# Oxygen uptake kinetics during two bouts of heavy cycling separated by fatiguing sprint exercise in humans

N. TORDI,<sup>1</sup> S. PERREY,<sup>3</sup> A. HARVEY,<sup>2</sup> AND R. L. HUGHSON<sup>2</sup>

<sup>1</sup>Laboratoire des Sciences du Sport, 25030 Besançon Cedex, France; <sup>2</sup>Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1; and <sup>3</sup>Faculté des Sciences du Sport, Unité de Formation et de Recherche en Sciences et Techniques des Activités Physiques et Sportives, 2991, 34090 Montpellier, France

Submitted 19 June 2002; accepted in final form 2 October 2002

Tordi, N., S. Perrey, A. Harvey, and R. L. Hughson. Oxvgen uptake kinetics during two bouts of heavy cycling separated by fatiguing sprint exercise in humans. J Appl *Physiol* 94: 533–541, 2003. First published October 11, 2002; 10.1152/japplphysiol.00532.2002.-We tested the hypothesis that  $O_2$  uptake ( $VO_2$ ) kinetics at the onset of heavy exercise would be altered in a state of muscle fatigue and prior metabolic acidosis. Eight well-trained cyclists completed two identical bouts of 6-min cycling exercise at >85% of peak Vo<sub>2</sub> separated by three successive bouts of 30 s of sprint cycling. Not only was baseline Vo<sub>2</sub> elevated after prior sprint exercises but also the time constant of phase II Vo<sub>2</sub> kinetics was faster (28.9  $\pm$  2.4 vs. 22.2  $\pm$  1.7 s; P < 0.05). CO<sub>2</sub> output (Vco<sub>2</sub>) was significantly reduced throughout the second exercise bout. Subsequently Vo<sub>2</sub> was greater at 3 min and increased less after this after prior sprint exercise. Cardiac output, estimated by impedance cardiography, was significantly higher in the first 2 min of the second heavy exercise bout. Normalized integrated surface electromyography of four leg muscles and normalized mean power frequency were not different between exercise bouts.  $\dot{V}_{02}$  and  $\dot{V}_{C02}$ kinetic responses to heavy exercise were markedly altered by prior multiple sprint exercises.

cardiac output; high-intensity cycling; muscle fatigue; electromyography

THE PULMONARY OXYGEN UPTAKE (VO2) response to constant-intensity exercise reflects the time course of the adjustment of muscle  $Vo_2$  toward a steady state (4, 40). Gerbino et al. (18) first demonstrated that  $Vo_2$  kinetics during heavy exercise were accelerated by a prior bout of heavy exercise and proposed that this speeding was the consequence of an increased vasodilatation and a greater O<sub>2</sub> availability during the second bout of heavy exercise. MacDonald et al. (31) demonstrated even faster adaptation of  $\dot{V}o_2$  in the first as well as the second bout of exercise when arterial  $O_2$  content was increased. Also of interest during the adaptation to heavy-intensity exercise is the "slow component" or the progressive increase in Vo<sub>2</sub> above that predicted for the work rate (33). The slow component has its origin in the working muscles (37), but the mechanism is unresolved (5, 41). The magnitude of this slow component is reduced by prior heavy intensity exercise (13, 18, 31), and this corresponds with less depletion of muscle phosphocreatine (41).

In contrast to the early reports by Gerbino et al. (18) and MacDonald et al. (31), some investigators have stated the higher  $\dot{V}o_2$  at the onset of a second bout of high-intensity exercise or after a single 30-s sprint was simply a function of increased recruitment of muscle fibers early in exercise (13), perhaps reducing the metabolic disturbance in individual fibers (11, 12, 26). Of particular interest is that these investigators have interpreted their data to indicate that although  $\dot{V}o_2$  is elevated early in exercise, the actual rate of increase as assessed by the phase II time constant was not altered (13, 26). Results such as these have led some investigators to propose that  $O_2$  delivery was not limiting the rate of increase in oxidative metabolism at the onset of heavy- to very heavy-intensity exercise (3, 11–13).

Recent direct investigations of O<sub>2</sub> transport during repeated bouts of high-intensity exercise demonstrated residual effects of the first bout of high-intensity exercise on forearm (30) and leg (28) acid-base status and other potential vasodilators in venous blood from the formerly working muscle. These factors might have contributed to elevated blood flow and greater  $O_2$  extraction early in a subsequent exercise bout. In light of these experiments (28, 30), we hypothesized that a greater increase in the level of metabolic acidosis during leg cycling might cause a further, and detectable, acceleration in Vo<sub>2</sub> kinetics in the second bout of heavy, constant-load exercise. To achieve this marked acidosis, we had subjects complete three all-out 30-s sprint cycle tests between the two bouts of heavy constant-load exercise. It has been suggested that the fiber recruitment, and by extension a delayed and additional slow component of Vo<sub>2</sub>, could be related to exercise intensity and the muscle pH (42). Therefore, we hypothesized that the slow component would occur earlier and be of larger magnitude in the second exercise bout after fatiguing sprint exercises.

Address for reprint requests and other correspondence: R. L. Hughson, Dept. of Kinesiology, University of Waterloo, Waterloo, ON, Canada N2L 3G1 (E-mail: hughson@uwaterloo.ca)

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

## **METHODS**

Subjects. Eight well-trained male cyclists (age  $29.8 \pm 1.9$  yr, height  $177.2 \pm 2.3$  cm, and weight  $71.3 \pm 2.4$  kg) participated in this study. After receiving complete verbal and written details of the protocol, each subject gave informed, written consent on a form approved by the office of research ethics at the University of Waterloo.

*Experimental design.* Each subject performed preliminary testing consisting of an incremental exercise to volitional fatigue in which the work rate increased as a ramp function by 30 W/min at a cycling frequency of 80 rpm. We defined fatigue as the point when the subject was unable to maintain a pedaling rate of 75 rpm, despite strong verbal encouragement. The gas-exchange data obtained from the ramp test were used to estimate the ventilatory threshold (VT) and the peak  $\dot{V}O_2$  ( $\dot{V}O_2$  peak). VT was determined from the point of increased minute ventilation ( $\dot{V}E$ )-to- $\dot{V}O_2$  ratio without an increase in  $\dot{V}E$ -to- $CO_2$  output ( $\dot{V}CO_2$ ) ratio.  $\dot{V}O_2$  peak was taken as the average of the highest five consecutive breaths attained in the last minute of exercise.

On the second test day, subjects performed two identical step transitions lasting 6 min after 4-min baseline cycling at 25 W. This work rate was estimated to require  $\dot{V}o_2$  equal to ~85% of  $\dot{V}o_2$  peak, although progressive increase in  $\dot{V}o_2$  above this value was expected. After the first submaximal exercise bout, subjects rested for 10 min before completing three 30-s bouts of all-out sprint cycling [Wingate test (16)]. The three sprint exercises were separated by 4 min of passive recovery. After the third sprint, subjects rested for 10 min divided in 6 min of passive recovery and 4 min of baseline cycling at 25 W, and then they performed the second step exercise.

All submaximal tests were conducted on the same electromagnetically braked cycle ergometer (Excalibur, Lode, Groningen, Netherlands). Seat and handlebar positions were kept constant for individual subjects during the course of the study and subjects used their own shoes and pedals. Pedal frequency was maintained at 80 rpm for both identical step transitions.

For the sprint exercises, the subjects performed a standard Wingate test (16). This consisted of 30 s of maximal effort on a mechanically braked cycle ergometer (model 841E, Monark, Varberg, Sweden) against a preset friction load of 7.5% of the subject's body weight. The subjects were encouraged to cycle at their maximal attainable pedaling frequency throughout the entire test duration.

Breath-by-breath ventilation and gas exchange were measured on a computerized system (First Breath, St Agatha, ON, Canada), which sampled inspired and expired volumes with a volume turbine (model VMM-110, Alpha Technologies, Laguna Beach, CA) and fractional concentrations of  $O_2$ ,  $CO_2$ ,  $N_2$  by mass spectrometry (model MGA-1100A, Marquette, Milwaukee, WI). The mass spectrometer was calibrated with precision-analyzed gas mixtures before each test.

Heart rate (HR) was continuously monitored via a threelead electrocardiograph (model 7803A, Hewlett-Packard, Fort Collins, CO), and mean HR over each breath was recorded.

Measurement of changes in thoracic impedance during the cardiac cycle provides an estimate of beat-to-beat changes in stroke volume and cardiac output. The impedance device (PhysioFlow PF-05, Manatec Biomedical, Paris, France) concept and the methodology used in the present study have been described in recent studies (15, 38). Two sets of electrodes (Ag-AgCl), one transmitting and one sensing, were applied above the supraclavicular fossa at the left base of the neck and along the xiphoid of each subject. Another set of two electrodes was used to monitor a single electrocardiographic signal (CM5 position). The estimate of cardiac output by the impedance technique was required to span a large range of values and might underestimate slightly values with a different technique (17); therefore, we normalized the data to the baseline cycling period during the first exercise bout.

Surface electromyography (EMG) was obtained from the vastus lateralis (VL), rectus femoris (RF), vastus medialis (VM), and gastrocnemius medialis (GM) by using bipolar surface electrodes (Ag-AgCl electrodes, 200 Medi-Trace, surface cup diameter 10 mm) with an interelectrode distance of 35 mm. All signals were collected on the subject's right leg, and the reference electrode was placed on the right anterior superior iliac spine. Electrode sites were determined by visualization and palpation to determine the most prominent part of the muscle belly. Before electrode placement, the skin was shaved and cleaned with alcohol. The electrodes and cables were then taped firmly in place to reduce artifact and to maintain position throughout the entire experiment. The myoelectrical signal was band-pass filtered (20-500 Hz), differentially amplified (gain 2,000 times, input impedance 2 M $\Omega$ , common mode rejection rate >90 dB), and sampled at 1,024 Hz during the last 15 s of each minute of exercise and continuously during the Wingate tests. The raw EMG signals were then converted from analog to digital by using a 12-bit analog-to-digital conversion board (MetraByte DAS-16). The digitized signal was displayed on computer using collection software and saved for later analysis.

Data analysis. Breath-by-breath data for  $\dot{V}o_2$  for each subject were linearly interpolated between breaths to give values at 1-s intervals and fit to a curve using a threecomponent exponential model starting at the onset of exercise. The computer model utilized to describe the kinetic response provides an estimate of the amplitude of baseline  $\dot{V}o_2$  kinetics ( $A_0$ ); amplitudes of phases I, II, and III of  $\dot{V}o_2$ kinetics ( $A_1$ ,  $A_2$ , and  $A_3$ ); time delays of phases I, II, and III of  $\dot{V}o_2$  kinetics ( $TD_1$ ,  $TD_2$ , and  $TD_3$ ), and time constants of phases I, II, and III of  $\dot{V}o_2$  kinetics ( $\tau_1$ ,  $\tau_2$ , and  $\tau_3$ ). The phase I fitting parameters were set to achieve a completed response before the start of phase II, thus allowing for complete comparison with other investigations that have omitted the phase I in curve fitting of heavy-intensity exercise (5, 13).

$$Vo_2 = A_0 + A_1[1 - e^{-(t - TD_1)/\tau_1}]\mu_1$$

$$+A_2[1-e^{-(t- ext{TD}_2)/ au_2}]\mu_2+A_3[1-e^{-(t- ext{TD}_3)/ au_3}]\mu_3$$

where

 $\begin{aligned} &\mu_1=0 \quad \text{for} \quad t < \text{TD}_1 \quad \text{and} \quad \mu_1=1 \quad \text{for} \quad t \geq \text{TD}_1 \\ &\mu_2=0 \quad \text{for} \quad t < \text{TD}_2 \quad \text{and} \quad \mu_2=1 \quad \text{for} \quad t \geq \text{TD}_2 \\ &\mu_3=0 \quad \text{for} \quad t < \text{TD}_3 \quad \text{and} \quad \mu_3=1 \quad \text{for} \quad t \geq \text{TD}_3 \end{aligned}$ 

Model parameters were determined by least squares nonlinear regression (except phase I as noted above) in which the best fit was defined by minimization of the residual sum of squares. The overall time course of the response was determined from the mean response time (MRT). The MRT is equivalent to the time required to achieve ~63% of the difference between  $A_0$  and the final Vo<sub>2</sub> value as previously used (31).

Beat-by-beat data for cardiac output were averaged every five beats and were fit to a curve by using a two-component exponential model starting at the onset of exercise. This fitting procedure allowed for the best-fit estimate and minimized random error. The values obtained at the end of each minute from the curve fitting were used to evaluate the cardiac output response profile for each subject.

The raw EMG signals were processed off-line (34) by fullwave rectification and digital low-pass filtering with a second-order Butterworth filter (cutoff frequency = 4 Hz) to produce a linear envelope. Five sequential bursts were then selected for each signal and integrated (iEMG). Mean power frequency (MPF) was determined using a fast Fourier transform algorithm over the 15-s window (cutoff frequency 512 Hz). Similar methods evaluated the EMG during the Wingate cycling. In this case, data were binned in 5-s intervals starting between 5 and 10 s of exercise and then expressed as a ratio relative to the power output in that same interval. Normalization of data in the constant-load tests was to the values at the first minute of the first exercise bout for each subject. In the Wingate tests, data were normalized to the second 5-s interval of the first all-out sprint. Muscle activation was evaluated for each muscle separately.

Mean values of Vo<sub>2</sub>, Vco<sub>2</sub>, HR, and VE were calculated during the final 2 min before the step increase in work rate, from 5 s before to 5 s after 3 min and during the last 10 s of exercise. In addition to the curve-fitting procedure, the  $\dot{V}o_2$ slow component was also computed as the difference in  $\dot{V}o_2$ between *minutes 3* and 6 ( $\Delta \dot{V}o_{2 \ 6-3}$ ) (18).

Statistical analysis. For simple comparisons of values that were present in only one condition for pre- vs. post-Wingate, paired *t*-tests were computed. This included analysis of respiratory variables and HR changes on baseline and at *minutes 3* and 6. Cardiac output means were compared by using the nonparametric Wilcoxon test. Significance was set at P < 0.05. All data are presented as means  $\pm$  SE.

The constant-load cycling EMG response was evaluated by a two-way repeated-measures ANOVA on the effects of condition (i.e., pre- and post-Wingate) and time. Both power output and the ratio of EMG to power output from the three Wingate tests were also evaluated by two-way repeatedmeasures ANOVA. If the normality or the equality of variance failed, the nonparametric Friedman test was used for post hoc comparisons.

#### RESULTS

Incremental test. The  $\dot{V}o_{2 \text{ peak}}$  of 65.5  $\pm$  0.8 ml·min<sup>-1</sup> ·kg<sup>-1</sup> was associated with a peak work rate of 441.2  $\pm$  9.7 W during the 30 W/min ramp incremental test. VT occurred at 76.5  $\pm$  1.6% of  $\dot{V}o_{2 \text{ peak}}$ .

Wingate tests. The peak power output decreased during the three successive Wingate tests (705.4  $\pm$  27.9, 638.5  $\pm$  29.3, and 598.8  $\pm$  26.1 W; P < 0.05). Power output declined during each successive Wingate test (Fig. 1; P < 0.05), and the ratio of EMG to power output was increased with time and from bout to bout in certain muscles (see Fig. 2).

Surface EMG during heavy exercise. Normalized MPF and iEMG (Fig. 3) did not change significantly with time within each exercise bout. Nor were there significant changes in MPF or iEMG between pre- and post-Wingate bouts of heavy exercise. The maximum deviation for MPF was <5% from the value observed at the first minute of the first exercise bout (data not shown).

Heavy cycling exercises. Vo<sub>2</sub> measured at the end of the pre- and post-Wingate heavy exercises correspond to 97.8  $\pm$  2.5 and 97.0  $\pm$  1.9% of the Vo<sub>2 peak</sub>, respectively (P > 0.05). Because the Vo<sub>2</sub> tended toward Vo<sub>2 peak</sub>, this exercise might be categorized as severe (36). The time courses of increase in Vo<sub>2</sub> for one subject



Fig. 1. Absolute power output during 3 repetitions of 30-s all-out sprint cycling (Wingate test). Values are means  $\pm$  SE. for Wingate test 1 (•), test 2 ( $\odot$ ), and test 3 (•). The decline in power output within a test was significant for each repetition (P < 0.05). Decline in power output between tests was different as indicated (\*P < 0.05 relative to Wingate test 1, #P < 0.05 relative to Wingate test 2).

(Fig. 4) and the group mean responses (with 95% confidence intervals, Fig. 5) reveal the faster  $Vo_2$  response in the post-Wingate tests. The model parameters for curve fitting of the  $\dot{V}o_2$  response to heavy exercise in pre- and post-Wingate are summarized in Table 1. After the Wingate tests, a significant (P < 0.05) increase in  $A_0$  was found compared with pre-Wingate (Table 1).  $A_1$  was not significantly affected by prior sprint exercise. The  $\tau_2$  was significantly faster, by 23% (P < 0.05), after the 30-s sprint repetitions. Neither  $A_2$ nor the sum of  $A_1 + A_2$  was affected, but Vo<sub>2</sub> was significantly greater at 3 min in post- compared with pre-Wingate tests (+124 ml/min; Table 2). The 3min  $Vo_2$  was influenced by the elevated  $A_0$  and the faster  $\tau_2$  as well as the earlier onset of phase III (TD<sub>3</sub>) in the post-Wingate bout of heavy exercise (Table 1). There were no differences in  $A_3$  or  $\tau_3$  between the two bouts of heavy exercise, but the mean response time obtained as a weighted average of the time constants was significantly faster post-Wingate (Table 1). Endexercise Vo<sub>2</sub> was not different between exercise bouts. The  $\Delta Vo_{2 6-3}$  was  $\sim 30\%$  less in post- compared with pre-Wingate tests (300.6  $\pm$  55.6 vs. 210.2  $\pm$  92.4 ml/ min, respectively; P < 0.05).

Pre- to post-Wingate comparisons at three different times (baseline, 3rd min, end exercise) showed significant changes in VE, HR, and VCO<sub>2</sub> (Table 2). During the 2-min baseline period preceding heavy exercise, HR was significantly higher in post-Wingate, whereas VE, and VCO<sub>2</sub> were not significantly different. At the third minute in post-Wingate test, VO<sub>2</sub>, VE, and HR were all higher, whereas VCO<sub>2</sub> was lower compared with pre-Wingate test. During the last minute of the post-Wingate test, despite the significant increase in VE the VO<sub>2</sub> was still significantly lower. HR in the last minute of exercise was not different between pre- and post-Wingate test. The different time course for VCO<sub>2</sub> is plotted for pre- and post-Wingate tests in Fig. 5.





Cardiac output was significantly greater during the baseline and the first 2 min of heavy exercise but not thereafter (Table 3).

# DISCUSSION

This study was designed to investigate the effects of preexisting metabolic acidosis on the adaptation of  $\dot{V}o_2$  at the onset of a second bout of heavy constant-load exercise. The study was also designed to investigate the effects of prior fatiguing exercise on the  $\dot{V}o_2$  and









Fig. 4. Superimposed  $O_2$  uptake ( $\dot{V}O_2$ ) data for a single subject from heavy exercise *bout* 1 ( $\odot$ ) and *bout* 2 after three repetitions of 30-s all-out sprint cycling ( $\bullet$ ) to show more rapid adaptation in *bout* 2.



Fig. 5.  $\dot{V}O_2$  and  $CO_2$  output ( $\dot{V}CO_2$ ) in baseline, during 6 min of heavy exercise, and in recovery for *bout 1* (black traces) and *bout 2* (gray traces). Values are mean  $\pm$  95% confidence intervals.

bout of exercise, but the prior fatiguing exercise had no significant effect on the indicator of muscle activation from the EMG signal. This latter observation was similar to that of a recent study that investigated a single muscle during repeated heavy exercise (42)

It has been consistently observed for several different exercise models that Vo<sub>2</sub> is elevated during the early phase of a heavy exercise bout when it was performed after previous heavy exercise (11–13, 18, 27, 28, 30, 31, 42). Initial reports from studies of leg cycling exercise similar to the present study did not confirm that this was a consequence of faster  $\tau_2$  but instead relied on reduction of the mean response time or of the  $\Delta Vo_{2,6-3}$  (18, 31). Both of these indicators were also significantly reduced in the second exercise bout in the present study. Several recent studies did not find significant acceleration of phase II kinetics after prior heavy exercise (10-13, 27, 42). We incorporated three bouts of all-out sprint cycling between the two bouts of heavy exercise to induce a greater metabolic acidosis (32, 44) that we reasoned might enhance vasodilatation and O<sub>2</sub> extraction at the onset of the second bout of exercise. Three bouts of sprint cycling rather than one bout as in a recent study (12) would have caused a greater and more sustained increase in plasma lactate. Whereas Burnley et al. (12) observed plasma lactate to be 5.6 mmol/l after their single bout of exercise, Mc-Cartney et al. (32) found plasma lactate to remain high (~20 mmol/l) for at least 20 min after repeated bouts of 30-s all-out sprint cycling. With the same exercise model, Spriet et al. (44) observed muscle H<sup>+</sup> to increase from baseline of 195  $\pm$  12 nmol/l to 274  $\pm$  19 after the first bout of sprinting to 315  $\pm$  24 (i.e., corresponding pH = 6.50) after the third bout of sprint cycling.

Previously, Gerbino et al. (18) and MacDonald et al. (31) postulated that enhanced O<sub>2</sub> delivery and possibly a right shift of the O<sub>2</sub>-hemoglobin dissociation curve in the second bout of heavy exercise would supply more  $O_2$  and allow faster adaptation of oxidative metabolism. Although we do not have data for leg blood flow in this study, two lines of evidence would support the proposal that O<sub>2</sub> delivery has increased. First, we did find significantly elevated HR and estimated cardiac output throughout the early phase of the second bout of exercise that were consistent with elevated bulk O<sub>2</sub> transport. Second, two recent studies that examined arm (30) and leg exercise (28) observed that higher muscle  $Vo_2$  in the second bout of exercise was a consequence of both higher blood flow and greater O<sub>2</sub> extraction early in the second bout of exercise. A potential increase in delivery of O<sub>2</sub> along with probable changes in muscle enzymes and/or metabolic intermediates (22) certainly contributed to our observation of faster  $\tau_2$ . Because we did not measure blood flow or its distribution we cannot exclude the possibility that temperature effects caused greater blood flow to skin for thermoregulation.

It was important to optimize experimental conditions to determine whether  $\tau_2$  would be altered (41). Recently, Hughson et al. (25) demonstrated from a computer simulation model that it is extremely difficult to detect either dynamic nonlinearities or differences in time constant even when they are present. It is possible that the recent investigations that did not find an effect on  $\tau_2$  (11–13, 27, 42) either did not have enough residual effect on local vasodilator influences to

Table 1.  $\dot{V}O_2$  kinetics parameters during heavy exercise pre- and post-Wingate tests

	Pre-Wingate	Post-Wingate
$\overline{A_0, ml/min}$	$970.5\pm37.9$	$1,119 \pm 50.3^{*}$
$A_1$ , ml/min	$666.8\pm50$	$660.0\pm45.4$
$TD_1$ , s	$1.1\pm0.4$	$1.2\pm0.5$
$\tau_1$ , s	$1.0\pm0.1$	$0.9\pm0.03$
$A_2$ , ml/min	$2,352.6 \pm 113.1$	$2,268.7 \pm 87.7$
$TD_2$ , s	$10.0 \pm 0.8$	$9.3\pm0.4$
τ <sub>2</sub> , s	$28.9 \pm 2.4$	$22.2 \pm 1.7^{*}$
$A_1 + A_2$ , ml/min	$3,020.4 \pm 97.9$	$2,928.7 \pm 65.1$
$A_3$ , ml/min	$624.0 \pm 89.4$	$534.4 \pm 44.8$
$TD_3$ , s	$120.9\pm6.4$	$96.0 \pm 3.1^{*}$
τ <sub>3</sub> , s	$154.2\pm17.1$	$127.1\pm12.2$
MRT, s	$73.6\pm7.3$	$56.1 \pm 3.8^{*}$

Values are means  $\pm$  SE for 8 subjects.  $A_0$ , amplitude of baseline  $O_2$  uptake ( $\dot{V}O_2$ ) kinetics;  $A_1$ ,  $A_2$ , and  $A_3$ , amplitude of phase I, II, and III of  $\dot{V}O_2$  kinetics, respectively;  $\tau_1$ ,  $\tau_2$ , and  $\tau_3$ , time constants of phase I, II, and III of  $\dot{V}O_2$  kinetics, respectively; TD<sub>1</sub>, TD<sub>2</sub>, and TD<sub>3</sub>, time delay of phase I, II, and III of  $\dot{V}O_2$  kinetics, respectively; MRT, mean response time. \*Significantly different from pre-Wingate, P < 0.05.

		Pre-Wingate			Post-Wingate		
	BL	3 min	6 min	BL	3 min	6 min	
Vo <sub>2</sub> , ml/min VE, l/min VCO <sub>2</sub> , ml/min HR, boats/min	$\begin{array}{c} 1,024.8\pm40.5\\ 27.7\pm1.4\\ 930.7\pm29.7\\ 79.1\pm4.4\end{array}$	$4,160.5 \pm 127.3 \\ 124.8 \pm 5.8 \\ 4,607.9 \pm 152.4 \\ 160.6 \pm 4.3$	$\begin{array}{c} 4,461.1\pm110.6\\ 142.5\pm8.5\\ 4,718.3\pm146.8\\ 174.0\pm4.4 \end{array}$	$1,157.1 \pm 51.1^{*}$ $35.5 \pm 4.1$ $868.7 \pm 55.5$ $111.5 \pm 3.1^{*}$	$4,284.6 \pm 120.5^{*}$ $143.4 \pm 9.5^{*}$ $4,146.5 \pm 83.0^{*}$ $170.4 \pm 2.8^{*}$	$\begin{array}{r} 4,494.8\pm108.5\\ 164.5\pm8.1^{*}\\ 4,519.7\pm139.3^{*}\\ 177.1\pm3.4\end{array}$	

Table 2. Respiratory variables and heart rate changes during heavy exercise pre- and post-Wingate tests

Values are means  $\pm$  SE for 8 subjects. BL, baseline level; VE, minute ventilation; HR, heart rate; VCO<sub>2</sub>, carbon dioxide output. \*Significantly different from pre-Wingate, P < 0.05.

achieve an effect, that an effect was present but was undetectable by the curve-fitting methods employed, or that the studies had insufficient statistical power to observe an effect. The observation that even heavyintensity prior arm exercise has an effect on subsequent heavy leg exercise  $Vo_2$  kinetics (8) suggests that changes in arterial blood acid-base can have an impact on cardiac output and/or local vasodilatation at the onset of exercise and that the effect, at least with the prior arm exercise, can be independent of altered muscle metabolic factors. Another argument often used to claim that  $\tau_2$  is independent of  $O_2$  delivery is the comparison between heavy (above ventilatory threshold) vs. moderate (below threshold) intensities of exercise (11, 13, 42). Some studies have shown very markedly slower mean values for  $\tau_2$  with heavy compared with moderate exercise [e.g., 24% (14) to 38% (42)] or progressive lengthening with heavier exercise (6), but they failed to find statistical significance. These observations again point to limited sensitivity of kinetic analysis (25) especially when the response becomes nonlinear with heavy exercise (41).

The present study and the recent investigation by Rossiter et al. (41) were designed to examine in detail the time constant for  $\dot{V}o_2$  during the phase II response. Both investigations found significant acceleration of phase II time constant by prior heavy exercise. Other research that observed elevated  $\dot{V}o_2$  but without faster phase II kinetics was taken to indicate an unchanged response on top of an existing baseline of higher  $\dot{V}o_2$  (13, 42). To account for this elevated baseline, Burnley et al. (13) subtracted the baseline for both first and second exercise bouts. The problem with this analysis was that end-exercise  $\dot{V}o_2$  was reduced in the

Table 3. Evolution of cardiac output in pre- and post-Wingate tests normalized to baseline values before the first bout of heavy exercise

	Pre-Wingate $(n = 7)$	Post-Wingate $(n = 6)$
BL, minute 3, and minute 4	1	$1.44 \pm 0.06*$
Minute 1	$2.22\pm0.11$	$2.46 \pm 0.15^{*}$
Minute 2	$2.35\pm0.12$	$2.61 \pm 0.18^{*}$
Minute 3	$2.43\pm0.13$	$2.72\pm0.19$
Minute 4	$2.48\pm0.13$	$2.79\pm0.20$
Minute 5	$2.53\pm0.14$	$2.84\pm0.22$
Minute 6	$2.56\pm0.15$	$2.88\pm0.23$

Values are means  $\pm$  SE; *n*, no. of subjects. \*Significantly different from pre-Wingate, P < 0.05.

second bout, suggesting improved efficiency and  $Vo_2$ recovered after the second bout of exercise to values below the baseline. More recently, Burnley et al. (11) allowed baseline  $Vo_2$  to recover completely by interposing 12-min of recovery rather than 6 min. Again they observed elevated  $Vo_2$  at the onset of the second bout of exercise but it was attributed to greater amplitude of phase II rather than faster kinetics. The mechanism proposed for this was that a greater number of muscle fibers were recruited early in exercise (10) and that oxidative metabolism in these fibers continued to adapt at the same rate, assumed to be independent of  $O_2$  but limited by metabolic control factors.

One objective of the present research was to investigate muscle recruitment patterns through surface EMG. If we first consider the EMG pattern during the completion of the Wingate tests, the characteristic pattern of muscle fatigue is evident. Within each 30-s all-out sprint, the ratio of iEMG to power output increased progressively, suggesting progressive fatigue of muscle fibers. In contrast with the findings from the successive all-out sprints, observations during the constant-load cycling tasks did not provide evidence of altered muscle function even after the fatiguing all-out sprints. Throughout the first constant-load heavy cycling bout, normalized iEMG did not deviate from the value observed at the end of the first minute of exercise. In the second bout of heavy exercise after the three 30-s all-out sprints, iEMG tended to be greater than that in the first bout, which would have been consistent with residual fatigue, but individual variability precluded statistically significant findings. As in the first exercise bout, there were no significant changes in iEMG over time within the exercise bout. The MPF of the EMG also showed a small trend to lower values, again a potential indicator of fatigue, but the difference was not statistically significant. The EMG is an indirect method of assessing muscle fiber recruitment. The limitations of the technique might be responsible for the variable observations where some researchers (9, 10, 43) have found changes in EMG corresponding to the onset of the phase III slow component. Other researchers did not find any effects on the EMG to correspond with the onset of the slow component during prolonged heavy exercise in trained cyclists (29) or during repeated bouts of heavy exercise (42). The fact that our subjects were well-trained cyclists and we were able to monitor only four muscles meant that any subtle difference in muscle recruitment

strategies probably introduced sufficient variability in individual muscle or combined muscle activity patterns to prevent us from observing the anticipated signatures of muscle fatigue.

Oxidative metabolism in the transition from rest or light to heavier exercise is regulated by the interaction of  $O_2$  and biochemical factors (45). To date the details of this interaction are not completely understood. In the human calf muscle, Richardson et al. (39) observed by magnetic resonance spectroscopy that intramuscular  $Po_2$  dropped within 20 s to values <5 Torr and that this value was relatively constant across a wide range of work rates. If it can be assumed that similar low, or lower, values occurred during heavy cycling exercise, then it is necessary for the biochemical factors that determine flux rate for ATP formation to adapt by increasing the phosphorylation potential (1, 46). The recent observations of Rossiter et al. (41) of less reduction of muscle phosphocreatine concentration in the second bout of heavy exercise coincided with faster Vo<sub>2</sub> kinetics and were consistent with less disturbance of the phosphorylation potential as a consequence of prior heavy exercise. Recent animal experiments have also explored models of two bouts of electrical stimulation. In the isolated *Xenopus* muscle fiber there was a faster decline in intracellular Po2 in the second compared with the first bout of stimulation, but this was due to a shorter delay to the onset of depletion of O<sub>2</sub> rather than faster time constant (22). These results suggested that a metabolic factor was modified by the previous stimulation. Observations of microvascular Po<sub>2</sub> in rat spinotrapezius muscle also found shorter time to onset of depletion of  $O_2$  in a second exercise bout of electrical stimulation (7). Unfortunately, in this latter study, muscle blood flow could not be measured in the transition from rest to stimulation so no comments could be made about  $O_2$  delivery except in the steady state except that the ratio of  $O_2$  delivery to  $O_2$  utilization did not change. Also this rat muscle model differed in that there was no preexisting metabolic acidemia or elevated blood flow (7). These animal experiments differ from observations in human muscle where the onset of oxidative metabolism at the onset of heavy or severe exercise was demonstrated to be very rapid (3). The more rapid adaptation of blood flow and O<sub>2</sub> transport in the second heavy exercise bout has parallels in other exercise models. When blood flow was higher in the early phase of forearm exercise by placing the arm below rather than above the heart (24) or by elevating mean arterial pressure before exercise (35) muscle Vo<sub>2</sub> adapted more rapidly. An additional biochemical adaptation that might have contributed to the increased V<sub>02</sub> in the second bout of exercise was the higher level of activity of the pyruvate dehydrogenase complex due to residual effects of prior exercise (19). Whether this would have allowed faster  $\dot{V}_{O_2}$  kinetics without a coincident increase in  $O_2$  delivery is not known. Recent experiments achieving greater activation of pyruvate dehydrogenase complex by dichloroacetate before the start of heavy exercise in dogs (20) and humans (2) found no acceleration of muscle Vo<sub>2</sub>.

Direct measurements of muscle  $\dot{V}o_2$  across working forearm (30) or knee-extensor muscles (28) have confirmed that the elevated  $\dot{V}o_2$  in a second bout of heavy to intense exercise occurred at the working muscle. In both of these experiments, muscle blood flow was higher and  $O_2$  extraction was greater early in the second bout of exercise. These findings confirmed the original hypothesis of Gerbino et al. (18). Interestingly the maximum  $O_2$  extraction in the two experiments was relatively low, reaching peak values of <155 ml/l (28, 30). Krustrup et al. (28) observed that once this peak value of extraction in these experiments was reached, any further increase in muscle  $\dot{V}o_2$  followed an increase in muscle blood flow.

Heavy prior exercise has a major impact on metabolism in the subsequent exercise bout (18, 28). The greater oxidative metabolism early in the second bout allowed markedly reduced anaerobic energy production (28). The consequence of this, perhaps in conjunction with a greater percentage of fat metabolism and altered metabolic respiratory quotient (21), was that Vco<sub>2</sub> was markedly lower throughout the entire second exercise bout. Changes in  $CO_2$  storage between the first and second bouts of exercise also contributed to the different time courses for adaptation of  $Vco_2$  in heavy exercise. CO<sub>2</sub> stores would have decreased with the metabolic acidosis of the first exercise bout (23). CO<sub>2</sub> stores would have been further decreased after the repeated 30-s all-out sprint cycling also as a function of the increased VE that contributed to the respiratory compensation for the metabolic acidosis. The mean value for  $V_{CO_2}$  in the second heavy exercise bout barely reached the lower 95% confidence interval of the first exercise bout. This observation might indicate that the elevated lactate from the repeated sprint cycling acted as a substrate for oxidative metabolism in the second bout, reducing the metabolic acidosis. This speculation remains to be confirmed under these experimental conditions.

In summary, the data from the present experiments demonstrated that for heavy cycling exercise,  $\tau_2$  was significantly faster when the exercise was preceded by three bouts of all-out sprint cycling. This prior intense exercise would have established a marked metabolic acidosis within the exercising muscles that remained through to the start of the subsequent heavy exercise bout (44). The acidosis probably contributed to enhanced vasodilatation early in exercise so that increased muscle blood flow and O<sub>2</sub> delivery were supported by the higher HR and cardiac output in the first minutes of the heavy exercise. The prior exercise and intracellular acidosis probably also affected metabolic pathways and substrates for oxidative phosphorylation so that these too contributed to the more rapid adaptation of Vo<sub>2</sub>. Thus consistent with the initial hypothesis of Gerbino et al. (18) and contrary to the conclusions of some recent investigations (13, 27), the more rapid adaptation of oxidative metabolism in heavy exercise that followed prior heavy or intense exercise was a function of improved  $O_2$  delivery. The functional significance of our results is that we have confirmed the nonlinear characteristics of  $Vo_2$  during the phase II response in the heavy exercise domain (18). From a physiological perspective our observations of whole body  $\dot{V}o_2$  suggest that adaptation of oxidative phosphorylation at the onset of heavy exercise is dependent on at least two factors that we have proposed previously (45) to be the interaction between  $O_2$  supply and  $O_2$  utilization (i.e., biochemical) mechanisms (1, 45). Surface recording of EMG from four different leg muscles was unable to detect significant differences in fiber recruitment patterns in these highly trained cyclists that might have coincided with the onset of the  $\dot{V}o_2$ slow component.

We thank David Northey for excellent technical assistance. This research was supported by the Natural Sciences and Engineering Research Council of Canada.

### REFERENCES

- 1. Arthur PG, Hogan MC, Bebout DE, Wagner PD, and Hochachka PW. Modeling the effects of hypoxia on ATP turnover in exercising muscle. *J Appl Physiol* 73: 737–742, 1992.
- Bangsbo J, Gibala MJ, Krustrup P, González-Alonso J, and Saltin B. Enhanced pyruvate dehydrogenase activity does not affect muscle O<sub>2</sub> uptake at onset of intense exercise in humans. Am J Physiol Regul Integr Comp Physiol 282: R273– R280, 2002.
- Bangsbo J, Krustrup P, González-Alonso J, Boushel R, and Saltin B. Muscle oxygen kinetics at onset of intense dynamic exercise in humans. Am J Physiol Regul Integr Comp Physiol 279: R899–R906, 2000.
- Barstow TJ, Buchthal S, Zanconato S, and Cooper DM. Muscle energetics and pulmonary oxygen uptake kinetics during moderate exercise. J Appl Physiol 77: 1742–1749, 1994.
- Barstow TJ, Jones AM, Nguyen PH, and Casaburi R. Influence of muscle fiber type and pedal frequency on oxygen uptake kinetics of heavy exercise. *J Appl Physiol* 81: 1642–1650, 1996.
- Barstow TJ and Mole PA. Linear and nonlinear characteristics of oxygen uptake kinetics during heavy exercise. J Appl Physiol 71: 2099–2106, 1991.
- Behnke BJ, Kindig CA, Musch TI, Sexton WL, and Poole DC. Effects of prior contractions on muscle microvascular oxygen pressure at onset of subsequent contractions. *J Physiol* 539: 927–934, 2002.
- 8. Bohnert B, Ward SA, and Whipp BJ. Effects of prior arm exercise on pulmonary gas exchange kinetics during high-intensity leg exercise in humans. *Exp Physiol* 83: 557–570, 1998.
- Borrani F, Candau R, Millet GY, Perrey S, Fuchslocher J, and Rouillon JD. Is the Vo<sub>2</sub> slow component dependent on progressive recruitment of fast-twitch fibers in trained runners? J Appl Physiol 90: 2212–2220, 2001.
- Burnley M, Doust JH, Ball D, and Jones AM. Effects of prior heavy exercise on Vo<sub>2</sub> kinetics during heavy exercise are related to changes in muscle activity. J Appl Physiol 93: 167–174, 2002.
- 11. Burnley M, Doust JH, Carter H, and Jones AM. Effects of prior exercise and recovery duration on oxygen uptake kinetics during heavy exercise in humans. *Exp Physiol* 86: 417–425, 2001.
- Burnley M, Doust JH, and Jones AM. Effects of prior heavy exercise, prior sprint exercise and passive warming on oxygen uptake kinetics during heavy exercise in humans. *Eur J Appl Physiol* 87: 424–432, 2002.
- Burnley M, Jones AM, Carter H, and Doust JH. Effects of prior heavy exercise on phase II pulmonary oxygen uptake kinetics during heavy exercise. J Appl Physiol 89: 1387–1396, 2000.
- 14. Carter H, Jones AM, Barstow TJ, Burnley M, Williams CA, and Doust JH. Oxygen uptake kinetics in treadmill running and cycle ergometry: a comparison. *J Appl Physiol* 89: 899–907, 2000.

- 15. Charloux A, Lonsdorfer-Wolf E, Richard R, Lampert E, Oswald M, Mettauer B, Geny B, and Lonsdorfer J. A new impedance cardiograph device for the non-invasive evaluation of cardiac output at rest and during exercise: comparison with the "direct" Fick method. *Eur J Appl Physiol* 82: 313–320, 2000.
- 16. Dotan R and Bar-Or O. Load optimization for the Wingate Anaerobic Test. *Eur J Appl Physiol* 51: 409–417, 1983.
- DuQuesnay MC, Stoute GJ, and Hughson RL. Cardiac output in exercise by impedance cardiography during breathholding and normal breathing. J Appl Physiol 62: 101–107, 1987.
- Gerbino A, Ward SA, and Whipp BJ. Effects of prior exercise on pulmonary gas-exchange kinetics during high-intensity exercise in humans. J Appl Physiol 80: 99–107, 1996.
- Gibala MJ, MacLean DA, Graham TE, and Saltin B. Tricarboxylic acid cycle intermediate pool size and estimated cycle flux in human muscle during exercise. Am J Physiol Endocrinol Metab 275: E235-E242, 1998.
- Grassi B, Hogan MC, Greenhaff PL, Hamann JJ, Kelley KM, Aschenbach WG, Constantin-Teodosiu D, and Gladden LB. Oxygen uptake on-kinetics in dog gastrocnemius in situ following activation of pyruvate dehydrogenase by dichloroacetate. J Physiol 538: 195–207, 2002.
- Green H, Houston M, Thomson J, and Reid P. Alterations in ventilation and gas exchange during exercise-induced carbohydrate depletion. *Can J Physiol Pharmacol* 57: 615-618, 1979.
- Hogan MC. Fall in intracellular Po<sub>2</sub> at the onset of contractions in *Xenopus* single skeletal muscle fibers. J Appl Physiol 90: 1871-1876, 2001.
- Hughson RL and Inman MD. Gas exchange analysis of immediate CO<sub>2</sub> storage at onset of exercise. *Respir Physiol* 59: 265– 278, 1985.
- Hughson RL, Shoemaker JK, Tschakovsky M, and Kowalchuk JM. Dependence of muscle Vo<sub>2</sub> on blood flow dynamics at the onset of forearm exercise. J Appl Physiol 81: 1619–1626, 1996.
- 25. Hughson RL, Tschakovsky ME, and Houston ME. Regulation of oxygen consumption at the onset of exercise. *Exerc Sport Sci Rev* 29: 129–133, 2001.
- Koppo K and Bouckaert J. In humans the oxygen uptake slow component is reduced by prior exercise of high as well as low intensity. *Eur J Appl Physiol* 83: 559–565, 2000.
- Koppo K and Bouckaert J. The effect of prior high-intensity cycling exercise on the VO<sub>2</sub> kinetics during high-intensity cycling exercise is situated at the additional slow component. Int J Sports Med 22: 21-26, 2001.
- Krustrup P, González-Alonso J, Quistorff B, and Bangsbo J. Muscle heat production and anaerobic energy turnover during repeated intense dynamic exercise in humans. J Physiol 536: 947–956, 2001.
- Lucia A, Hoyos J, and Chicharro JL. The slow component of Vo<sub>2</sub> in professional cyclists. Br J Sports Med 34: 367–372, 2000.
- 30. MacDonald MJ, Naylor HL, Tschakovsky ME, and Hughson RL. Evidence that peripheral circulatory factors limit the rate of increase in muscle O<sub>2</sub> uptake at the onset of heavy exercise. J Appl Physiol 90: 83–89, 2001.
- MacDonald MJ, Pedersen PK, and Hughson RL. Acceleration of Vo<sub>2</sub> kinetics in heavy submaximal exercise by hyperoxia and prior high-intensity exercise. J Appl Physiol 83: 1318–1325, 1997.
- McCartney N, Spriet LL, Heigenhauser GJ, Kowalchuk JM, Sutton JR, and Jones NL. Muscle power and metabolism in maximal intermittent exercise. J Appl Physiol 60: 1164–1169, 1986.
- Paterson DH and Whipp BJ. Asymmetries of oxygen uptake transients at the on- and offset of heavy exercise in humans. *J Physiol* 443: 575-586, 1991.
- Patla AE. Measurement of the electromyogram. J Mot Behav 17: 443-461, 1985.
- Perrey S, Tschakovsky ME, and Hughson RL. Muscle chemoreflex elevates muscle blood flow and O<sub>2</sub> uptake at exercise onset in nonischemic human forearm. J Appl Physiol 91: 2010–2016, 2001.
- Poole DC. Role of exercising muscle in slow component of Vo<sub>2</sub>. Med Sci Sports Exerc 26: 1335–1340, 1994.

- Poole DC, Schaffartzik W, Knight DR, Derion T, Kennedy B, Guy HJ, Prediletto R, and Wagner PD. Contribution of exercising legs to the slow component of oxygen uptake kinetics in humans. J Appl Physiol 71: 1245–1253, 1991.
- Richard R, Lonsdorfer-Wolf E, Charloux A, Doutreleau S, Buchheit M, Oswald M, Lampert E, Mettauer B, and Geny B. Non-invasive cardiac output evaluation during a maximal progressive exercise test, using a new impedance cardiograph device. Eur J Appl Physiol 85: 202–207, 2001.
- Richardson RS, Noyszewski EA, Kendrick KF, Leigh JS, and Wagner PD. Myoglobin O<sub>2</sub> desaturation during exercise evidence of limited O<sub>2</sub> transport. J Clin Invest 96: 1916–1926, 1995.
- 40. Rossiter HB, Ward SA, Doyle VL, Howe FA, Griffiths JR, and Whipp BJ. Inferences from pulmonary O<sub>2</sub> uptake with respect to intramuscular [phosphocreatine] kinetics during moderate exercise in humans. J Physiol 518: 921–932, 1999.
- 41. Rossiter HB, Ward SA, Kowalchuk JM, Howe FA, Griffiths JR, and Whipp BJ. Effects of prior exercise on oxygen uptake

and phosphocreatine kinetics during high-intensity knee-extension exercise in humans. J Physiol 537: 291–303, 2001.

- 42. Scheuermann BW, Hoetling BD, Noble ML, and Barstow TJ. The slow component of O<sub>2</sub> uptake is not accompanied by changes in muscle EMG during repeated bouts of heavy exercise in humans. *J Physiol* 531: 245–256, 2001.
- Shinohara M and Moritani T. Increase in neuromuscular activity and oxygen uptake during heavy exercise. Ann Physiol Anthropol 11: 257-262, 1992.
- 44. Spriet LL, Lindinger MI, McKelvie RS, Heigenhauser GJF, and Jones NL. Muscle glycogenolysis and H<sup>+</sup> concentration during maximal intemittent cycling. J Appl Physiol 66: 8-13, 1989.
- 45. Tschakovsky ME and Hughson RL. Interaction of factors determining oxygen uptake at the onset of exercise. J Appl Physiol 86: 1101–1113, 1999.
- Wilson DF and Rumsey WL. Factors modulating the oxygen dependence of mitochondrial oxidative phosphorylation. Adv Exp Med Biol 222: 121-131, 1988.



541