise.

Conflict of Interest

None of the authors of this paper has a competing interest.

References


Author contributions
Conception and design of the work: B.S.D. and P.C.N.S. Acquisition, analysis and interpretation of data for the work: P.C.N.S, K.M.S, R.D.L and B.S.D. Drafting the work or revising it critically for important intellectual content: P.C.N.S, K.M.S, R.D.L, L.G.A.G and B.S.D. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.
### Table 1 Peak exercise values obtained during ramp incremental cycling.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean ± SD</th>
<th>Lower bound</th>
<th>Upper bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO$_{2\text{peak}}$ (L.min$^{-1}$)</td>
<td>4.2 ± 0.5</td>
<td>3.9</td>
<td>4.5</td>
</tr>
<tr>
<td>VO$_{2\text{peak}}$ (ml.kg.min$^{-1}$)</td>
<td>51.5 ± 7.4</td>
<td>47.0</td>
<td>56.0</td>
</tr>
<tr>
<td>HR$_{\text{max}}$ (bpm)</td>
<td>181 ± 12</td>
<td>174</td>
<td>188</td>
</tr>
<tr>
<td>P$_{\text{max}}$ (W)</td>
<td>378 ± 39</td>
<td>355</td>
<td>402</td>
</tr>
<tr>
<td>[La]$_{\text{peak}}$ (mmol.L$^{-1}$)</td>
<td>10.6 ± 2.3</td>
<td>9.2</td>
<td>11.9</td>
</tr>
</tbody>
</table>

CI = confidence interval. SD = standard deviation. VO$_{2\text{peak}}$ = peak of oxygen uptake. HR$_{\text{max}}$ = heart rate maximal values. P$_{\text{max}}$ = maximal power output. [La]$_{\text{peak}}$ = blood lactate peak values.
Table 2: VO\textsubscript{2} parameters during the square-wave bouts with and without prior exercise.

<table>
<thead>
<tr>
<th></th>
<th>VH\textsubscript{CON}</th>
<th>VH\textsubscript{EXP}</th>
<th>t value</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{VO}_2\text{baseline}) (L.min(^{-1}))</td>
<td>1.27 ± 0.17 (95%CI: 1.17-1.38)</td>
<td>1.33 ± 0.17(*) (95%CI: 1.23-1.43)</td>
<td>2.99</td>
<td>0.02</td>
</tr>
<tr>
<td>(A) (L.min(^{-1}))</td>
<td>2.28 ± 0.27 (95%CI: 2.12-2.44)</td>
<td>2.48 ± 0.39(*) (95%CI: 2.25-2.72)</td>
<td>2.82</td>
<td>0.02</td>
</tr>
<tr>
<td>(\text{Gain}) (ml.W(^{-1}).min(^{-1}))</td>
<td>8.8 ± 0.7 (95%CI: 8.4-9.2)</td>
<td>9.5 ± 0.6(*) (95%CI: 9.2-9.9)</td>
<td>2.65</td>
<td>0.02</td>
</tr>
<tr>
<td>(A_{\text{TOTAL}}) (L.min(^{-1}))</td>
<td>3.55 ± 0.38 (95%CI: 3.33-3.78)</td>
<td>3.81 ± 0.48(*) (95%CI: 3.52-4.10)</td>
<td>4.33</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>(\tau) (s)</td>
<td>24.5 ± 7.9 (95%CI: 19.8-29.3)</td>
<td>27.8 ± 6.8 (95%CI: 23.8-31.9)</td>
<td>1.40</td>
<td>0.19</td>
</tr>
<tr>
<td>(\text{TD}) (s)</td>
<td>13.4 ± 4.8 (95%CI: 10.5-16.2)</td>
<td>9.6 ± 4.3(*) (95%CI: 7.1-12.2)</td>
<td>2.25</td>
<td>0.04</td>
</tr>
<tr>
<td>(\text{VO}_2\text{SC} 3\text{ min})</td>
<td>0.28 ± 0.23 (95%CI: 0.14-0.42)</td>
<td>0.12 ± 0.11(*) (95%CI: 0.05-0.18)</td>
<td>4.78</td>
<td>0.04</td>
</tr>
<tr>
<td>(\text{VO}_2\text{SC} 8\text{ min})</td>
<td>0.63 ± 0.30 (95%CI: 0.45-0.80)</td>
<td>0.34 ± 0.18(*) (95%CI: 0.23-0.45)</td>
<td>4.78</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>(\text{VO}_{2\text{SC}\text{trajectory}})</td>
<td>0.11 ± 0.04 (95%CI: 0.08-0.13)</td>
<td>0.06 ± 0.03(*) (95%CI: 0.04-0.08)</td>
<td>4.66</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>(\text{TD}_s) (s)</td>
<td>144 ± 46 (95%CI: 116-171)</td>
<td>162 ± 33 (95%CI: 142-182)</td>
<td>1.19</td>
<td>0.26</td>
</tr>
<tr>
<td>(\text{VO}_{2\text{END}})</td>
<td>4.18 ± 0.50 (95%CI: 3.88-4.48)</td>
<td>4.15 ± 0.52 (95%CI: 3.83-4.46)</td>
<td>0.67</td>
<td>0.51</td>
</tr>
</tbody>
</table>

\(\text{VH}_{\text{CON}}\): control condition. \(\text{VH}_{\text{EXP}}\): experimental condition. \(\text{VO}_2\text{baseline}\) = the average value over the one min of resting baseline; \(A\) = the amplitude for the fundamental phase; \(A_{\text{TOTAL}} = A + \text{VO}_2\text{baseline}\); \(\tau\) = the time constant; \(\text{TD}\) = the time delay; \(\text{VO}_{2\text{SC}}\) = slow component of \(\text{VO}_2\); \(\text{TD}_s\) = TD of \(\text{VO}_{2\text{SC}}\) phase; \(\text{VO}_{2\text{END}}\) = the average \(\text{VO}_2\) value over the last 15 s at 3 or 8 min of exercise.
Table 3: Peak torque and peak power output measured during the maximal isokinetic effort performed previous or following square-wave bouts with and without prior exercise.

<table>
<thead>
<tr>
<th>Condition</th>
<th>VH&lt;sub&gt;CON&lt;/sub&gt;</th>
<th>VH&lt;sub&gt;EXP&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-exercise</td>
<td>3min</td>
</tr>
<tr>
<td>Peak Torque (Nm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VH&lt;sub&gt;CON&lt;/sub&gt;</td>
<td>136 ± 26</td>
<td>114 ± 28</td>
</tr>
<tr>
<td>VH&lt;sub&gt;EXP&lt;/sub&gt;</td>
<td>(95%CI: 120-151)</td>
<td>(95%CI: 97-131)</td>
</tr>
<tr>
<td>Peak Torque (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VH&lt;sub&gt;CON&lt;/sub&gt;</td>
<td>100</td>
<td>84 ± 6</td>
</tr>
<tr>
<td>VH&lt;sub&gt;EXP&lt;/sub&gt;</td>
<td>(95%CI: 80-87)</td>
<td>(95%CI: 72-84)</td>
</tr>
<tr>
<td>Peak Power (W)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VH&lt;sub&gt;CON&lt;/sub&gt;</td>
<td>1705 ± 34</td>
<td>1347 ± 393</td>
</tr>
<tr>
<td>VH&lt;sub&gt;EXP&lt;/sub&gt;</td>
<td>(95%CI: 1497-1913)</td>
<td>(95%CI: 1110-1585)</td>
</tr>
<tr>
<td>Peak Power (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VH&lt;sub&gt;CON&lt;/sub&gt;</td>
<td>100</td>
<td>78 ± 8</td>
</tr>
<tr>
<td>VH&lt;sub&gt;EXP&lt;/sub&gt;</td>
<td>(95%CI: 73-82)</td>
<td>(95%CI: 65-69)</td>
</tr>
</tbody>
</table>

VH<sub>CON</sub>: control condition. VH<sub>EXP</sub>: experimental condition. There was a significant effect on time within subjects for both variables. Different letters showed significant differences p<0.01.
Table 4: Peak torque and peak power output measured during the maximal isokinetic effort performed previous or 1 min after very heavy-intensity exercise until exhaustion (pilot study).

<table>
<thead>
<tr>
<th>Very heavy exercise</th>
<th>Pre-exercise</th>
<th>Post-exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak Torque (Nm)</td>
<td>136 ± 25</td>
<td>132 ± 31</td>
</tr>
<tr>
<td></td>
<td>(95%CI: 121-152)</td>
<td>(95%CI: 113-151)</td>
</tr>
<tr>
<td>Peak Power output (W)</td>
<td>1722 ± 335</td>
<td>1616 ± 399</td>
</tr>
<tr>
<td></td>
<td>(95%CI: 1520-1925)</td>
<td>(95%CI: 1375-1857)</td>
</tr>
</tbody>
</table>

There was no significant effect on time within subjects for both variables.
Figures

**Figure 1.** Experimental design of study. GET = Gas exchange threshold. MIE = Maximal isokinetic effort. Upper panel = control exercise condition. Lower Panel = Prior exercise condition

**Figure 2.** Mean group values of oxygen uptake (VO\(_2\)) in square-wave exercise transitions set to deplete 70% W\(^*\). Triangle symbols represent the control condition (VH\(_{CON}\)). Open circle symbols represent the prior exercise condition (VH\(_{EXP}\)). The gray triangle and the black circle represent the slow component of VO\(_2\) (VO\(_{2SC}\)) at 3 min during VH\(_{CON}\) and VH\(_{EXP}\) condition, respectively. The dotted vertical lines represent begin of exercise and the start of VO\(_{2SC}\). The continued lines showed the fit and residuals (gray for VH\(_{CON}\) and black for VH\(_{EXP}\)). Note that the VO\(_{2SC}\) was significant different between conditions and the VO\(_2\) maximal values (VO\(_{2peak}\)) were attained

**Figure 3.** VH\(_{CON}\): control condition. VH\(_{EXP}\): experimental condition. There was a significant effect condition x time for blood lactate concentration ([La]) and \(\Delta[La]\) (\(F = 68.33, p <0.01;\) \(F = 14.41, p <0.01\), respectively). Different letters showed significant differences p< 0.05. Open triangles represent the VH\(_{CON}\); open circles represent the VH\(_{EXP}\)

**Figure 4.** VH\(_{CON}\): control condition. VH\(_{EXP}\): experimental condition. There was no significant interaction condition x time for \(\Delta\)torque \(F = 1.09, p = 0.35\); but a significant effect on time within subjects; \(F = 83.4, p <0.01\). Different letters showed significant differences

**Figure 5.** VO\(_{2SC}\) = slow component of oxygen uptake. Panels A and B: Control condition. Panels C and D: prior exercise condition. There was significant temporal relationship between VO\(_{2SC}\) magnitude at 3 min (white circles) or 8 min (black circles) and \(\Delta\) torque or \(\Delta\) power at 3rd and 8th min. The bigger white and black circles represent mean values at 3 min and 8 min of exercise, respectively
Figure 1. Experimental design of study. GET = Gas exchange threshold. MIE = Maximal isokinetic effort. Upper panel = control exercise condition. Lower Panel = Prior exercise condition.
Figure 2. Mean group values of oxygen uptake (VO2) in square-wave exercise transitions set to deplete 70% W’. Triangle symbols represent the control condition (VHCON). Open circle symbols represent the prior exercise condition (VHEXP). The gray triangle and the black circle represent the slow component of VO2 (VO2SC) at 3 min during VHCON and VHEXP condition, respectively. The dotted vertical lines represent begin of exercise and the start of VO2SC. The continued lines showed the fit and residuals (gray for VHCON and black for VHEXP). Note that the VO2SC was significant different between conditions and the VO2 maximal values (VO2peak) were attained.
Figure 3. VHCON: control condition. VHEXP: experimental condition. There was a significant effect condition x time for blood lactate concentration ([La]) and Δ[La] (F = 68.33, p < 0.01; F = 14.41, p < 0.01, respectively). Different letters showed significant differences p < 0.05. Open triangles represent the VHCON; open circles represent the VHEXP.
Figure 4. VHCON: control condition. VHEXP: experimental condition. There was no significant interaction condition x time for ∆torque F = 1.09, p = 0.35; but a significant effect on time within subjects; F = 83.4, p <0.01. Different letters showed significant differences.
Figure 5. VO2SC = slow component of oxygen uptake. Panels A and B: Control condition. Panels C and D: prior exercise condition. There was significant temporal relationship between VO2SC magnitude at 3 min (white circles) or 8 min (black circles) and Δ torque or Δ power at 3rd and 8th min. The bigger white and black circles represent mean values at 3 min and 8 min of exercise, respectively.