The slow component of pulmonary O₂ uptake accompanies peripheral muscle fatigue during high-intensity exercise

Daniel A. Keir,1,2 David B. Copithorne,1,2 Michael D. Hodgson,1,2 Silvia Pogliaghi,5 Charles L. Rice,1,2,4 and John M. Kowalchuk1,2,3

1Canadian Centre for Activity and Aging, The University of Western Ontario, London, Ontario, Canada; 2School of Kinesiology, The University of Western Ontario, London, Ontario, Canada; 3Department of Physiology and Pharmacology, The University of Western Ontario, London, Ontario, Canada; 4Department of Anatomy and Cell Biology, The University of Western Ontario, London, Ontario, Canada; and 5Department of Neuroscience, Biomedicine and Movement Sciences, University of Verona, Italy

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Keir DA, Copithorne DB, Hodgson MD, Pogliaghi S, Rice CL, Kowalchuk JM. The slow component of pulmonary O₂ uptake accompanies peripheral muscle fatigue during high-intensity exercise. J Appl Physiol 121: 493–502, 2016. First published June 23, 2016; doi:10.1152/japplphysiol.00249.2016.—During constant-power output (PO) exercise above lactate threshold (LT), pulmonary O₂ uptake (V˙O₂p) features a developing slow component (V˙O₂pSC). This progressive increase in O₂ cost of exercise is suggested to be related to the effects of muscle fatigue development. We hypothesized that peripheral muscle fatigue as assessed by contractile impairment would be associated with the V˙O₂pSC. Eleven healthy men were recruited to perform four constant-PO tests at an intensity corresponding to ∼60% (very heavy, VH) where Δ is 60% of the difference between LT and peak V˙O₂p. The VH exercise was completed for each of 3, 8, 13, and 18 min (i.e., VH3, VH8, VH13, VH18) with each preceded by 3 min of cycling at 20 W. Peripheral muscle fatigue was assessed via pre- vs. postexercise measurements of quadriceps torque in response to brief trains of electrical stimulation delivered at low (10 Hz) and high (50 Hz) frequencies. During exercise, breath-by-breath V˙O₂p was measured by mass spectrometry and volume turbine. The magnitude of V˙O₂pSC increased (P < 0.05) from 224 ± 81 ml/min at VH3 to 520 ± 119, 625 ± 134, and 678 ± 156 ml/min at VH8, VH13, and VH18, respectively. The ratio of the low-to-high frequency (10/50 Hz) response was reduced (P < 0.05) at VH3 (−12 ± 9%) and further reduced (P < 0.05) at VH8 (−25 ± 11%), VH13 (−42 ± 19%), and VH18 (−46 ± 16%), mirroring the temporal pattern of V˙O₂pSC development. The reduction in 10/50 Hz ratio was correlated (P < 0.001, r² = 0.69) with V˙O₂pSC amplitude. The temporal and quantitative association of decrements in muscle torque production and V˙O₂pSC suggest a common physiological mechanism between skeletal muscle fatigue and loss of muscle efficiency.

NEW & NOTEWORTHY

Quadriceps muscle torque production in response to electrically stimulated contractions (a measure of peripheral muscle fatigue) progressively decreases with greater durations of high-intensity constant-load cycling, and the time course and magnitude of this response mirrors that of the pulmonary oxygen uptake (V˙O₂p) slow component. The quantitative and temporal association between the V˙O₂p slow component and peripheral muscle fatigue suggests that mechanisms contributing to muscle fatigue also contribute to an increased O₂ cost of exercise.

FOLLOWING THE ONSET OF CONSTANT-power output (PO) exercise below the lactate threshold (LT; the exercise intensity associated with net blood lactate accumulation), pulmonary O₂ uptake (V˙O₂p) achieves a steady state after a brief adjustment period of 2–3 min (63). This steady-state V˙O₂p may be predicted from the linear V˙O₂p-PO relationship established below the LT. However, during supra-LT exercise, there is a marked increase in V˙O₂p for a given PO (reflecting a reduced work efficiency) such that V˙O₂p exceeds that expected based on extrapolation of the linear V˙O₂p-PO relationship (66). The extra V˙O₂p measured above that predicted by the sub-LT V˙O₂p-PO relationship is referred to as the “V˙O₂p slow component” (V˙O₂pSC) because it is considered to be of delayed onset and contributes to a longer delay before a steady-state V˙O₂p is attained (by approximately 10–15 min) (5, 45). With greater exercise intensities above LT, the magnitude of the V˙O₂p slow component and the time before attaining a new steady state increases (44). However, at intensities above critical power (CP; the highest exercise intensity at which a steady-state V˙O₂p can be achieved), V˙O₂p no longer reaches a plateau, but rather continues to rise and, if tolerated, maximal V˙O₂p is reached (64). Because this increased V˙O₂p cost of exercise occurs at intensities at which muscle fatigue has been reported (3, 13, 39, 50), the intensity-dependent decline in muscle efficiency is thought to be associated with the progressive development of muscle fatigue (12, 65).

Approximately 85% of the V˙O₂p slow component is known to originate from within the active muscle (47). Mechanistically, its appearance has been linked to 1) reductions in the efficiency of skeletal muscle contraction (increased ATP cost of force production) or mitochondrial energy production (increased O₂ cost of ATP resynthesis) (10, 36, 58), or both; and 2) a delayed recruitment of less oxidatively efficient, larger motor units to compensate for attenuated force production in those already active motor units (23, 29, 37). These mechanisms can be generalized on the basis of motor unit recruitment (either initial activation of less-efficient muscle fibers or delayed muscle fiber activation) and muscle fatigue (fatigued muscle fibers continue to consume O₂ without contributing to force production requirements or do so at a higher O₂ cost), however, there is a paucity of evidence directly linking these phenomena and the V˙O₂p slow component in humans.

Cannon et al. (12) were among the first to directly examine the relationship between muscle fatigue and \( \text{V}_\text{O}_2 \text{p} \) slow component development. Using 8-min bouts of cycling exercise at three different POs corresponding to below LT (i.e., moderate exercise), between LT and CP (i.e., heavy exercise), and above CP (very heavy exercise), Cannon et al. (12) reported that fatigue (as inferred by reductions in peak PO measured during maximal isokinetic cycling pre- vs. postexercise) was related to the magnitude of the \( \text{V}_\text{O}_2 \text{p} \) slow component [see Fig. 4 in Cannon et al. (12)]. In that study, however, reductions in velocity-specific peak PO already were established by 3 min of exercise (i.e., before a significant development of a \( \text{V}_\text{O}_2 \text{p} \) slow component) with no additional time-dependent reductions in velocity-specific peak power between 3 and 8 min of exercise, suggesting that the time course of muscle fatigue development was unrelated to \( \text{V}_\text{O}_2 \text{p} \) slow component progression. Furthermore, the authors interpreted these data to suggest that additional recruitment of muscle was not obligatory for \( \text{V}_\text{O}_2 \text{p} \) slow component development, but that a reduction in mechanical efficiency of fatigued fibers was involved. However, it is difficult to reconcile how a reduction in mechanical efficiency of muscle might occur without additional fatigue development.

A challenge associated with tracking the development of muscle fatigue in response to dynamic cycling exercise is that it is difficult to assess during exercise. Furthermore, muscle fatigue may arise from factors related to neurological transmission (central fatigue), factors from within the muscle (peripheral fatigue) (20), or some combination depending on the task (8), making it difficult to quantify fatigue while isolating etiology. Although both central and peripheral factors likely play a role in muscle fatigue development during cycling exercise (56), only peripheral factors should have an effect on muscle metabolism. In contrast to the isokinetic power model adopted by Cannon et al. (12), depression of muscle force in response to low and high frequencies of electrical stimulation are considered standard measures for peripheral muscle fatigue assessment (15, 16). Importantly, by focusing on elicited contractile responses, this approach excludes any confounding influences related to changes in centrally mediated force or PO. Using this method, previous work indicated that the time course of peripheral fatigue during high-intensity, constant-PO cycling (13) and time-trial based knee-extension exercise (21) exhibited an exponential increase toward an asymptotic value. Interestingly, this profile bears resemblance to that of the \( \text{V}_\text{O}_2 \text{p} \) slow component development during high-intensity cycling exercise (12, 17) and thus it remains tenable that the time course of skeletal muscle fatigue is related to \( \text{V}_\text{O}_2 \text{p} \) development.

Therefore, this study was designed to 1) determine the time course and magnitude of muscle fatigue development, as defined by impairment in contractile capability during very heavy-intensity cycling exercise, by comparing measures of voluntary and involuntarily contractile properties of quadriceps muscle torque pre- and postexercise; and 2) relate the anticipated peripheral muscle fatigue to changes in the \( \text{V}_\text{O}_2 \text{p} \text{SC} \) over the time course of constant-PO exercise. It was hypothesized that peripheral muscle fatigue would develop with a similar time course as that of the \( \text{V}_\text{O}_2 \text{p} \) slow component during 18 min of very heavy constant-PO exercise and that the magnitude of peripheral fatigue would be associated with \( \text{V}_\text{O}_2 \text{p} \) slow component amplitude. Here, “peripheral muscle fatigue” is defined as a reduction in knee extensor torque output in response to electrically stimulated muscle contraction at frequencies of 10 and 50 Hz.

**Materials and Methods**

**Ethical Approval**

The study was conducted according to the Declaration of Helsinki and all procedures were approved by The University of Western Ontario Ethics Committee for Research on Human Subjects. Procedures and risks were explained to each subject, and all participants volunteered and gave informed written consent to participate before the start of the study.

**Participants**

Eleven healthy young adult men (age 26 ± 4 yr, mean ± SD; body mass 81 ± 8 kg; height 182 ± 6 cm; \( \text{V}_\text{O}_2 \text{peak} \) 45.9 ± 4.8 ml·kg\(^{-1}\)·min\(^{-1}\)) participated in the study. Participants were nonsmokers with no known musculoskeletal, respiratory, cardiovascular, or metabolic conditions, and none were taking any medications that might influence cardiopulmonary or metabolic responses to exercise.

**Experimental Protocol**

Each exercise test consisted of 1) a preexercise neuromuscular assessment, 2) the exercise intervention, and 3) a postexercise neuromuscular assessment.

**Exercise Testing.** Participants reported to the laboratory and initially performed a symptom-limited ramp-incremental exercise cycling test to the limit of tolerance (50 W baseline for 4 min followed by a 25 W/min ramp). Participants were allowed to select a preferred cadence but thereafter were encouraged to maintain this cadence to within ± 10 revolutions per minute (rpm; mean = 71 ± 5, range 65–80). The results of the ramp-incremental test were used to estimate each participant’s lactate threshold (LT), respiratory compensation point (RCP), and \( \text{V}_\text{O}_2 \text{peak} \) and to calculate POs for the subsequent tests. This preliminary test also served as a familiarization session for all neuromuscular testing procedures.

The participants then completed 1) one constant-PO test at an intensity corresponding to 80%LT (moderate, MOD) for 18 min (MOD18); and 2) five constant-PO tests at an intensity of \( \Delta \text{60} \) (very heavy, VH), which is the PO corresponding to 60% of the difference in \( \text{V}_\text{O}_2 \) between LT and \( \text{V}_\text{O}_2 \text{peak} \). An intensity of \( \Delta \text{60} \) was chosen so that all participants were above their maximal lactate steady-state critical power ensuring that a metabolic steady state would not be achieved (34). The VH condition was completed for each of 3, 8, 13, and 18 min (i.e., VH3, VH8, VH13, and VH18), and a second 18-min cycling trial was completed, during which the researchers collected electromyographic (EMG) data. All constant-PO trials were preceded by 3 min of cycling at 20 W. A VH18 condition preceded all other trials. This was done to ensure that the \( \Delta \text{60} \) intensity elicited the targeted physiological responses of very heavy exercise. If end-exercise \( \text{V}_\text{O}_2 \) was not greater than the \( \text{V}_\text{O}_2 \) at the RCP and \( \text{V}_\text{O}_2 \text{p} \) and blood lactate reached a steady state, the PO was adjusted and the VH18 trial was repeated. After the target VH intensity was verified, all other trials were completed in a randomized order.

All tests were conducted in an environmentally controlled laboratory at a similar time of day, 2 to 3 h after a standardized meal (composed of 500 ml of water and 2–3 g/kg body mass of low-glycemic-index carbohydrates). Exercise protocols were performed on an electromagnetically braked cycle ergometer (Velotron; RacerMate, Seattle, WA). Participants were instructed to abstain from vigorous physical activity in the 24 h preceding each test and to avoid caffeine consumption on the day of testing. All testing sessions were separated by a minimum of 48 h.

**Neuromuscular Testing.** Neuromuscular testing was performed before and immediately following each constant-PO cycling protocol.
(posttesting commenced within ~45 s after each exercise bout and was completed within ~3 min). Participants performed voluntary and involuntary isometric knee extension contractions using the left leg while seated in a Humac-Norm Cybex dynamometer (Computer Sports Medicine, Stoughton, MA), with hips and ankle joints at 90 degrees, and a knee joint angle of 90 degrees for isometric voluntary and involuntary tests, and for the beginning of the isotonic voluntary knee extensions. The lever length was adjusted so that the resistance pad rested comfortably on each participant’s leg just proximal to the malleoli with the lever’s center of rotation aligned with the rotational axis of the knee. Participants were secured using shoulder and waist belts. Two custom-made aluminum foil electrodes (~20 × 5 cm) wrapped in paper towel and soaked with a conductive brine were tightly taped over the anterior thigh just proximal to the inguinal fold, and the second electrode was placed on the distal thigh ~7 cm superior to the patella (49). The electrodes were attached to a constant current muscle stimulator (DS7AH: Digitimer, Welwyn Garden City, Hertfordshire, UK) to elicit involuntary contractions. In contrast to the preexercise protocol, in the postexercise protocol involuntary contractions (1 Hz twitch, 10 Hz and 50 Hz), were completed prior to the MVC. This was done to ensure that the 1-, 10-, and 50-Hz involuntary contractions were rapidly completed following exercise cessation to minimize any time-dependent recovery. The order and timing associated with the pre- and postexercise neuromuscular assessment protocols were identical for all trials.

Data Collection

Exercise responses. During each trial, breath-by-breath gas-exchange measurements were made as previously described (35). Briefly, inspired and expired volumes and flow rates were measured using a low-dead-space bidirectional turbine (VMM 110; Alpha Technologies, Laguna Hills, CA) and pneumotach (4813; Hans Rudolph, Shawnee, KS). Respired air was continuously sampled at the mouth and analyzed by mass spectrometry (AMIS 2000; Innovision, Shawnee, KS). Respired air was continuously sampled at the sampling inlet and its detection by the mass spectrometer was instantaneous square-wave change in fractional gas concentration at known volume (3 liters) over a range of flow rates, and the pneumotach was used to resolve inspiratory-expiratory phase transitions, and to transfer volume signals as measured by the turbine. Flow from the pneumotach was used to resolve inspiratory-expiratory phase transitions, and the turbine was used for volume measurement. The computer executed a peak-detection program to determine end-tidal PO2, end-tidal PCO2, and inspired and expired volumes and durations to build a profile of each breath. Breath-by-breath alveolar gas exchange was calculated using the algorithms of Swanson (56).

During MOD and VH18, blood lactate concentration (mM) was measured in arterialized-capillary blood samples (~5 μl) taken from a participant’s heated earlobe using a Lactate Scout (Sports Resource Group, Hawthorne, NY). Samples were obtained at 20 W and during the 3rd, 8th, 13th, and 18th minutes and analyzed immediately. Heart rate was monitored continuously using a Polar Transmitter (Polar Electro, Lachine, QC, Canada) and data were collected using PowerLab Chart version 7.3.1 (ADInstruments, Colorado Springs, CO).

Neuromuscular tests. Isometric torque (Nm) and maximal velocity isotonic knee extensions (deg/s) were recorded before and after each exercise protocol. Torque data were collected and displayed on a computer using Spike 2 version 7.02 (Cambridge Electronic Design, Cambridge, UK). Torque was sampled at a frequency of 50 Hz. ISOMETRIC MEASURES. Doublet stimulation (pulse separation 10 ms; pulse width 200 μs; 400 V, range 250–650 mA) was used to establish the maximal knee extensor twitch torque (Nm), defined as the point at which increases in stimulation intensity (mA) no longer resulted in an increase in torque production. Stimulation intensity was then increased by 20% to elicit supramaximal stimulation. A minimum of two maximal voluntary contractions (MVCs) lasting ~3 s were completed, and a third MVC was completed if the first two MVCs differed by more than 10%. Two minutes of rest was provided between each attempt. Participants were provided with visual feedback and strong verbal encouragement during all MVCs. A supramaximal doublet was elicited during (superimposed twitch) and succeeding (potentiated twitch) each MVC. This was used to calculate voluntary activation (VA = superimposed twitch/potentiated twitch) (7). All postexercise stimulation settings were identical to those established preexercise.

The quadriceps muscle was next stimulated at tetanic frequencies of 10 and 50 Hz for 1 s [see Edwards et al. (16)] using a 50-μs pulse width (400 V, range 250–475 mA) at an intensity that achieved ~50% MVC at 50 Hz (see Fig. 1). The 10-Hz stimulation was elicited at the same stimulator settings as the 50-Hz stimulation. Postexercise tetanic stimulation intensities (mA) were identical to those used preexercise.

Fig. 1. Comparison of torque measurements of a representative participant during maximal voluntary contraction (MVC, A) and electrical muscle stimulation for 1 s at 50 Hz and 1 s at 10 Hz (B and C, respectively) before (black line) and after (gray line) 18 min of very heavy constant-power output exercise (VH18). The depression in MVC torque is consequent to the superimposed doublet stimulation. Note the reduction in torque tracings following exercise. Also note change in y-axis scale in C.

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ISOTONIC MEASURES. Maximal knee extension isotonic velocity was assessed with a resistance of 20% of the preexercise MVC torque. Participants were encouraged to contract as rapidly as possible during three attempts (kicks) with 2 s of rest between attempts. A fourth kick was performed if peak velocity varied by more than 10% during the three kicks. Velocity was recorded in degrees per second (deg/s). Isotonic power (W) was determined as the product of angular velocity in radians/s and torque (Nm).

SURFACE ELECTROMYOGRAPHY. During one of the VH18 trials, EMG data were recorded over the vastus lateralis muscle of the left thigh using one pair of Ag-AgCl surface electrodes (interelectrode distance ~3 cm) placed over the belly of the muscle, with a ground electrode placed over the fibula. This area was shaved and cleaned using isopropyl alcohol prior to electrode placement. EMG recordings were amplified (1 K) with a bandwidth frequency ranging from 10 Hz to 10 KHz. The raw EMG data were sampled at a frequency of 5,000 Hz, rectified and computed as RMS amplitude, and normalized to the EMG data recorded during an isometric MVC performed prior to exercise at a knee angle of 90 degrees. The last 10 cycling bursts of vastus lateralis EMG data leading up to the 3rd, 8th, 13th, and 18th minutes of exercise were averaged and reported.

Data Analyses

Breath-by-breath VO2p data were edited on an individual basis by removing aberrant data that lay 3 SD from the local mean (38). After editing, trial repetitions were linearly interpolated on a second-by-second basis, ensemble-averaged, and time-aligned such that time “zero” represented the onset of exercise. The fundamental (phase II) VO2p kinetics were isolated using the criteria outlined previously (33) and were fit with a monoexponential function: VO2p(t) = VO2pASL + ΔVO2SS·(1 − e−(t−TD)/τ), where VO2p(t) is the value of VO2p at any time during the transition, VO2pASL is the pretransition baseline value, ΔVO2SS is the steady-state increase in VO2p above the baseline value, τ is the time constant of the response, and TD is the time delay. Identification of the end of the phase II fitting window and “onset” of the VO2p slow component were established using the criteria outlined by Murgatroyd et al. (41). The VO2p at the time points corresponding to 3, 8, 13, and 18 min were determined from a 20-s bin average. The VO2pSC amplitude at each of these time points was determined from the difference between the VO2p at that time and the VO2p correspond- ing to projected phase II “steady-state” amplitude (see Fig. 2). The VO2p at each time point also was normalized to the “VO2p slow component reserve,” which was calculated as the difference between the projected phase II VO2p steady-state amplitude and VO2 peak and expressed as %VO2p slow component reserve.

Both the estimated LT and RCP were independently determined by three reviewers with expertise in reviewing gas exchange data and identifying threshold responses. The average of the three values was used provided estimated agreements agreed to within 200 ml/min. In instances in which after further review one of the three estimates remained outside this limit, an average of the two in closest agreement was used. The LT was estimated by visual inspection as the VO2p at which CO2 output (VCO2p) began to increase out of proportion in relation to VO2p, with the ventilatory equivalent of VCO2p (V̇E/V̇CO2p) and end-tidal PO2 were stable (6). RCP was determined as the point where CO2 output (VCO2p) began to increase during the period of isocapnia (62). This point was confirmed by examining V̇E/VCO2p plotted against VO2p, and by identifying the second breakpoint in the V̇E-to-V̇O2p relationship.

To provide an indication of neuromuscular fatigue, both voluntary and involuntary muscle fatigue measures were analyzed and compared pre- vs. postexercise for each individual participant and trial. Voluntary measures included peak MVC torque, VA, and maximal isometric power. Involuntary measures included the average torque generated during the 10- and 50-Hz tetanic contractions, and a ratio of low-to-high frequency (10/50 Hz) was computed. All changes were expressed in absolute and relative units.

The magnitude of change in all involuntary muscle contraction variables at VH3, VH8, VH13, and VH18 were compared with the VO2p slow component amplitude (in absolute terms and normalized to %VO2p slow component reserve) at those time points to examine whether the magnitude of peripheral muscle fatigue was associated with the amplitude of the VO2p slow component.

Statistical Analysis

Data are presented as means ± SD. A one-way ANOVA with repeated measures was used to compare select variables across time points. When significant main effects were found, a Tukey’s post hoc analysis was performed for multiple comparisons testing. Effect sizes (partial eta squared, ηp2) were calculated for changes in muscle fatigue measurements across time. Paired t-tests were used to compare differences in variables between VH18 and MOD18 exercise conditions. The relationship between muscle fatigue measurements and VO2p slow component magnitude was assessed with Pearson correlation coefficient while controlling for repeated measures within the same subjects as outlined by Glantz and Slinker (25). All statistical analyses were performed using SigmaPlot version 11.0 (Systat Software, San Jose, CA). Statistical significance was accepted at α < 0.05.

RESULTS

The group mean PO2peak from the ramp-incremental exercise test was 354 ± 38 W. This resulted in a mean VO2 peak of 3.7 ± 0.4 l/min (45.9 ± 4.8 ml·kg−1·min−1) and peak heart rate of 187 ± 6 beats per minute. The average LT and RCP of participants was 2.18 ± 0.3 l/min (59 ± 6%VO2 peak) and 3.12 ± 0.4 l/min (84 ± 4%VO2 peak), respectively. The mean PO corresponding to MOD (80%LT) and VH (~Δ60) were 113 ± 15 and 246 ± 40 W, respectively.

Fig. 2. Pulmonary oxygen uptake (VO2p) response profile (white circles) of a representative participant during a transition to very heavy (VH) exercise. A monoexponential model (black line) was fitted to the exponential region (phase II) with residuals (gray line) displayed about y = 0 (see inset graph displaying truncated response). Narrow residuals confirm goodness of fit. The phase III (VO2p slow component onset, VO2pSC) occurred at 142 s (see end of residuals line) and the phase II fit was extrapolated to the end of the exercise. The magnitude of the VO2pSC was determined at 3, 8, 13, and 18 min of exercise as the difference (see double arrows) between the VO2p at those time points (enlarged black circles) and the extrapolated phase II VO2p steady state (VO2pSS, thick dashed black line). The VO2pSC reserve was calculated as the difference between maximal VO2p (VO2max) and the extrapolated phase II VO2pSS (see bracket). The VO2pSC reserve was used to normalize the VO2pSC response for each individual (see text for details).
Exercise Measurements

The group mean VO_{2}\text{peak}, blood lactate, and heart rate responses to MOD and VH exercise protocols are displayed in Figure 3, A–C. In MOD18, there was no evidence of any sustained blood lactate accumulation; however, a small transient increase was discernible early in the exercise (see Fig. 3B). The difference in VO_{2}\text{peak} between the 3rd and 18th minutes of MOD18 was 29 ± 50 ml/min, which was not different from zero (P > 0.05), and consistent with a VO_{2}\text{peak} steady state. The VO_{2}\text{peak} at the end of MOD18 was 1.72 ± 0.27 l/min, which was ~460 ± 130 ml/min (range 250–710 ml/min, 79 ± 5%VO_{2}\text{peak} at LT) below the VO_{2}\text{peak} associated with LT. In VH, both lactate and heart rate increased (P < 0.05) over the course of the 18 min (Fig. 3, B and C). For each participant the end-exercise VO_{2}\text{peak} was greater than their respective VO_{2}\text{peak} at RCP (by 364 ± 152 ml/min, range 150–611 ml/min) and within 218 ± 156 ml/min (range 26–461 ml/min) of VO_{2}\text{peak} (corresponding to 94 ± 4%VO_{2}\text{peak}). Also, end-exercise heart rate was within 96 ± 4% of peak heart rate.

For VH exercise, the group mean fundamental τVO_{2}\text{peak} was 28 ± 17 s (95% confidence interval 3 ± 1 s), and the phase II VO_{2}\text{peak} was 2.80 ± 0.34 l/min. The VO_{2}\text{peak} "onset" occurred at 117 ± 17 s on average. The VO_{2}\text{peak} amplitude increased (P < 0.05) from 224 ± 81 ml/min at the 3rd minute to 520 ± 119, 625 ± 134, and 678 ± 156 ml/min at the 8th, 13th, and 18th minutes, respectively (Fig. 3A). This represented 25 ± 8, 59 ± 12, 71 ± 15, and 77 ± 15% of VO_{2}\text{peak} reserve for 3, 8, 13, and 18 min, respectively.

During the repeated VH18 trial, EMG data of the vastus lateralis muscle increased (P < 0.01) from 9 ± 4% of RMS from MVC at baseline (20 W of cycling) to 48 ± 15% at the 3rd minute, with no further change (P > 0.05, n^2_p = 0.79) at the 8th (46 ± 16%), 13th (48 ± 19%), or 18th minutes (45 ± 19%).

Muscle Fatigue Measurements

The absolute and relative peak torque responses (expressed as % of the preexercise values) during voluntary and electrically stimulated contractions measured before and immediately postexercise at VH3, VH8, VH13, VH18, and MOD18 are presented in Table 1. There were no differences (P > 0.05) in preexercise (“baseline”) values for all muscle contractile properties across all exercise trials (data not shown).

Absolute and relative changes in MVC peak torque and isotonic power pre- vs. postexercise are displayed in Table 1. There was a main effect of time (P < 0.001) for both MVC (n^2_p = 0.66, Fig. 4C) and isotonic power (n^2_p = 0.40), with both progressively decreasing with time during VH. The relative change in MVC and isotonic power was greater (P < 0.01) at VH18 compared with MOD18 exercise. Voluntary activation at baseline ranged from 88 to 90% across protocols, and was lower (P < 0.05) than baseline at VH8 but was not depressed further in subsequent protocols (n^2_p = 0.35). Furthermore, there was no difference (P = 0.09) in the percent change of VA between VH18 and MOD18.

Changes in peak torque in response to stimulated contractions at 10 and 50 Hz as well as 10/50 Hz ratio are displayed in Fig. 4, B and C. All peak torque values decreased progressively (main effect, P < 0.001) over time with VH exercise (effect sizes n^2_p = 0.84, 0.50, 0.77, 0.79, 0.57 for 10, 50, 10/50 ratio, 1-Hz twitch, and potentiated twitch, respectively). The reduction in MVC across protocols was reflected by a very similar reduction in the 50-Hz torque response, whereas the 10-Hz responses were substantially lower. This resulted in significant changes in the 10/50 Hz ratio. Specifically, the 10-Hz and 10/50 Hz ratio responses were less (P < 0.05) in the VH3, VH8, and VH13 tests but were not different between VH13 and VH18 (10 Hz P = 0.54 and 10/50 Hz P = 0.94).
The 50-Hz tetanic torque was reduced at VH8 ($P < 0.05$) and again at VH13. The % change in peak torque was greater ($P < 0.05$) after VH18 compared with MOD18 for all peripheral fatigue measurements (see Table 1).

**Peripheral Muscle Fatigue and Oxygen Uptake**

The $\dot{V}O_{2p}$ slow component amplitude was correlated with the % reduction (from baseline) in the 10 Hz ($r^2$ adjusted = 0.75, $P < 0.01$), 50 Hz ($r^2$ adjusted = 0.31, $P < 0.05$), and 10/50 Hz ratio ($r^2$ adjusted = 0.69, $P < 0.001$; Fig. 5A). The $\dot{V}O_{2p}$ slow component amplitude also was correlated ($r^2$ adjusted = 0.31, $P < 0.05$) with the % reduction in maximal isotonic power. Also, all of these variables were correlated ($P < 0.01$) when the $\dot{V}O_{2p}$ slow component was expressed in %$\dot{V}O_{2p}\text{RC}$ reserve (see Fig. 5B for 10/50 Hz ratio; $r^2$ adjusted = 0.64, $P < 0.01$).

**DISCUSSION**

A direct association between skeletal muscle fatigue and the $\dot{V}O_{2p}$ slow component is often assumed but has yet to be verified experimentally in humans. The current study specifically assessed the time course of peripheral muscle fatigue during constant-PO exercise above the respiratory compensation point to test the hypothesis that the $\dot{V}O_{2p}$ slow component increases in unison with skeletal muscle fatigue. The main findings were as follows: 1) the time course of electrically stimulated muscle torque loss (a measure of peripheral muscle fatigue) coincided with the profile of the $\dot{V}O_{2p}$ slow component; 2) the largest $\dot{V}O_{2p}$ slow component amplitudes were associated with largest reductions in electrically stimulated muscle torque; and 3) voluntary neural activation of the vastus lateralis muscle (as assessed by surface EMG data) was constant during the period in which the $\dot{V}O_{2p}$ slow component and muscle fatigue developed. The quantitative and temporal association between the $\dot{V}O_{2p}$ slow component and loss of force-generating capability of the exercising muscle suggest that the mechanisms contributing to muscle fatigue also contribute to the increased $O_2$ cost of exercise.

Electrical stimulation of muscle is often used to assess peripheral factors of neuromuscular function following muscle contractions (16, 32); however, it currently is not possible to assess muscle contractile properties with this method in humans during dynamic cycling without temporarily interrupting the exercise protocol. Rather than attempting to track the muscle fatigue response over time during a single exercise bout, the present study used multiple bouts of exercise of varying durations performed on separate days to construct the muscle fatigue-time relationship for exercise at a constant, very heavy-intensity PO. This approach established changes in muscle contractile function at discrete time points (from 3 to 18 min; that is, VH3 to VH18) and allowed for tendable comparisons between these changes and the development of the $\dot{V}O_{2p}$ slow component.

Few studies have specifically examined the progression of muscle fatigue during constant-PO cycling. In the present study, with longer durations of VH exercise, a linear decrease in MVC was observed from VH3 to VH18 concomitant with a relatively small linear reduction in voluntary activation (VA, approximately $-9\%$ at VH18). These data indicate that centrally mediated limitations contributed minimally to the loss of maximal voluntary torque production (MVC, approximately $-22\%$ at VH18). The reduction in MVC across protocols was reflected by a very similar linear reduction in the 50 Hz torque response (approximately $-26\%$), whereas the reduction in 10 Hz stimulation was substantially greater (approximately $-59\%$) and curvilinear (Fig. 4, B and C). As a result, the 10/50 Hz ratio demonstrated a curvilinear reduction with time (Fig. 4C). The greater depression of force when tested at low frequencies (e.g., 10 Hz) compared with higher frequencies (e.g., $\geq 50$ Hz) is a hallmark of high-intensity muscle fatigue (15, 16, 31) and is related specifically to mechanisms that occur at or beyond the neuromuscular junction (32). The reduction in 10/50 Hz ratio with time in the present study indicates that the progressive failure of the muscle to generate torque was mediated by peripheral mechanisms supporting that muscle fatigue (as measured) was induced by the dynamic contractions.
The low-to-high frequency fatigue ratio has been extensively used to assess peripheral muscle fatigue following dynamic muscle contraction (4, 13, 15, 28, 59). For this reason, the change in 10/50 Hz ratio was chosen as the primary variable to associate with the development of the VO_{2p} slow component. A significant relationship ($r^2$ adjusted = 0.69, Fig. 5A) was observed between the VO_{2p} slow component amplitude (see Fig. 4A) and the fall in 10/50 Hz ratio (see Fig. 4C) indicating that larger VO_{2p} slow component amplitudes accompanied greater increases in peripheral muscle fatigue. Although this association does not establish cause and effect, it is possible that both phenomena evolve from common mechanisms. Indeed, reductions in the contractile properties of muscle previously have been related to changes that occur in the muscle metabolic environment (27, 57). For example, inorganic phosphate (P_i) is known to accumulate in the cytosol during very heavy exercise (30). As a consequence of increases in muscle [P_i], deficits in both metabolic and contractile protein coupling efficiency have been established via reductions in the free energy yield from ATP hydrolysis (1, 26) and impairments in calcium exchange from the sarcoplasmic reticulum (14, 22, 60), respectively. Furthermore, accumulation of other metabolic byproducts such as H^+, K^+, and ADP_{free} also have been linked to both reductions in metabolic efficiency (26) and factors known to cause low-frequency fatigue (i.e., those related to decrements in excitation-contraction coupling and action potential propagation) (4, 32). That a link between muscle fatigue and the progressive reduction in work efficiency was observed during high-intensity exercise in the present study supports the hypothesis that a common effector may lead to simultaneous reductions in force output capabilities of the muscle and increases in the VO_{2} required at a given PO.

Alternatively, it is thought that with progressive attenuation in force-generating capabilities of active muscle fibers during locomotor exercise, additional recruitment of previously inactive motor units is necessary to maintain PO, causing a net increase in VO_{2p} (23, 29, 37). The bulk of the evidence supporting this “progressive recruitment” hypothesis stems from studies reporting increases in quadriceps muscle surface EMG data by measurement of mean power frequency during constant-PO exercise where a VO_{2p} slow component is present (9, 43, 46, 52, 54). However, numerous studies, including the current study, have not found this relationship (11, 24, 40, 48, 53). In the present study, RMS of the vastus lateralis muscle was constant from the 3rd to 18th minutes of very heavy exercise (constituting less than 50% of RMS generated during MVC), whereas PO and cadence remained unchanged. It has been reported that cycling exercise at 75%VO_{2max} requires less than 40% maximal dynamic force production (2, 12, 51), which raises the question whether increased recruitment of additional motor units or enhanced motor neuron firing rates, or both, are requisite to compensate for the developing fatigue within active muscle fibers. It is possible that at these relatively submaximal POs any fatigue-induced decline in force-producing capability of the active muscle may have negligible effects on the ability of that muscle to sustain the force requirements of the task (61). Furthermore, it must be recognized in any of these studies that have used surface EMG measures during dynamic fatiguing exercise there are technical and physiological limitations to making specific interpretations about motor unit properties (18, 19). However, despite possible limitations with the measure of surface electromyographs, increases in VO_{2p} were reported concurrently with reductions in PO and
muscle activation during sprint-cycling in humans (58), and a V\textsubscript{O2p} slow component has been observed in the absence of progressive recruitment in electrically stimulated dog muscle (65). These results largely refute a role for progressive motor unit recruitment in relation to V\textsubscript{O2p} slow component development. The present observation of a strong association between V\textsubscript{O2p} slow component amplitude and the magnitude of peripheral muscle fatigue in the absence of any changes in muscle activation (at least for the muscles studied here) favors a mechanism whereby the fatiguing fiber pool remains capable of generating the required power to continue constant-PO cycling but does so at a greater O\textsubscript{2} cost.

During 8 min of very heavy constant-PO exercise, Cannon et al. (12) reported that maximal isokinetic cycling power (an index of muscle fatigue) was reduced by the 3rd minute but was not depressed further at 8 min. Because the major fraction of the V\textsubscript{O2p} slow component evolved between the 3rd and 8th minutes in this condition, it was concluded that muscle fatigue precedes the V\textsubscript{O2p} slow component but that the two variables are not temporally related. This implicates muscle fatigue as a possible mechanism for initiation but not progression of the V\textsubscript{O2p} slow component. The present study modified the protocol and added to the findings of Cannon et al. (12) by evaluating the V\textsubscript{O2p} slow component and muscle fatigue over a longer time period (18 vs. 8 min) and by assessing the development of fatigue using a protocol that focused specifically on the time course of “peripheral” fatigue via reductions in electrically stimulated muscle torque and power development. Our finding that the time course of muscle fatigue development and the development of the V\textsubscript{O2p} slow component were related implicates muscle fatigue not only as an initiator of the V\textsubscript{O2p} slow component but also a mechanism by which it progresses with time.

Surprisingly, significant low-frequency fatigue was observed after just 18 min of moderate cycling (in the absence any V\textsubscript{O2p} slow component), although high-frequency (50 Hz) fatigue, MVC, VA, and isometric power were unaltered. Peripheral fatigue has been reported following moderate-intensity cycling exercise (39, 42); however, these changes were observed following very long durations (>1.5 h). This may be attributable to a “total work” effect because both the reduction in torque production with 10-Hz tetanus and 10/50 Hz ratio were different following MOD18 and VH8, in which total work was approximately equal. The clear distinctions in both the metabolic and physiological responses to working at these different intensities makes it difficult to speculate as to the physiological significance. Indeed, both VA and 50 Hz peak torque were reduced further in VH8 compared with MOD18 (P < 0.05) despite different exercise durations, suggesting that intensity affected the neuromuscular and contractile properties of the muscle differently in both conditions.

Limitations. Although care was taken to ensure that postexercise neuromuscular testing was completed immediately after exercise, some time was required (~45 s from end exercise) for the participants to dismount the cycle ergometer and begin neuromuscular assessment on the knee extension dynamometer. All postexercise measurements were completed within 3 min of exercise cessation. This timing, in addition to the order of the testing paradigms, was kept constant and so it is unlikely that this would have a large influence on the results, and indeed differences might have been enhanced if recovery time due to transitioning was minimized. Additionally, the fatigue-time relationship was constructed over several visits. Thus day-to-day variability of the muscle contractile properties could have contributed to variability in the neuromuscular data. However, several control measures were taken to limit any day-to-day variability on both neuromuscular and exercise responses including standardizing preexercise meals/hydration, having individuals exercise at a similar time of day, ensuring a 48-h gap between test sessions, and advising against consumption of caffeinated products prior to exercise. Furthermore, testing was not completed if an individual’s preexercise MVC torque was not within 10% of that of previous test days. Finally, force measures, as assessed, were not specific to dynamic cycling. Rather, postcycling isometric torque production was investigated using various paradigms at a fixed angle of knee flexion. Although these likely provide a good approximation of the contractile and neuromuscular properties of the muscle, it is
not necessarily identical to dynamic function. Therefore, the isotonic knee extensions were included to provide some indication of similarities, and the magnitude of the reduction in maximal isotonic torque was correlated to the VO₂p slow component amplitude.

Conclusion. The ability to maintain PO during prolonged cycling is limited by ability to resist fatigue. Furthermore, the appearance and magnitude of the V˙O₂p slow component has been positively associated with exercise intolerance (41). This study provides the first direct evidence in humans of an association between fatigue that develops within active muscle and amplitude of the VO₂p slow component. In the absence of any changes in muscle activation over the course of the exercise, these data indicate that as exercising muscle fatigues, it concurrently requires more VO₂ to sustain the same power output. Thus the appearance and evolution of the VO₂p slow component may stem from the same mechanisms that cause the development of skeletal muscle fatigue.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


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