

## Article

# Physiological, Perceptual, and Neuromuscular Responses to $\dot{V}O_2$ -Clamp Cycle Ergometry Exercise

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**Abstract:** Recommendations for endurance exercise prescription are often based on percentages of heart rate (HR) or the volume of oxygen consumption ( $\dot{V}O_2$ ) maximum or reserve that is extrapolated to a power output (P) or velocity. Previous work has demonstrated dissociations of the expected responses to exercise anchored to the critical heart rate (CHR) compared with the P associated with CHR. However, it is unclear if similar dissociations due to reductions in P to maintain the designated intensity would be present during exercise anchored to the  $\dot{V}O_2$  associated with CHR ( $\dot{V}O_{2CHR}$ ). The purpose of this study was to examine the patterns in physiological ( $\dot{V}O_2$ , HR, P, respiration rate [RR], muscle oxygen saturation [%SmO<sub>2</sub>]), neuromuscular (electromyographic and mechanomyographic amplitude [EMG AMP, MMG AMP], mean power frequency [EMG MPF, MMG MPF]), and perceptual (rating of perceived exertion [RPE]) responses during exercise at  $\dot{V}O_{2CHR}$  ( $\dot{V}O_2$ -clamp). On separate days, ten participants (age:  $25 \pm 4$  yr) performed a graded exercise test and four constant P trials at 85–100% of peak P (PP) to derive CHR and  $\dot{V}O_{2CHR}$ . Responses were recorded during a trial to exhaustion at  $\dot{V}O_{2CHR}$  ( $32.86 \pm 7.12$  mL·kg<sup>−1</sup>·min<sup>−1</sup>;  $T_{Lim} = 31.31 \pm 21.37$  min) and normalized in 10% intervals of  $T_{Lim}$  to their respective values at PP. The one-way repeated-measures ANOVA with post hoc, Bonferroni-corrected, pairwise comparisons indicated differences ( $p < 0.001$ ) from baseline for HR (mean  $\pm$  SD %change =  $8 \pm 3\%$ ), RR ( $43 \pm 38\%$ ), P ( $-15 \pm 5\%$ ), EMG MPF ( $10 \pm 8\%$ ), and RPE ( $65 \pm 38\%$ ), but no differences ( $p = 0.077$ – $0.955$ ) for %SmO<sub>2</sub> ( $-17 \pm 53\%$ ), EMG AMP ( $-3 \pm 16\%$ ), MMG AMP ( $40 \pm 61\%$ ), and MMG MPF ( $1 \pm 7\%$ ). The loss in performance observed during  $\dot{V}O_2$ -Clamp exercise may provide a quantification of the inefficiency associated with the  $\dot{V}O_2$  slow component phenomenon. The neuromuscular responses suggested constant muscle excitation despite the reductions in P, but the metabolic and perceptual responses suggested a combination of feedforward and feedback mechanisms regulating  $T_{Lim}$ . Future studies should further examine responses to the  $\dot{V}O_2$ -Clamp exercise at a uniform threshold.

**Keywords:** cycling; endurance exercise; fatigue



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## 1. Introduction

The current recommendations for exercise prescription to improve cardiorespiratory endurance often rely on percentages of oxygen uptake ( $\dot{V}O_2$ ) and heart rate (HR) maximum or reserve ( $\dot{V}O_{2R}$  and  $HRR$ ) that are low enough to be maintained for 20 to 60 min but high enough to elicit the desired metabolic and cardiorespiratory responses [1]. The threshold (% $\dot{V}O_2$  or  $HR_{max}$ ) for improving cardiorespiratory fitness is dependent upon an individual's training status, where intensities of 65–80%  $\dot{V}O_{2max}$  in moderately trained

individuals and 95–100%  $\dot{V}O_{2\max}$  in highly trained individuals may be required to elicit adaptations [2]. However, the current understanding of physiological and perceptual responses to continuous, dynamic exercise is centered primarily on measures taken when exercise intensity is anchored by power output and not  $\dot{V}O_2$  or HR [3]. During exercise maintained at a constant power output above the ventilatory threshold (VT), there are predictable, intensity-dependent increases in HR,  $\dot{V}O_2$ , respiration rate (RR), muscle excitation, measured via electromyographic amplitude (EMG AMP), and ratings of perceived exertion (RPE) [3–12]. Additionally, fatiguing, constant power exercise is characterized by decreases in action potential conduction velocity along the sarcolemma, measured via EMG mean power frequency (EMG MPF), as a result of the accumulation of metabolic byproducts [9–12]. The motor unit excitation strategies are further characterized by changes in motor unit recruitment, measured via mechanomyographic amplitude (MMG AMP), and the global motor unit firing rate, measured via mechanomyographic mean power frequency (MMG MPF) [9,10,12]. The specific MMG AMP and MMG MPF responses have been shown to vary depending on the power output [9,10,12], but in conjunction with EMG measures, these further delineate the EMG AMP (muscle excitation) into factors related to the recruitment and firing rate of motor units due to muscular fatigue [9–12]. Thus, exercise anchored by power output is described by specific responses that reflect increases in the metabolic and cardiovascular intensity throughout the work bout and, at higher power outputs ( $>VT$ ), may result in evidence of neuromuscular fatigue. Depending on the power output at which the exercise is performed, it is possible for  $\dot{V}O_2$  and HR to increase during the exercise bout to values that are greater than the prescribed ranges for the training session.

Previously, researchers have explored the physiological and perceptual responses when exercise was anchored to a perceptual (RPE-Clamp) or physiological parameter ( $\dot{V}O_2$ -Clamp, HR-Clamp), that is, an exercise where the perceptual (RPE) or physiological ( $\dot{V}O_2$  or HR) parameter is held constant by altering the external load (i.e., speed or power output) [13–17]. Under these conditions, the physiological responses no longer followed the predictable patterns described for constant power output exercise. For example, during the HR-Clamp exercise, RPE and RR increased, but power output,  $\dot{V}O_2$ , and minute ventilation ( $\dot{V}_E$ ) decreased [15–17]. Similarly, during the RPE-Clamp exercise,  $\dot{V}O_2$ , respiratory exchange ratio (RER), and power output decreased [13]. In addition, muscle excitation during exercise anchored by a perceptual or physiological parameter did not follow the expected pattern of fatigue during constant power output exercise but instead tracked the decreases in power output [13,15–17]. Thus, the current evidence indicates there are divergent patterns of responses for physiological and perceptual parameters when exercise is anchored to a physiological (HR) or perceptual (RPE) parameter compared with exercise anchored to a power output.

The application of HR to the critical power (CP) model to derive the critical heart rate (CHR) [18] has been suggested to provide an individualized, physiologically based threshold that meets the intensity (83–94% HR of  $\dot{V}O_{2\text{peak}}$ ) and duration ( $>24$  min) recommendations for cardiorespiratory endurance training [16,17,19]. The CHR is derived from the linear regression of the total heartbeats ( $HB_{\text{Lim}}$ ) versus  $T_{\text{Lim}}$  from multiple exhaustive work bouts at various power outputs. Previous work [16,17,19] has shown that the decreases in work rate required to maintain HR at CHR results in more sustainable intensities (i.e., longer times to exhaustion ( $T_{\text{Lim}}$ )) than those anchored by power output yet elicit a metabolic stimulus within the recommended range [16–18]. Specifically, exercise at or just below (minus 5  $\text{b}\cdot\text{min}^{-1}$ ) CHR was sustained from ~12 to 60 min with metabolic responses maintained (64–88%  $\dot{V}O_{2\text{peak}}$ ) within the desired ranges for improving cardiorespiratory fitness [17,19]. Succi et al. [19] directly compared exercise at CHR to exercise at the power output associated with CHR and demonstrated that CHR was more sustainable (longer  $T_{\text{Lim}}$ ) than constant power output exercise. These differences in sustainability may potentially result in disparate physiological stimuli depending on

the anchoring scheme. Specifically, during exercise anchored to both a constant HR and a constant power output, subjects demonstrated  $\dot{V}O_2$ , HR, and RPE values that were within the American College of Sports Medicine (ACSM) exercise prescription guidelines [1,19]. However, during constant power output exercise, participants demonstrated progressive increases in  $\dot{V}O_2$ , HR, and RPE that reached near maximal values, leading to the premature termination of exercise prior to or shortly after achieving the recommended duration of the exercise of at least 20 min [19]. Conversely, during the HR-Clamp exercise, participants were able to sustain exercise for longer, thus incurring a potentially greater time stimulus. In addition, it was demonstrated that exercise at CHR resulted in lower  $\dot{V}O_2$ , RPE, muscle excitation, and higher muscle oxygen saturation (%SmO<sub>2</sub>) responses compared with constant power output exercise [19]. Therefore, the changes in power output to maintain the designated HR at CHR result in the differing sustainability, metabolic, neuromuscular, and perceptual responses versus exercise anchored to the power output associated with CHR [19]. Notably, these alterations in power output resulted in a mirror image of the  $\dot{V}O_2$  slow component phenomenon is typically seen during constant power exercise [19,20]. That is, during sufficiently intense constant power output exercise ( $>VT$ ), there is a progressive increase ( $>200 \text{ mL} \cdot \text{min}^{-1}$ ) in  $\dot{V}O_2$  greater than what would be expected due to the recruitment of less efficient, higher threshold motor units [20,21]. However, the change of power output during HR-Clamp exercise has been suggested to reflect the performance loss (i.e., power output or velocity decrease) as a result of this muscular inefficiency [20]. Thus, examining the responses to  $\dot{V}O_2$ -Clamp exercise at the  $\dot{V}O_2$  associated with the CHR may provide additional insights into the dissociations of responses between exercise at a constant physiological parameter and constant power output exercise.

The moderate, heavy, and severe intensity domains are well-defined by specific physiological and perceptual responses [3–12] for constant power output exercise. Typically, within the moderate intensity domain ( $<VT$ ), there are increases in  $\dot{V}O_2$ , HR, and RPE, which plateau, and exercise is able to be tolerated for an extended period of time ( $>60 \text{ min}$ ) [3]. Within the heavy intensity domain ( $>VT$ ,  $<CP$ ), there are increases in blood lactate and further increases in  $\dot{V}O_2$ , HR, and RPE with the presence of the  $\dot{V}O_2$  slow component phenomenon due to the recruitment of less efficient, higher threshold motor units [3,20]. As a result of this inefficiency, these values take longer to reach a plateau [3,20]. Finally, within the severe intensity domain, the  $\dot{V}O_2$  slow component phenomenon is further exacerbated, resulting in further increases in  $\dot{V}O_2$ , HR, blood lactate, and RPE, which may [3] or may not [22] reach their respective maximums as exhaustion is reached. Conversely, the typical responses within these intensity domains are less clear when exercise is anchored by the physiological parameters (HR and  $\dot{V}O_2$ ) used to derive the current intensity recommendations for improving CE. Furthermore, the specific physiological and perceptual responses to  $\dot{V}O_2$ -Clamp exercises are not well-defined. Previous work from Ribeiro [14] demonstrated that there were general decreases in power output over time to maintain a constant  $\dot{V}O_2$ , with a plateau in HR after an initial increase [14]. However, the authors did not examine the specific time course of responses, nor any neuromuscular or perceptual responses. In addition, it is unclear how the physiological, neuromuscular, and perceptual responses to  $\dot{V}O_2$ -Clamp exercise compares to that of previous work examining the responses to exercise anchored to another physiological parameter such as HR [19]. Thus, the examination of  $\dot{V}O_2$ -Clamp exercise, specifically the  $\dot{V}O_2$  associated with the CHR ( $\dot{V}O_{2CHR}$ ) is warranted to further elucidate the typical patterns of responses for exercise at constant physiological parameters and potential mechanisms underpinning the divergent responses between exercise anchored to a physiological parameter versus constant power output. Therefore, the purpose of this study was to examine the time course of changes in cardiovascular, metabolic, neuromuscular, and perceptual responses during  $\dot{V}O_2$ -Clamp exercise at  $\dot{V}O_{2CHR}$ . It was hypothesized that, based on previous work examining responses to exercise anchored to a constant physiological parameter [17,19],

$\dot{V}O_2$ -Clamp trials would result in an increase of HR, RR, and RPE, decreases in power output, EMG AMP, and MMG MPF, and no change in EMG MPF or MMG AMP.

## 2. Methods

Ten physically active subjects (men:  $n = 6$ ; women:  $n = 4$ ; mean  $\pm$  SD age:  $25.10 \pm 3.73$  years; height:  $173.09 \pm 11.90$  cm; body mass:  $74.66 \pm 8.51$  kg) completed this study. Physically active was defined as completing 30 min of physical activity per day for 5 days per week on most weeks for the past 6 months [1]. Physical activity included walking ( $n = 4$ ), running ( $n = 7$ ), cycling ( $n = 2$ ), swimming ( $n = 3$ ), high-intensity interval training ( $n = 3$ ), resistance training ( $n = 7$ ), and yoga ( $n = 2$ ). The subjects were screened for cardiovascular, pulmonary, metabolic, muscular, and/or coronary heart disease via a health history questionnaire. The subjects were instructed to abstain from caffeine consumption 4 h prior to testing and refrain from exercise 24 h prior to testing. This study was approved by the university's Institutional Review Board for Human Subjects (IRB# 51399), meeting the ethical standards of the Declaration of Helsinki, and all subjects provided a written informed consent document prior to testing.

### 2.1. Experimental Design

This study was based on previous work examining CHR [19]. This study was a repeated measures design that included six visits, with each visit separated by at least 24 h. On visit one, the subjects completed a graded exercise test (GXT) to exhaustion on a cycle ergometer to determine peak oxygen consumption ( $\dot{V}O_{2peak}$ ), HR ( $HR_{max}$ ), respiration rate ( $RR_{max}$ ), peak power output (PPO), and the power at  $\dot{V}O_{2max}$  ( $p\dot{V}O_{2max}$ ). During visits two through five, HR and  $\dot{V}O_2$  were measured during four randomly ordered, continuous rides to exhaustion at 85%, 90%, 95%, and 100% of  $p\dot{V}O_{2peak}$ . The total work versus time to exhaustion ( $T_{Lim}$ ) mathematical model used to determine the critical power (CP) was applied to derive the critical heart rate (CHR), explained in greater detail below [18]. Linear regression of HR versus  $\dot{V}O_2$  was used to determine the  $\dot{V}O_2$  associated with CHR ( $\dot{V}O_{2CHR}$ ). Visit six consisted of a trial to exhaustion at  $\dot{V}O_{2CHR}$  with  $\dot{V}O_2$ , HR, RR, RPE, power output, % $SmO_2$ , RPE, EMG AMP, MMG AMP, EMG MPF and MMG MPF responses were recorded.

### 2.2. Electromyographic and Mechanomyographic Measurements

The EMG signals were measured from the vastus lateralis (VL) on the dominant leg, as determined via which leg the subject would kick a ball with, during the GXT and the tests at  $\dot{V}O_{2CHR}$ . For all subjects, the dominant leg was the right leg. Prior to electrode placement, the skin was shaved with a disposable razor, abraded with sandpaper, and then cleaned with isopropyl alcohol. A bipolar electrode (circular 24 mm, Kendall disposable EMG electrodes, Covidien Ltd., Gosport Hampshire, UK) arrangement (30 mm interelectrode distance) was placed according to the SENIAM Project for EMG electrode placement recommendations [23]. A reference line was drawn one third the distance between the lateral superior border of the patella and the anterior superior iliac spine. The electrode site was located 5 cm lateral to the reference line to ensure the electrodes were over the VL muscle [24]. Lastly, a goniometer (Smith and Nephew Rolyan, Inc., Menomonee Falls, WI, USA) was used to position the EMG electrode at a  $20^\circ$  angle to the reference line to approximate the pennation angle of the VL muscle fibers [25]. The EMG signal was collected using a wired signal (MP150, Biopac Systems, Inc., Santa Barbara, CA, USA) and amplified (gain:  $1000\times$ ) using differential amplifiers (EMG 150, Biopac Systems, Inc., Santa Barbara, CA, USA, bandwidth = 10–500 Hz). The MMG signals were recorded using an accelerometer (Model: EGAS-S704-10 Rev C, Measurement Specialties, Les Clayes Sous Bois, France, gain  $200\times$ , bandwidth 0–200 Hz, dimensions:  $1.0 \times 1.0 \times 0.5$  cm, mass 1.0 g, sensitivity  $10 \text{ mV}\cdot\text{g}^{-1}$ ) placed on the VL between the EMG electrodes using double-sided adhesive tape. The signal was analyzed from the sensitive axis of the accelerometer

positioned perpendicular to the skin surface. The alternative sensitive axis was positioned along the longitudinal axis of the muscle.

### 2.3. Signal Processing

The raw EMG and MMG signals were digitized at 2000 Hz and stored in a personal computer for analysis and processed with a custom program written in LabVIEW programming software (version 7.1, National Instruments, Austin, TX, USA). The EMG and MMG signals were zero-phase bandpass-filtered (fourth-order Butterworth) at 10–500 Hz and 5–100 Hz, respectively. The EMG (root-mean-squared,  $\mu\text{V}_{\text{rms}}$ ) and MMG ( $\text{m}\cdot\text{s}^{-2}$ ) AMP and EMG and MMG MPF (Hz) values were calculated. For the MPF analysis, each data segment was processed with a Hamming window and a discrete Fourier transform (DFT) algorithm. The MPF was selected to represent the power spectrum based on the recommendations of Diemont et al. [26] and Hermens [23] and was calculated as described by Kwatny and colleagues [27].

### 2.4. Muscle Oxygenation Measurements

A NIRs (MOXY, Fortiori Design LLC, Hutchinson, MN, USA) device was placed on the VL of the left thigh using SENIAM guidelines, approximately one-third the distance between the lateral superior border of the patella and the anterior superior iliac spine. Prior to placement, the skin was shaved with a disposable razor, abraded with sandpaper, and then cleaned with isopropyl alcohol. The MOXY uses four wavelengths of NIRs light at 680, 720, 760, and 800 nm, and the sensor contains a single LED and two detectors placed 12.5 and 25.0 mm from the source. The %SmO<sub>2</sub> was continuously measured during all trials to exhaustion. During each session, the system was connected to a personal computer using the manufacturer's software program (Peripedal©, Version 1.5.5) to provide a graphic display of the data. Data were acquired at 2 Hz and obtained from the sensor's internal memory. Data were extracted from the Moxy device using the Moxy PC application.

### 2.5. Determination of $\dot{V}\text{O}_{2\text{peak}}$ and Ventilatory Threshold

The subjects performed a GXT to exhaustion on a calibrated cycle ergometer (Lode, Corival, Groningen, The Netherlands) to determine the  $\dot{V}\text{O}_{2\text{peak}}$ ,  $\text{HR}_{\text{peak}}$ ,  $\text{RR}_{\text{peak}}$ ,  $\text{p}\dot{V}\text{O}_{2\text{peak}}$ , %SmO<sub>2peak</sub>, EMG AMP<sub>peak</sub>, EMG MPF<sub>peak</sub>, MMG AMP<sub>peak</sub>, and MMG MPF<sub>peak</sub>. The subject was fitted with a nose clip, mouthpiece mounted to a headset (2700; Hans Rudolph, Kansas City, MO, USA), and heart rate monitor (Polar Heart Watch system, Polar Electro Inc., Lake Success, New York, NY, USA). The ergometer seat height was adjusted so the subject's leg was near full extension at the bottom of the pedal revolution. Expired gas samples were collected and analyzed using a calibrated TrueMax 2400 metabolic cart (Parvo Medics, Sandy, UT, USA). Prior to testing, the gas analyzers were calibrated to room air and gases of known concentration, and the NIRs device, EMG electrodes, and MMG accelerometer were placed as previously described. The NIRs, EMG, and MMG signals were recorded throughout the test. The oxygen (O<sub>2</sub>) and carbon dioxide (CO<sub>2</sub>) parameters were expressed as 20 s averages [28]. The HR was recorded continuously and expressed as 5 s averages. Each subject was asked to give an RPE during the last 10 s of each min using the Borg 6–20 RPE scale [29]. The test began at 50 W at a pedal cadence of 70 rev·min<sup>−1</sup>, and the power output was increased by 30 W every two min until the subject could no longer maintain the 70 rev·min<sup>−1</sup> cadence. The  $\dot{V}\text{O}_{2\text{peak}}$ ,  $\text{HR}_{\text{peak}}$ , and  $\text{RR}_{\text{peak}}$  were defined as the highest 20 s average  $\dot{V}\text{O}_2$ , HR, and RR values, respectively, were recorded during the test. Following completion of the test, the subject performed a cool down on the cycle ergometer for a self-determined period of time. The VT was determined using the V-slope method [30], where the VT was defined as the power output associated with  $\dot{V}\text{O}_2$  corresponding to the point of intersection of two separately derived regression lines of the  $\dot{V}_E$  versus  $\dot{V}\text{O}_2$  plot.

## 2.6. Determination of CP and $\dot{V}O_2$ CHR

The CHR was determined from four randomly ordered constant power output rides to exhaustion at 85%, 90%, 95%, and 100%  $p\dot{V}O_{2peak}$ . The cycle ergometer was set up for each subject as previously described, and the subject was fitted with a Polar Heart Rate monitor, respiration rate/ECG monitor, EMG electrodes, and MMG accelerometer. The RPE, HR,  $\dot{V}O_2$ , RR, and %SmO<sub>2</sub> were measured as previously described. The rides were performed to exhaustion at 70 rev·min<sup>-1</sup>, and the power outputs were selected so that the time to exhaustion ( $T_{Lim}$ ) ranged from 3 to 15 min. The HR values were taken every 5 s ( $HR_{avg}$ ). The total number of heartbeats ( $HB_{Lim}$ ) during each of the rides to exhaustion was calculated as the product of  $T_{Lim}$  (min) and  $HR_{avg}$  (b·min<sup>-1</sup>) [18]. The  $HB_{Lim}$  for each trial was plotted against  $T_{Lim}$ . The slope coefficient of the regression of the  $HB_{Lim}$  versus  $T_{Lim}$  relationship was defined as the CHR. Similarly, the CP was defined as the slope coefficient of the regression of the total work done during each constant power trial versus the  $T_{Lim}$  relationship. The  $\dot{V}O_2$ CHR was determined using the linear regression equation of the HR versus  $\dot{V}O_2$  relationship to find the  $\dot{V}O_2$  associated with CHR.

## 2.7. Continuous Rides to Exhaustion at $\dot{V}O_2$ CHR

The  $\dot{V}O_2$ , HR, RR, RPE, power output, %SmO<sub>2</sub>, EMG and MMG AMP and MPF, and time to exhaustion ( $T_{Lim}$ ) were examined during continuous rides at  $\dot{V}O_2$ CHR. Prior to testing, the NIRs device, EMG electrodes, and MMG accelerometer were placed as previously described. The subjects completed a five min warm-up at 50 W on the cycle ergometer. After one min of passive rest, the power was increased to elicit the  $\dot{V}O_2$  values at the  $\dot{V}O_2$ CHR. If the  $\dot{V}O_2$ CHR was not achieved within 5 min, then the power output was gradually increased to elicit the designated  $\dot{V}O_2$ . The cadence was maintained at 70 rev·min<sup>-1</sup>, and the test was terminated when the subject was unable to maintain the designated cadence for 10 s despite strong verbal encouragement or when they completed 60 min of exercise. The  $\dot{V}O_2$ , HR, RR, and power output were recorded as 20 s averages. The %SmO<sub>2</sub> was recorded every 2 s and expressed as 20 s averages. The RPE values were taken every min. The EMG and MMG signals were recorded continuously throughout the rides. The time spent in each exercise intensity domain was calculated as the sum of the time that the power output corresponded to severe (>CP), heavy (<CP, >VT), and moderate (<VT), including the time to reach the designated threshold. However, the time to reach the designated threshold was not included in the total time to exhaustion. To synchronize the data from the various instruments, the NIRs device was turned on 1 min prior to the start of the test to match with the starting timestamp within the metabolic cart and the first pedal revolution shown via the neuromuscular signals.

## 2.8. Statistical Analyses

The composite responses for each variable ( $T_{Lim}$ ,  $\dot{V}O_2$ , HR, RR, power output, RPE, EMG AMP, EMG MPF, MMG AMP, and MMG MPF) were recorded during the trial to exhaustion at  $\dot{V}O_2$ CHR and normalized as a percentage of the maximal value from the GXT. The %SmO<sub>2</sub> was expressed as the absolute measure during the trial [31,32]. Because each subject had a different time to exhaustion, time was normalized as a percentage of  $T_{Lim}$  and 11 data points (0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100%) were used for all metabolic data and 10 data points (0–10, 10–20, 20–30, 30–40, 40–50, 50–60, 60–70, 70–80, 80–90, 90–100%) were used for the neuromuscular data. The time to achieve  $\dot{V}O_2$ CHR was omitted from the analyses to account for the neuromuscular and metabolic kinetic response at the beginning of the exercise. Sphericity was assessed using Mauchly's test of sphericity, and normality was assessed via the Shapiro–Wilk test, visual inspection of histograms, and Q-Q plots. Separate one-way repeated measures ANOVA and post hoc pairwise comparisons with a Bonferroni-corrected alpha level were used to examine the metabolic ( $\dot{V}O_2$ , HR, RR,

power output, %SmO<sub>2</sub>), perceptual (RPE), and neuromuscular (EMG and MMG AMP and MPF) responses across time relative to the initial value. An alpha level of  $p \leq 0.05$  was used to determine statistical significance for all ANOVAs. The Bonferroni-corrected alpha for the metabolic and neuromuscular comparisons were set at  $p \leq 0.005$  and  $p \leq 0.0056$ , respectively. All statistical analyses were performed using the Statistical Package for the Social Sciences software (v.25.0 IBM SPSS Inc., Chicago, IL, USA).

### 3. Results

The results of the GXT demonstrate a mean  $\pm$  SD  $\dot{V}O_{2\max}$   $41.50 \pm 8.81 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ,  $HR_{\max}$  of  $188 \pm 11 \text{ b}\cdot\text{min}^{-1}$ , a  $RR_{\max}$  of  $52.11 \pm 9.76 \text{ breaths}\cdot\text{min}^{-1}$ , and  $p\dot{V}O_{2\max}$  of  $269 \pm 63 \text{ W}$ . Furthermore, VT and CP occur at  $145.89 \pm 31.02 \text{ W}$  and  $194.15 \pm 48.07 \text{ W}$ , respectively. The  $T_{\text{Lim}}$  and power outputs for the determination of CHR range from 2.68–11.75 min and 170–380 W, respectively. The mean  $\pm$  SD CHR is  $169.65 \pm 14.26 \text{ b}\cdot\text{min}^{-1}$  and the  $\dot{V}O_{2\text{CHR}}$  occur at  $32.86 \pm 7.12 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  which represents  $79.4 \pm 0.08\%$   $\dot{V}O_{2\max}$  from the GXT.

During the trial at  $\dot{V}O_{2\text{CHR}}$ , the subjects achieved the designated  $\dot{V}O_2$  within 2.63–7.62 min ( $4.55 \pm 1.65 \text{ min}$ ) and were able to sustain exercise for 8.66–60 min ( $31.31 \pm 21.37 \text{ min}$ ). The mean  $\dot{V}O_2$  maintained throughout the trial at  $\dot{V}O_{2\text{CHR}}$  was  $32.17 \pm 6.81 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  ( $77.8\% \pm 0.08\%$  of  $\dot{V}O_{2\text{peak}}$ ). The range of percent changes (% $\Delta$ ) from the time  $\dot{V}O_{2\text{CHR}}$  was reached at the end of the trial for HR, RR, power output, RPE, and %SmO<sub>2</sub> were 5–14% ( $8 \pm 3\%$ ), 8–125% ( $43 \pm 38\%$ ),  $-7$ – $-23\%$  ( $-15 \pm 5\%$ ), 33–150% ( $65 \pm 38\%$ ), and  $-24$ – $156\%$  ( $-17 \pm 53\%$ ), respectively. The results of the one-way repeated measures ANOVAs and post hoc Bonferroni-corrected pairwise comparisons indicate there were significant differences among the time points for HR, RR, power output, and RPE ( $F(10, 90) = 4.339$ – $26.14$ ,  $p < 0.001$ ,  $\text{p}\eta^2 = 0.325$ – $0.744$ ) (Figure 1; Table 1). There was no difference among the time points for %SmO<sub>2</sub> ( $F(10, 90) = 43.026$ ,  $p = 0.474$ ,  $\text{p}\eta^2 = 0.097$ ). While there was a difference among the time points for  $\dot{V}O_2$  ( $F(10, 90) = 2.836$ ,  $p = 0.004$ ,  $\text{p}\eta^2 = 0.240$ ), the Bonferroni-corrected pairwise comparisons indicate there is no difference in  $\dot{V}O_2$  from start to finish (Figure 1). However, there is a significant difference where 50%  $T_{\text{Lim}}$  is lower than the initial time point. This result is most likely the result of a time lag in  $\dot{V}O_2$  response to power output adjustments, which caused an undershoot in the  $\dot{V}O_2$  below the designated  $\dot{V}O_2$  (Figure 1A).

During the trial at  $\dot{V}O_{2\text{CHR}}$ , the % $\Delta$  in EMG AMP, EMG MPF, MMG AMP, and MMG MPF were  $-23$ – $33\%$  ( $-3 \pm 16\%$ ),  $2$ – $27\%$  ( $10 \pm 8\%$ ),  $-3$ – $130\%$  ( $40 \pm 61\%$ ), and  $-6$ – $14\%$  ( $1 \pm 7\%$ ). The one-way repeated-measures ANOVA and Bonferroni-corrected pairwise comparisons indicate there is a difference among the time points only for EMG MPF ( $F(10, 90) = 10.768$ ,  $p < 0.001$ ,  $\text{p}\eta^2 = 0.545$ ), and no differences for EMG AMP ( $F(10, 90) = 1.822$ ,  $p = 0.077$ ,  $\text{p}\eta^2 = 0.168$ ) and MMG AMP ( $F(10, 90) = 0.349$ ,  $p = 0.955$ ,  $\text{p}\eta^2 = 0.037$ ), or MMG MPF ( $F(10, 90) = 0.560$ ,  $p = 0.826$ ,  $\text{p}\eta^2 = 0.059$ ) (Figure 2; Table 2).

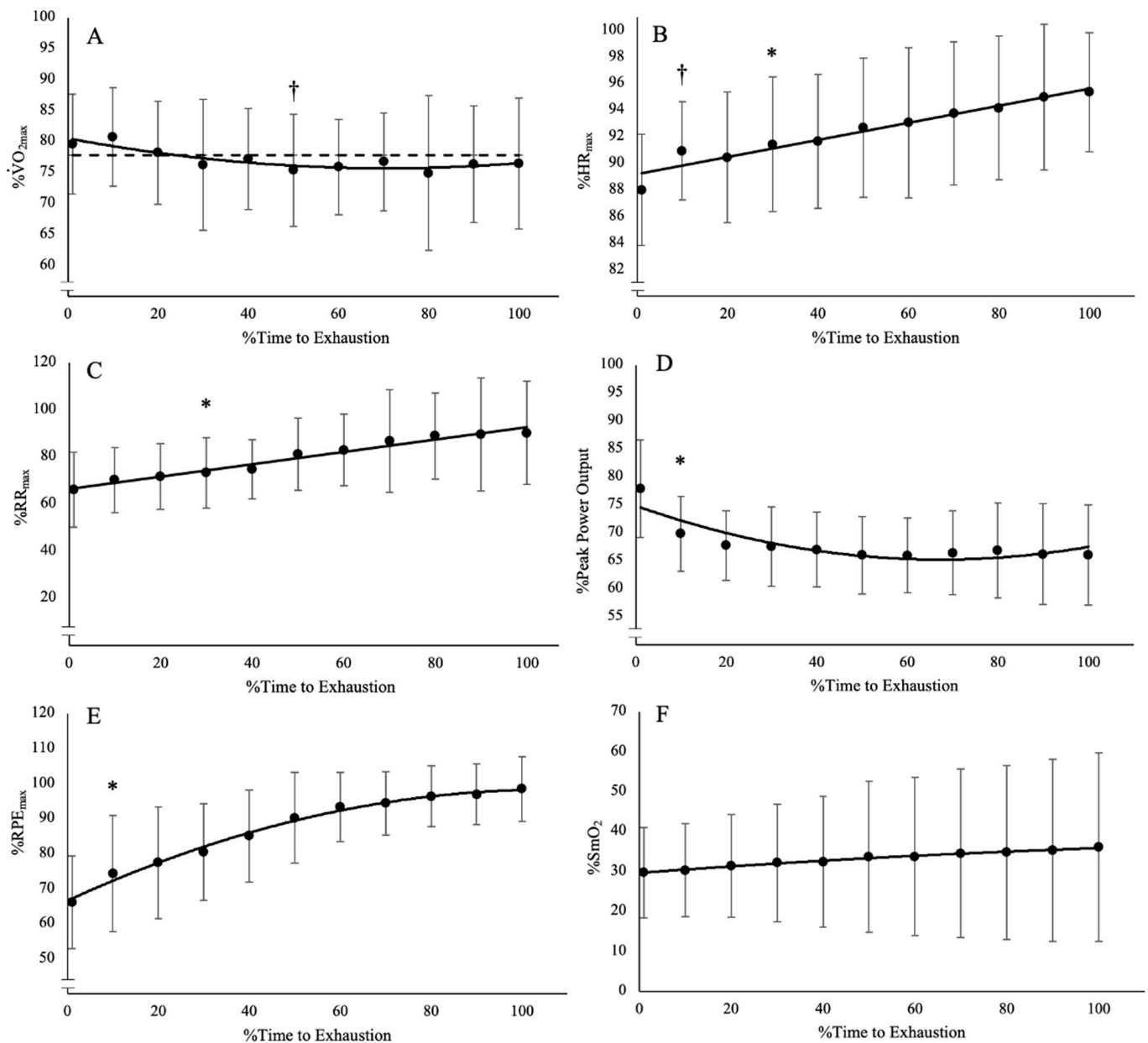


**Figure 1.** Time course of changes from the post hoc Bonferroni-corrected pairwise comparisons ( $p < 0.005$ ) for (A)  $\dot{V}O_2$ , which was lower than baseline at 50%  $T_{Lim}$  but was not different at any other time point; (B) heart rate (HR), which demonstrated an increase from baseline after 10%  $T_{Lim}$ ; (C) respiration rate (RR), which demonstrated an increase from baseline after 30%  $T_{Lim}$ ; (D) power output, which demonstrated a decrease from baseline after 10%  $T_{Lim}$ ; (E) rating of perceived exertion, which demonstrated an increase from baseline after 10%  $T_{Lim}$ ; and (F) muscle oxygen saturation (%SmO<sub>2</sub>), which was not different from among any of the time points. † Indicates differences from baseline at a single point. \* Indicates differences from baseline from that point onward for the rest of the trial. Dashed line indicates mean  $\dot{V}O_2$  at CHR.

**Table 1.** Relative mean and 95% confidence interval (CI) for oxygen uptake ( $\dot{V}O_2$ ), heart rate (HR), respiration rate (RR), power output, muscle oxygen saturation (%SmO<sub>2</sub>), rating of perceived exertion (RPE) at each time point, and the mean difference and 95% CI of the follow-up pairwise comparisons of each time point with the baseline value (0%T<sub>Lim</sub>).

| %T <sub>Lim</sub> | $\dot{V}O_2$<br>(95% CI)            | Mean<br>Difference<br>(95% CI) | HR<br>(95% CI)                      | Mean<br>Difference<br>(95% CI) | RR<br>(95% CI)                       | Mean<br>Difference<br>(95% CI) | Power<br>Output<br>(95% CI)         | Mean<br>Difference<br>(95% CI) | %SmO <sub>2</sub><br>(95% CI) | Mean<br>Difference<br>(95% CI) | RPE<br>(95% CI)                      | Mean<br>Difference<br>(95% CI) |
|-------------------|-------------------------------------|--------------------------------|-------------------------------------|--------------------------------|--------------------------------------|--------------------------------|-------------------------------------|--------------------------------|-------------------------------|--------------------------------|--------------------------------------|--------------------------------|
| 0                 | 79.5<br>(73.8–85.3)                 | -                              | 88.0<br>(85.0–91.0)                 | -                              | 66.0<br>(54.6–77.3)                  | -                              | 77.9<br>(71.7–84.1)                 | -                              | 29.7<br>(21.6–37.8)           | -                              | 66.1<br>(56.7–75.6)                  | -                              |
| 10                | 80.7<br>(75.0–86.4)                 | -1.2<br>(-2.6–0.2)             | <b>90.9 †</b><br><b>(88.3–93.6)</b> | -2.9<br>(-4.3–-1.5)            | 70.1<br>(60.3–80.0)                  | -4.2<br>(-9.2–0.9)             | <b>69.8 *</b><br><b>(65.0–74.6)</b> | 8.1<br>(4.7–11.5)              | 30.3<br>(22.0–38.6)           | -0.6<br>(-3.0–1.8)             | <b>74.4 *</b><br><b>(62.5–86.2)</b>  | -8.3<br>(-12.6–-4.0)           |
| 20                | 78.2<br>(72.2–84.1)                 | 1.3<br>(-1.6–4.2)              | 90.4<br>(86.9–94.0)                 | -2.4<br>(-4.1–-0.8)            | 71.6<br>(61.6–81.6)                  | -5.6<br>(-11.1–-0.2)           | <b>67.8 *</b><br><b>(63.4–72.2)</b> | 10.1<br>(7.2–13)               | 31.4<br>(22.2–40.5)           | -1.7<br>(-5.4–2.1)             | <b>77.4 *</b><br><b>(66.1–88.8)</b>  | -11.3<br>(-15.1–-7.5)          |
| 30                | 76.2<br>(68.7–83.8)                 | 3.3<br>(-0.4–7.0)              | <b>91.4 *</b><br><b>(87.8–95.0)</b> | -3.4<br>(-5.1–-1.7)            | <b>73.2 *</b><br><b>(62.5–83.9)</b>  | -7.2<br>(-11.5–-3.0)           | <b>67.5 *</b><br><b>(62.4–72.6)</b> | 10.4<br>(8.3–12.5)             | 32.2<br>(21.6–42.7)           | -2.5<br>(-7.6–2.6)             | <b>80.5 *</b><br><b>(70.6–90.4)</b>  | -14.3<br>(-18.0–-10.7)         |
| 40                | 77.2<br>(71.3–83.0)                 | 2.4<br>(-0.0–4.8)              | <b>91.7 *</b><br><b>(88.1–95.3)</b> | -3.7<br>(-5.1–-1.7)            | <b>74.7 *</b><br><b>(65.8–83.6)</b>  | -8.7<br>(-12.7–-4.8)           | <b>66.9 *</b><br><b>(62.1–71.8)</b> | 11.0<br>(8.7–13.3)             | 32.4<br>(20.7–44.1)           | -2.7<br>(-9.8–4.4)             | <b>85.1 *</b><br><b>(75.7–94.4)</b>  | -18.9<br>(-23.7–-14.2)         |
| 50                | <b>75.4 †</b><br><b>(68.9–81.8)</b> | 4.2<br>(2.0–6.3)               | <b>92.7 *</b><br><b>(89.0–96.4)</b> | -4.7<br>(-6.9–-2.4)            | <b>81.1 *</b><br><b>(70.2–92.0)</b>  | -15.2<br>(-23.5–-6.8)          | <b>66.0 *</b><br><b>(61.0–70.9)</b> | 11.9<br>(10.1–13.7)            | 33.7<br>(20.1–47.2)           | -4.0<br>(-13.2–5.2)            | <b>90.2 *</b><br><b>(80.9–99.5)</b>  | -24.1<br>(-30.7–-17.5)         |
| 60                | 75.9<br>(70.4–81.4)                 | 3.7<br>(1.1–6.2)               | <b>93.0 *</b><br><b>(89.0–97.1)</b> | -5.0<br>(-7.6–-2.5)            | <b>82.9 *</b><br><b>(72.0–93.7)</b>  | -16.9<br>(-26.0–-7.8)          | <b>66.0 *</b><br><b>(61.2–70.7)</b> | 11.9<br>(9.9–14)               | 33.7<br>(19.5–47.9)           | -4.0<br>(14.1–6.0)             | <b>93.3 *</b><br><b>(86.3–100.3)</b> | -27.2<br>(-34.9–-19.5)         |
| 70                | 76.7<br>(71.1–82.4)                 | 2.8<br>(1.0–4.6)               | <b>93.8 *</b><br><b>(89.9–97.6)</b> | -5.8<br>(-8.2–-3.3)            | <b>86.7 *</b><br><b>(71.2–102.2)</b> | -20.7<br>(-32.2–-9.3)          | <b>66.4 *</b><br><b>(61.0–71.8)</b> | 11.5<br>(9.5–13.6)             | 34.5<br>(19.4–49.6)           | -4.9<br>(-16.0–6.8)            | <b>94.3 *</b><br><b>(87.9–100.8)</b> | -28.2<br>(-37.5–-18.9)         |
| 80                | 74.9<br>(66.0–83.8)                 | 4.6<br>(-0.0–9.3)              | <b>94.2 *</b><br><b>(90.3–98.0)</b> | -6.1<br>(-8.5–-3.7)            | <b>88.9 *</b><br><b>(75.8–101.9)</b> | -22.9<br>(-35.4–-10.4)         | <b>66.8 *</b><br><b>(60.7–72.9)</b> | 11.1<br>(8.9–13.4)             | 34.7<br>(19.2–50.3)           | -5.1<br>(-16.9–6.8)            | <b>96.4 *</b><br><b>(90.2–102.5)</b> | -30.2<br>(-39.1–-21.3)         |
| 90                | 76.3<br>(69.6–83.1)                 | 3.2<br>(0.3–6.2)               | <b>95.0 *</b><br><b>(91.1–98.9)</b> | -7.0<br>(-9.4–-4.5)            | <b>89.6 *</b><br><b>(72.4–106.7)</b> | -23.6<br>(-37.4–-9.8)          | <b>66.2 *</b><br><b>(59.8–72.6)</b> | 11.7<br>(8.8–14.6)             | 35.3<br>(19.0–51.6)           | -5.6<br>(-18.1–6.9)            | <b>96.9 *</b><br><b>(90.7–103.1)</b> | -30.8<br>(-39.2–-22.3)         |
| 100               | 76.4<br>(68.9–84.0)                 | 3.1<br>(-0.1–6.3)              | <b>95.4 *</b><br><b>(92.2–98.6)</b> | -7.4<br>(-9.2–-5.5)            | <b>90.2 *</b><br><b>(74.5–105.8)</b> | -24.2<br>(-36.1–-12.3)         | <b>66.0 *</b><br><b>(59.6–72.4)</b> | 11.9<br>(8.8–15.0)             | 36.1<br>(19.2–53.0)           | -6.4<br>(-19.5–6.7)            | <b>98.4 *</b><br><b>(91.8–105.1)</b> | -32.3<br>(-39.9–-24.7)         |

† Indicates difference from baseline at a single point. \* Indicates differences from baseline from that point onward for the rest of the trial.

**Table 2.** Relative mean and 95% confidence interval (CI) for electromyographic amplitude (EMG AMP), mean power frequency (EMG MPF), mechanomyographic amplitude (MMG AMP), mean power frequency (MMG MPF) at each time point, and the mean difference and 95% CI of the follow-up pairwise comparisons of each time point with the baseline value (0–10%T<sub>Lim</sub>).

| %T <sub>Lim</sub> | EMG AMP<br>(95% CI) | Mean Difference<br>(95% CI) | EMG MPF<br>(95% CI)                   | Mean Difference<br>(95% CI) | MMG AMP<br>(95% CI)  | Mean Difference<br>(95% CI) | MMG MPF<br>(95% CI)   | Mean Difference<br>(95% CI) |
|-------------------|---------------------|-----------------------------|---------------------------------------|-----------------------------|----------------------|-----------------------------|-----------------------|-----------------------------|
| 0–10              | 79.7<br>(71.3–88.1) | -                           | 99.8<br>(92.0–107.6)                  | -                           | 85.9<br>(63.0–108.8) | -                           | 98.4<br>(79.7–117.2)  | -                           |
| 10–20             | 75.2<br>(66.4–84.0) | 4.5<br>(0.8–8.2)            | 101.3<br>(92.6–109.9)                 | −1.4<br>(−3.6–0.7)          | 87.8<br>(61.4–114.3) | −1.9<br>(−9.1–5.3)          | 103.1<br>(78.3–127.8) | −4.6<br>(−15.6–6.4)         |
| 20–30             | 74.8<br>(66.5–83.0) | 4.9<br>(1.7–8.1)            | 102.2<br>(93.5–111.0)                 | −2.4<br>(−4.7–−0.1)         | 90.9<br>(61.0–120.8) | −4.9<br>(−15.6–5.7)         | 97.9<br>(80.1–115.6)  | 0.5<br>(−3.2–4.2)           |
| 30–40             | 74.8<br>(67.7–81.9) | 4.9<br>(0.4–9.4)            | 104.0<br>(95.5–112.6)                 | −4.2<br>(−7.2–−1.3)         | 86.2<br>(61.7–110.6) | −0.3<br>(−5.8–5.3)          | 100.0<br>(82.0–118.0) | −1.6<br>(−8.3–5.2)          |
| 40–50             | 74.9<br>(66.9–82.9) | 4.8<br>(−0.2–9.8)           | 105.8<br>(95.4–116.1)                 | −6.0<br>(−9.9–−2.1)         | 89.2<br>(66.0–112.4) | −3.3<br>(−11.9–5.4)         | 101.3<br>(84.1–118.5) | −2.9<br>(−9.6–3.8)          |
| 50–60             | 74.8<br>(66.3–83.4) | 4.9<br>(−0.7–10.4)          | 105.5<br>(95.7–115.3)                 | −5.7<br>(−9.4–−1.9)         | 88.5<br>(68.1–108.8) | −2.5<br>(10.4–5.3)          | 100.7<br>(83.3–118.2) | −2.3<br>(−8.9–4.3)          |
| 60–70             | 75.6<br>(66.3–84.9) | 4.1<br>(−1.0–9.2)           | <b>107.1 *</b><br><b>(97.4–116.9)</b> | −7.3<br>(−11.2–−3.5)        | 89.4<br>(67.9–110.9) | −3.5<br>(−12.3–5.3)         | 100.9<br>(83.4–118.4) | −2.5<br>(−10.0–5.0)         |
| 70–80             | 74.6<br>(65.5–83.7) | 5.1<br>(−1.4–11.6)          | <b>108.5 *</b><br><b>(98.1–118.9)</b> | −8.7<br>(−13.7–−3.7)        | 89.3<br>(67.4–111.2) | −3.4<br>(−14.0–7.3)         | 102.0<br>(85.2–118.8) | −3.6<br>(−10.6–3.4)         |
| 80–90             | 74.4<br>(64.2–84.7) | 5.3<br>(−0.7–11.2)          | <b>108.6 *</b><br><b>(98.4–118.9)</b> | −8.8<br>(−14.1–−3.5)        | 90.3<br>(68.7–112.0) | −4.4<br>(−12.2–3.4)         | 100.3<br>(82.6–117.9) | −1.9<br>(−8.2–4.4)          |
| 90–100            | 73.3<br>(63.2–83.5) | 6.4<br>(0.5–12.2)           | <b>109.9 *</b><br><b>(98.6–121.1)</b> | −10.1<br>(−15.7–−4.5)       | 88.5<br>(66.2–110.9) | −2.6<br>(−11.3–6.0)         | 102.7<br>(85.2–120.1) | −4.3<br>(−10.5–1.9)         |

\* Indicates significant differences from baseline from that point onward for the rest of the trial.

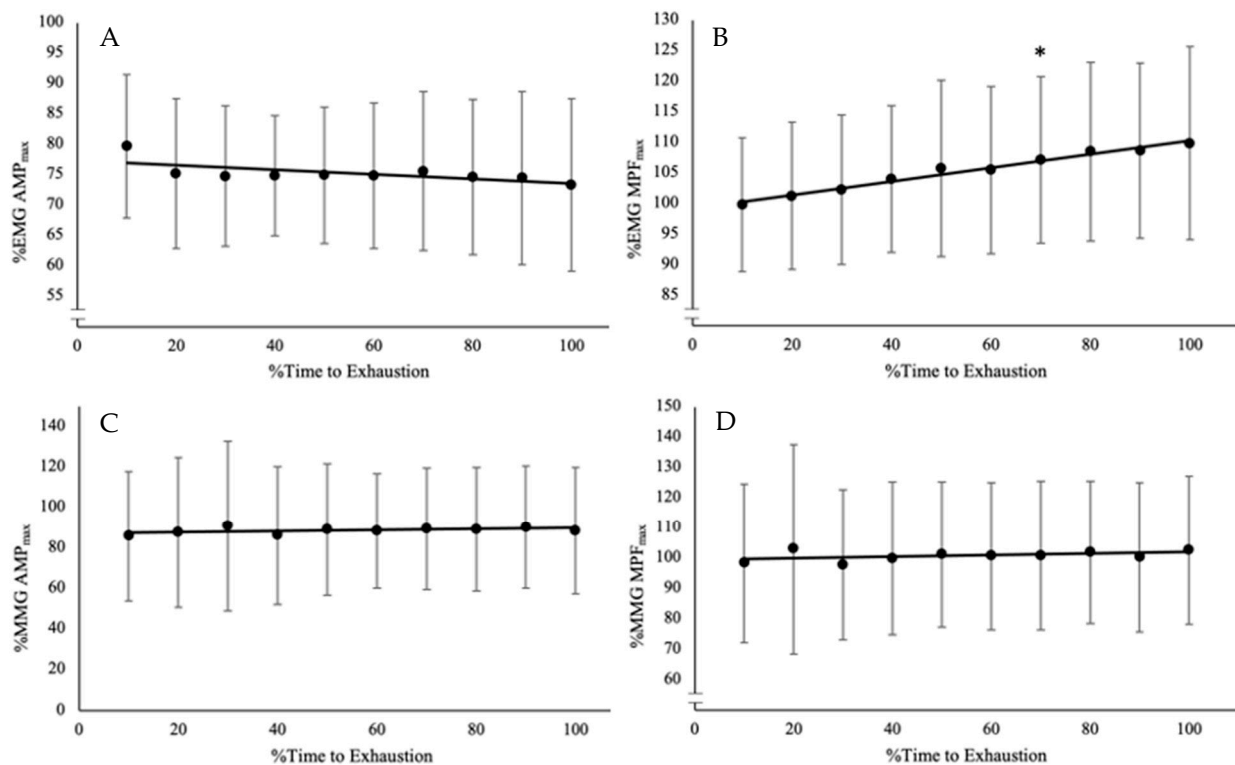


**Figure 2.** Time course of changes from the post hoc Bonferroni-corrected pairwise comparisons ( $p < 0.0056$ ) for (A) electromyographic amplitude (EMG AMP), which did not have any significant differences among the time points; (B) EMG mean power frequency (EMG MPF), which increased from baseline after 70%  $T_{Lim}$ ; (C) mechanomyographic amplitude (MMG AMP), which did not have any differences among the time points; and (D) MMG mean power frequency (MMG MPF), which did not have any differences among the time points. \* Indicates significant differences from baseline from that point onward for the rest of the trial.

#### 4. Discussion

Traditional examinations of the physiological responses that define the exercise intensity domains have often used constant power output models in which the intensity domains are defined by the changes in physiological variables, such as  $\dot{V}O_2$ , HR, and blood lactate concentration, but the power output is clamped [3]. However, in the current study, the  $\dot{V}O_2$ -Clamp model was used to better understand the interactions among physiological and performance variables. Allowing the power output to fluctuate while clamping another parameter, such as RPE [13], HR [15,17,19], or  $\dot{V}O_2$  [14], has provided a different viewpoint from which to infer mechanisms of fatigue during exercise. The  $\dot{V}O_2$ -Clamp model allows for the examination of the responses to exercise at  $\dot{V}O_{2CHR}$ , which theoretically represents a physiologically based threshold that reflects individual performance characteristics [15,17,19]. In this study, the average initial power output used to elicit  $\dot{V}O_{2CHR}$  ( $76 \pm 9\%$  PPO) represented an intensity within the severe intensity domain ( $>CP$ ) at approximately 16% of the difference between CP ( $194 \pm 48$  W) and PPO ( $269 \pm 63$  W). However, three of the ten subjects had initial power outputs that were in the heavy intensity domain ( $>VT$ ,  $<CP$ ), while the remaining seven were in the severe intensity domain. On average, during the  $\dot{V}O_2$ -Clamp trials, the power output decreased to  $66 \pm 9\%$  PPO ( $179 \pm 57$  W), which was greater than the power output associated with the VT ( $146 \pm 31$  W) and was, therefore, within the heavy intensity domain. While the average  $T_{Lim}$  ( $31.31 \pm 21.37$  min) indicated that exercise at  $\dot{V}O_{2CHR}$  represented a sustainable intensity, the examination of the individual differences makes that conclusion less clear.

Three of the ten subjects were able to sustain exercise at  $\dot{V}O_2$ CHR for the entire 60 min, with two of those three being subjects who were initially in the heavy intensity domain. Two subjects were able to sustain exercise for just over 30 min ( $32.27 \pm 1.80$  min), and the remaining five subjects were unable to sustain exercise for at least 30 min ( $13.71 \pm 4.62$  min). Therefore, the initial power output, relative to the exercise intensity domains, required to maintain the  $\dot{V}O_2$ CHR most likely determines the sustainability of exercise anchored to  $\dot{V}O_2$ .

Similar to the responses that have been demonstrated during constant HR exercise at CHR [19], exercise at  $\dot{V}O_2$ CHR appears to be predicated on time spent in each exercise intensity domain. Specifically, two of the three subjects who were able to maintain exercise for the full 60 min were, based on power output, within the moderate intensity domain ( $<VT$ ) for the majority of the trial ( $87.45\text{--}91.3\%$   $T_{Lim}$ ), with almost no time spent in the severe intensity domain ( $>CP$ ) (Table 3). The one other subject that maintained exercise for the full 60 min remained in the heavy intensity domain for the entire trial ( $>VT$ , but  $<CP$ ). The seven subjects who were unable to maintain exercise for the entire 60 min began an exercise in the severe or heavy intensity domains and were unable to transverse into the moderate intensity domain. While five of these seven subjects were able to transverse from the severe into the heavy intensity domain, the results of this study support previous work [33,34] that suggests that the amount of work performed in the severe intensity domain ( $>CP$ ) does not change regardless of pacing strategy or partial recovery of metabolic disturbances. This may indicate that  $\dot{V}O_2$  was not sensitive enough to elicit a sufficient decrease in power output to get into the moderate intensity domain before a significant accumulation of metabolic disturbances [35]. Thus, based on the individual responses, exercise at  $\dot{V}O_2$ CHR is dependent on the exercise intensity domain in which the initial power output is located. Individuals who were able to do minimal work in the severe intensity domain, such as those who start or transverse into the moderate intensity domain, were able to sustain exercise for 60 min. Conversely, those subjects that spent more time in the severe intensity domain most likely accumulated a significant amount of metabolic disturbances that ultimately led to fatigue within 30 min. However, future studies should examine the  $\dot{V}O_2$ -Clamp exercise at a  $\dot{V}O_2$ -based threshold, such as the critical  $\dot{V}O_2$  [36], to further evaluate  $\dot{V}O_2$ -Clamp exercise as a tool to examine various mechanisms of fatigue.

**Table 3.** Individual, mean, and SD time to exhaustion ( $T_{Lim}$ ), and the time spent in each exercise intensity domain at the  $\dot{V}O_2$  associated with critical heart rate ( $\dot{V}O_2$ CHR).

| Subject | Total $T_{Lim}$ (min) | Severe (min) | Heavy (min) | Moderate (min) |
|---------|-----------------------|--------------|-------------|----------------|
| 1       | 60.00                 | 0.00         | 60.00       | 0.00           |
| 2       | 18.34                 | 21.78        | 0.00        | 0.33           |
| 3       | 8.66                  | 9.90         | 3.96        | 0.00           |
| 4       | 9.01                  | 11.88        | 4.95        | 0.00           |
| 5       | 17.57                 | 6.60         | 14.52       | 0.00           |
| 6       | 60.00                 | 0.00         | 10.23       | 52.47          |
| 7       | 33.54                 | 1.98         | 31.68       | 0.00           |
| 8       | 60.00                 | 0.66         | 4.29        | 54.78          |
| 9       | 14.98                 | 0.00         | 15.18       | 0.00           |
| 10      | 30.99                 | 31.02        | 0.00        | 0.00           |
| Average | 31.30                 | 8.38         | 14.48       | 10.76          |
| SD      | 21.37                 | 10.65        | 18.59       | 22.60          |

Above critical power (CP) = severe intensity domain; below CP but greater than ventilatory threshold (VT) = heavy intensity domain; below VT = moderate intensity domain. Time within each exercise intensity domain includes the time required to reach  $\dot{V}O_2$ CHR that was not included in the total  $T_{Lim}$ .

Our current understanding of the response to exercise is based on constant power output/velocity trials where the response depends on the relative intensity with respect to the exercise intensity domains. While Ribeiro et al. [14] reported general decreases in power output during exercise at a constant  $\dot{V}O_2$ , the magnitude and time course of these decreases remain unclear. In the current study, there were significant decreases, ranging from 7–23% (mean = 15%), in power output after 10% of  $T_{Lim}$ . This sharp decline at the beginning of the trial was most likely due to a delayed  $\dot{V}O_2$  response when ramping to  $\dot{V}O_{2CHR}$ , which may have caused an initial overshoot in  $\dot{V}O_2$ . Following the sharp initial decline in power output, there was a stabilization after 20% $T_{Lim}$  that demonstrated a plateau for the rest of the duration of the trial (Figure 1D). This pattern of response in the power output demonstrates a similar response to that seen in self-paced exercise with a “fast-start” phase followed by an “even-pace” phase [34]. However, despite these decreases in power output, there was no significant difference in the EMG AMP, MMG AMP, MMG MPF, and %SmO<sub>2</sub> from the time the subjects achieved the designated  $\dot{V}O_2$  to the end of the trial. This suggests there may have been a consistent level of muscle excitation (motor unit recruitment, firing rate, and synchronization) and muscle oxygen saturation throughout the trial. This lack of difference throughout the trial was irrespective of whether the individual subject was able to sustain exercise for >30 min or not. This finding is in contrast with the response demonstrated during self-paced trials, where it was reported there was a decrease in muscle excitation after the “fast-start” [37]. This lack of change in muscle excitation may have impaired muscle oxygen saturation due to intramuscular pressure from the active musculature hampering the perfusion response [38,39]. The lack of change in muscle excitation may have also caused metabo-sensory feedback from group III/IV muscle afferents from the working skeletal and respiratory muscles [40–42]. Despite this afferent feedback, which has been shown to trigger the metaboreflex to cause increased perfusion to the exercising muscle [43–45], there was no change in the muscle oxygen saturation from the time the designated  $\dot{V}O_2$  was achieved at the end of the trial. This lack of change may be the result of the sustained level of muscle excitation and intramuscular pressure [46,47] but may also be a result of differences in muscle architecture, muscle stiffness, fiber type composition, intramuscular fluid pressure, or other morphological or methodological differences that were not examined in the current investigation [48–51].

It has also been hypothesized that during constant HR running [17], group III/IV’s muscle afferent feedback from an increase in RR was the main mediator of the increase in RPE. In the current study, there were significant increases in RR after 30% of  $T_{Lim}$ . Notably, four of the five subjects who were unable to sustain exercise for at least 30 min demonstrated increases in RR to maximal or supramaximal levels during trials at  $\dot{V}O_{2CHR}$  (98–134%  $RR_{peak}$ ), while those that could maintain exercise for at least 30 min demonstrated, on average, more moderate increases (56–96%  $RR_{peak}$ ). This increase in RR also coincided with similar increases in RPE (100–105%  $RPE_{peak}$ ). However, the increases in RPE occurred earlier (10%  $T_{Lim}$ ) than the increases in RR and were more consistent with the increases in HR (10%  $T_{Lim}$ ). The individual responses indicated that the subjects who were unable to sustain 30 min of exercise had higher starting (90–93% versus 80–88%  $HR_{peak}$ ) and ending (96–103% versus 87–97%  $HR_{peak}$ ) HR responses. Based on the original development of the RPE scale [29], it is not surprising that HR and RPE demonstrated similar responses, as it has been suggested that HR afferent feedback is also mediated through group III/IV afferent fibers [40,52]. While there is conflicting evidence that suggests HR is not a mediator of RPE [53], the evidence in the current study, along with previous work examining exercise anchored by different physiological parameters [13,17,19], points to multiple mechanisms that regulate the perception of effort and are likely a combination of feedforward [53] and feedback [40] mechanisms. Specifically, the mechanism of fatigue may be intensity- and anchoring-dependent. Future studies are required to examine the responses to exercise using physiologically based anchoring schemes at different intensities. For exercise anchored at  $\dot{V}O_{2CHR}$ , the decreases in power output were not sufficient to

cause a decruitment of the active musculature and still resulted in significant increases in RR, HR, and RPE, which together may help to explain the variability in sustainability.

## 5. Conclusions

While most studies use a constant power output to examine thresholds, the  $\dot{V}O_2$ -Clamp method was used in the current study to allow for the examination of the responses to  $\dot{V}O_{2CHR}$ , an individually derived, performance-based threshold. During exercise at  $\dot{V}O_{2CHR}$ , the reductions in power output constituted a loss in performance of 7–23% (mean = 15%). Calculating the area under the curve of the power output versus time relationship for the actual versus starting power output during the  $\dot{V}O_2$ -Clamp trial (Figure 3) potentially provides a quantifiable estimate of the loss of power output. These losses in performance may help to further quantify the inefficiency associated with the sustained recruitment of type II muscle fibers, which has been suggested to evoke the  $\dot{V}O_2$  slow component response [20]. The EMG, MMG, and %SmO<sub>2</sub> responses suggested continued muscle excitation throughout the trial and pointed to afferent feedback as a potential driver of performance. Meanwhile, the HR, RR, and RPE responses suggested a combination of feedforward and feedback mechanisms regulating the sustainability of exercise at  $\dot{V}O_{2CHR}$ . Thus, the  $\dot{V}O_2$ -Clamp exercise demonstrates a novel method for examining mechanisms of fatigue and the  $\dot{V}O_2$  slow components. Future studies should examine these responses with a uniform threshold, such as the respiratory compensation point or critical  $\dot{V}O_2$ , to further examine the utility of the  $\dot{V}O_2$ -Clamp exercise as a powerful tool in the examination of fatigue.

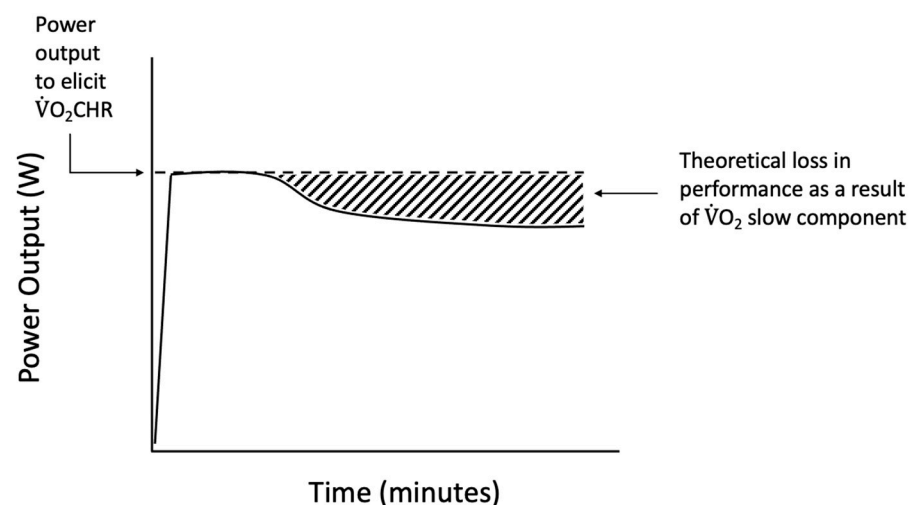


**Figure 3.** Theoretical representation of the performance loss during  $\dot{V}O_2$ -Clamp exercise as a result of inefficiencies such as the  $\dot{V}O_2$  slow component phenomenon.

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