Paper:

Blood Flow and Metabolic Control at the Onset of Heavy Exercise

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The rate of increase in oxygen uptake (VO_2) at the onset of a bout of heavy exercise is faster if it is preceded by a similar bout of heavy exercise. We tested the hypothesis during heavy leg exercise that leg blood flow (LBF) and VO_2 would both be elevated during the adaptive phase. On three separate days, six healthy young men completed two bouts of 6-minutes of knee extension / flexion exercise at about 85% $\text{VO}_{2\text{peak}}$ separated by 5-minutes 0-watt exercise on an electrically braked ergometer. LBF was determined by Doppler ultrasound. In the second exercise bout, LBF and VO_2 were significantly elevated in the baseline before exercise and throughout the exercise. Both the mean response time (time to 63% of difference between baseline and calculated end value) and the difference in VO_2 between minutes 3 and 6 of exercise indicated significantly faster attainment of the end exercise value in the second heavy exercise bout. These data showing the elevated LBF in the second bout of heavy exercise support the link between O_2 delivery and the adaptation of oxidative metabolism at the onset of heavy exercise.

Keywords: oxygen uptake, exercise, blood flow, muscle metabolism

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1. Introduction

It is well established that during constant load exercise, the delivery of oxygen is matched to the metabolic demand by an appropriate increase in muscle blood flow [Laughlin MH, Korthuis RJ, Duncker DJ and Bache RJ.(1996); Laughlin MH, Korthuis RJ, Duncker DJ and Bache RJ.(1996)]. Over a wide range of metabolic rates, it has been proposed that "oxygen delivery to the tissues, not blood flow per se, is the controlled variable producing exercise hyperemia" [Laughlin MH, Korthuis RJ, Duncker DJ and Bache RJ.(1996). pp. 709-710] although myogenic, endothelial and baroreflex factors can also influence blood flow. Indeed, in heavy whole body exercise, it might be this latter factor that actually restricts blood flow to the muscle. While these principles appear to be well accepted during the "relative steady state" of constant load exercise there are suggestions that delivery of blood flow at the onset of heavy to very heavy exercise is in excess of the metabolic demand for O_2 . Bangsbo et al. studied muscle blood flow and leg VO_2 in humans during very heavy single leg exercise that would have caused fatigue within four minutes. They concluded

that their "studies clearly point in the direction of a local limitation of muscle oxygen utilization and that oxygen availability to contracting muscles does not influence VO_2 kinetics at the onset of dynamic exercise". Although they did not measure muscle blood flow, Burnley et al. concluded "that the primary VO_2 kinetics are not limited by O_2 availability during heavy exercise".

Many studies have recently been conducted to investigate VO_2 kinetics with repeated bouts of heavy exercise [Tordi N, Perrey S, Harvey A and Hughson RL.(2003)]. In this model, exercise (at a work rate between the ventilatory threshold (T_{vent}) and peak VO_2) is conducted in two bouts separated by an interval that does not allow complete recovery before the second bout. The first report by Gerbino and colleagues that VO_2 increased more rapidly in the second of two bouts of heavy exercise was confirmed by MacDonald et al. who also demonstrated that VO_2 increased even more rapidly when the subjects breathed a hyperoxic gas mixture. These two investigations both suggested that blood flow and O_2 delivery were limiting in the first bout of exercise. Gerbino et al. proposed

that blood flow and O_2 delivery increased more rapidly in the second bout of exercise due to the residual acidosis that permitted more rapid vasodilation and a greater O_2 off-loading from the red blood cells due to the Bohr effect. MacDonald et al. tested this hypothesis during repeated bouts of forearm exercise and confirmed that blood flow and O_2 extraction were greater in the early phase of the second bout of exercise [MacDonald MJ, Naylor HL, Tschakovsky ME and Hughson RL.(2001)].

In the current study, we challenged our subjects with two bouts of heavy dynamic two-leg exercise involving both knee flexion and extension. This model has a sufficient metabolic demand that the central circulatory system is challenged to a greater extent than in the forearm [MacDonald MJ, Naylor HL, Tschakovsky ME and Hughson RL.(2001)] or the single-leg knee extension models [Bangsbo J, Krstrup P, González-Alonso J, Boushel R and Saltin B.(2000); Krstrup P, González-Alonso J, Quistorff B and Bangsbo J.(2001)]. We hypothesized that both leg blood flow and whole body VO_2 would be elevated across the transition phase in the second bout of heavy exercise.

2. Methods

2.1. Subjects

Six healthy men (age 23 ± 2 yrs, height 181 ± 3 cm, weight 79 ± 3 kg, mean $\pm$ SE) volunteered to participate in this study. The study was approved by the Office of Research Ethics at the University of Waterloo. The subjects gave written informed consent after receiving complete written and verbal details of the protocol. On each test day the subjects reported to the laboratory at least 2 hours after their last meal. They refrained from alcohol, caffeine and strenuous exercise for 24 hours before each test.

2.2. Protocol

The subjects practiced the knee extension/flexion exercise on an electrically braked ergometer on three separate occasions to become familiar with the activity prior to any data collection. The subjects were seated with the hips extended 120° to allow for stable access to the femoral artery for blood flow measurements [MacDonald MJ, Tarnopolsky MA and Hughson RL.(2000)]. Exercise was performed at approximately 50 knee extension/flexion cycles per minute.

Each subject performed an incremental test to exhaustion or functional limit as defined by the upper work rate that could be attained without excessive body motion. The test protocol consisted of 4- minutes

at 15W, followed by a ramp increase in work rate of 15W every minute. Breath-by-breath gas exchange data were collected [MacDonald M, Pedersen PK and Hughson RL.(1997)] for determination of VO_{2peak} and T_{VENT} . T_{VENT} was selected as the point at which the ventilatory equivalent for $VO_2(V_E/VO_2)$ increased without a change in the ventilatory equivalent for CO_2 output. The work rate for the constant load tests was established halfway between T_{VENT} and VO_{2peak} . The constant load heavy exercise tests were conducted on three separate days with at least two-days between the tests. Throughout these tests, breath-by-breath gas exchange and leg blood flow data were collected.

Leg blood flow (LBF) was determined as the product of femoral artery cross-sectional area measured by echo Doppler ultrasound (model SSH-140A, Toshiba Inc., Tochigi-Ken, Japan) times the mean blood velocity (MBV) measured by pulsed Doppler ultrasound (model 500V, Multigon Industries, Mt. Vernon, N.Y.) [MacDonald MJ, Tarnopolsky MA and Hughson RL.(2000)]. A continuous image of the common femoral artery was recorded from one leg to video tape using a 7.5-MHz linear array probe held approximately 2-3 cm distal to the inguinal ligament. An average of three diameter measurements was obtained three times during loadless baseline exercise, every 10 seconds during the first minute of high intensity exercise, and then at 1 minute intervals. A nonlinear regression was applied to the individual diameter estimates to obtain a continuous estimate of femoral artery diameter and to minimize impact of random variation in measurements. Continuous MBV was recorded from the common femoral artery of the other leg using a 4-MHz probe. MBV data were averaged over a 5s-time period and across the three trials for each subject.

Mean arterial pressure (MAP) was measured at the level of the femoral artery with a pneumatic finger cuff (Ohmeda 2300, Finapres, Englewood, CO). Heart rate (HR) was continuously determined from a three-lead electrocardiograph (ECG) (7803 A, Hewlett-Packard, USA).

Surface electromyography (EMG) was obtained from the vastus lateralis (VL), rectus femoris (RF), vastus medialis (VM), and right external oblique (EO) using bipolar surface electrodes (Ag-AgCl electrodes, 200 Medi-Trace, surface cup diameter 10mm) with an inter-electrode distance of 35mm. All signals were collected on the subject's right leg and the reference electrode was placed on the right anterior superior iliac spine. Visual inspection and palpation for the most prominent part of the muscle belly determined electrode sites. Prior to electrode placement, the skin was shaved and cleaned with alcohol. The electrodes and cables were then taped firmly in place to reduce artifact

Table 1. Parameters from fitting three-component exponential model to VO₂ responses during first and second heavy exercise bouts.

Bout	A ₀ ml/min	A ₁ ml/min	TD ₁ s	τ ₁ s	A ₂ ml/min	TD ₂ s	τ ₂ s	A ₃ ml/min	TD ₃ s	τ ₃ s	MRT s	A _{TOT} ml/min
1	845 ± 18	273 ± 41	1.7 ± 0.3	2.9 ± 0.3	662 ± 80.9	16.0 ± 1.5	32.8 ± 4.0	367 ± 31	125.9 ± 9.3	206.6 ± 25.8	124.0 ± 14.3	1303 ± 105
2	923 ± 29*	256 ± 67	2.1 ± 0.6	3.3 ± 0.6	715 ± 64	14.5 ± 1.4	27.4 ± 2.2	308 ± 18	100.1 ± 4.3	158.9 ± 11.9	88.3 ± 5.5*	1279 ± 98

Amplitude terms, time constants (τ) and time delays as described in Methods. A_{TOT} = A₁ + A₂ + A₃. * P<0.05 between exercise bouts. Values are mean ± SE, n = 6.

and maintain position throughout the experiment. The myoelectrical signal was band-pass filtered (20-500Hz), differentially amplified (gain 2000 times, input impedance 2M ohms, common mode rejection rate >90 dB) and sampled at 1024 Hz during the last 15 seconds of each minute of exercise. The raw EMG signals were then converted from analog to digital using a 12-bit A/D conversion board (MetraByte DA-16). The digitized signal was displayed on a computer using collection software and saved for later analysis. Following the removal of any bias, off line processing of EMG was performed using software developed in our laboratory. The raw EMG signals were full wave rectified and digitally low-pass filtered with a second order Butterworth filter (cut-off frequency = 4 Hz) to produce a linear envelope. Five sequential bursts were then selected for each signal to obtain integrated (iEMG). Mean power frequency (MPF) was determined using a fast Fourier transform algorithm over the 15-second window (cut-off frequency 512 Hz) and the resulting frequency spectrum. The iEMG values were expressed relative to the first minute of the first exercise bout for each subject. iEMG and MPF signals were analyzed as mean of the leg muscles (VL, VM and RF) and separately for each muscle.

Venous blood samples were obtained from the antecubital vein (cubital fossa) twice during the first four minutes of loadless exercise, at 1- and 5- minute of both high intensity exercise bouts and at the 4-minute point between the two bouts. The blood was collected in a 1 ml heparinized syringe. The samples were immediately analyzed for blood lactate content (NovaStat Profile Plus 9, Waltham, MA).

2.3. Kinetic Analysis

Breath-by-breath VO₂ data were linearly interpolated between points to give values at 1-s intervals then averaged across the three repetitions. A three-component exponential model was used to fit to the data using

a least squares procedure. The model had a baseline component (A₀), three amplitude terms (A₁, A₂, A₃), three time constants (τ₁, τ₂, τ₃), and three time delays (TD₁, TD₂, TD₃).

$$\begin{aligned} \dot{V}O_2(t) &= G_0 + G_1[1 - e^{-(t-TD_1)/\tau_1}] \cdot u_1 \\ &\quad + G_2[1 - e^{-(t-TD_2)/\tau_2}] \cdot u_2 + G_3[1 - e^{-(t-TD_3)/\tau_3}] \cdot u_3 \end{aligned}$$

Where

$$\begin{aligned} u_1 &= 0 \text{ for } t < TD_1 \text{ and } u_1 = 1 \text{ for } t \geq TD_1, \\ u_2 &= 0 \text{ for } t < TD_2 \text{ and } u_2 = 1 \text{ for } t \geq TD_2, \\ u_3 &= 0 \text{ for } t < TD_3 \text{ and } u_3 = 1 \text{ for } t \geq TD_3. \end{aligned}$$

The overall time course of the response was determined from the mean response time (MRT) which is the time required achieving approximately 63% of the total amplitude of the response from the baseline to the final plateau. It was calculated as a weighted sum of the time delay and time constant for each component.

$$\begin{aligned} MRT &= (G_1/(G_1+G_2+G_3)) \times (TD_1 + \tau_1) \\ &\quad + (G_2/(G_1+G_2+G_3)) \times (TD_2 + \tau_2) \\ &\quad + (G_3/(G_1+G_2+G_3)) \times (TD_3 + \tau_3). \end{aligned}$$

2.4. Statistical Analysis

The effects of prior exercise on VO₂ and LBF were analyzed using a one-way repeated measures analysis of variance. iEMG and MPF measures were analyzed for prior exercise and time effect using a two-way repeated measures analysis of variance. Significant main effect (P<0.05) were followed by a Student Newman-Keuls post hoc test. All data are presented as mean ± standard error (SE).

Table 2. VO_2 at minute 3 and 6 during first and second heavy exercise bouts with indicator of VO_2 slow component.

	1 st bout	2 nd bout
VO_2 at 3 min (ml/min)	1860 ± 102	$2014 \pm 113^*$
VO_2 at 6 min (ml/min)	2026 ± 110	$2141 \pm 117^*$
$\Delta\text{VO}_{2, 6-3}$ (ml/min)	166 ± 19	$127 \pm 6^*$

$\Delta\text{VO}_{2, 6-3}$ is difference between VO_2 at minutes 6 and 3. * $P < 0.05$ between exercise bouts. Values are mean $\pm$ SE, $n=6$.

Table 3. Heart rate and arterial blood pressure at the femoral artery during first and second heavy exercise bouts.

	Baseline		6 min	
	Bout 1	Bout 2	Bout 1	Bout 2
HR (bpm)	82 ± 4	$88 \pm 3^*$	118 ± 3	$127 \pm 5^*$
MAP (mmHg)	122 ± 1.6	122 ± 3.7	$150 \pm 3.8^\dagger$	$152 \pm 2.0^\dagger$

* $P < 0.05$ between exercise bouts. $^\dagger P < 0.05$ from baseline to heavy exercise. Values are mean $\pm$ SE, $n=6$.

Fig. 1. Continuous record of VO_2 (dotted line) and leg blood flow (solid line) response of a single subject during baseline exercise at 0 W and during the two bouts of heavy exercise. **Fig. 2.** Group mean responses for VO_2 showing the first bout (solid line) and second (dotted line) bouts of heavy exercise ($n=6$). Exercise began at $t=0$.

3. Results

3.1. Incremental Test

The $\text{VO}_{2\text{peak}}$ during the incremental tests (2460 ± 222 ml/min) was associated with a peak work rate of 173 ± 9 W, and a peak heart rate of 155 ± 7 bpm. The T_{VENT} occurred at 1763 ± 160 ml/min. A work rate of 114 ± 7 W was chosen for the high intensity exercise and resulted in $84 \pm 3\%$ and $88 \pm 3\%$ of the peak oxygen consumption in the first and second bout respectively (Table 1).

3.2. VO_2 Kinetics

VO_2 was elevated at the baseline and throughout the second exercise bout (Figs.1,2, Tables 1,2). With the exception of a faster response for MRT in the second exercise bout, there were no differences in the kinetic parameters (Table 1). The VO_2 slow component indicated by the difference in VO_2 between minutes 6 and 3 of each exercise bout was significantly smaller for the second exercise bout (Table 2).

3.3. Leg Blood Flow

LBF increased by $\sim 50\%$ in the first 0.5-min of heavy exercise and had almost doubled by 1-min of exercise

Fig. 3. Group mean responses for leg blood flow in the first (solid line and symbols) and second (dotted line and open symbols) bouts of heavy exercise. Values are mean $\pm$ SE, $n=6$, * $P<0.05$ between exercise bouts.

Fig. 4. Group mean responses for heart rate in the first (solid line and symbols) and second (dotted line and open symbols) bouts of heavy exercise. Values are mean, $n=6$, $P<0.05$ between exercise bouts as indicated in Table 3.

(Fig.3). LBF continued to increase to 3-min and showed a small further increase to the end of the exercise bout. LBF was elevated in the baseline and throughout the entire second heavy exercise bout.

3.4. Heart Rate and Mean Arterial Pressure

The heart rate was significantly elevated in the baseline and at 6-min of exercise (Table 3). At the start of exercise there was a very rapid increase of about 20 beats/min followed by a slower increase through the remainder of the exercise (Fig. 4). Mean arterial pressure, measured at the level of the femoral artery, was not different between exercise bouts but it did increase significantly during the heavy exercise compared to the baseline (Table 3).

Fig. 5. Blood lactate concentration presented as a continuous plot across the complete exercise protocol with exercise at 0 W and two bouts of heavy exercise. Values are mean $\pm$ SE, $n = 6$. * $P<0.05$ for comparison between the second exercise bout (solid symbols) and the similar time point in first exercise bout (open symbols).

Fig. 6. Integrated EMG for the first (solid line and symbols) and second (dotted line, open symbols) of heavy exercise. Values were normalized to the first minute of the first bout of exercise. Values are mean $\pm$ SE, $n = 6$. * $P<0.05$ for comparison to the first minute of each exercise bout.

3.5. Blood Lactate

Blood lactate measured from the forearm vein increased by the end of the first bout of heavy exercise and remained elevated during the loadless exercise between the heavy exercise bouts (Fig.5). There was a small drop at the beginning of the second heavy exercise bout then a further small increase. Each sample point in the second exercise was significantly higher than the corresponding time point in the first exercise bout (Fig.5).

3.6. Electromyographic Response

The iEMG taken as the average for the three leg muscles increased progressively in both the first and

second heavy exercise bout relative to the value at 1-min (**Fig.6**). Analysis of the individual muscles revealed that the vastus medialis and the vastus lateralis both increased significantly while the rectus femoris did not. The external oblique muscle did not show a significant change in iEMG across time or between trials. There was a very small but significant ($P < 0.05$) decrease in MPF for the leg muscles from the first (67 ± 0.4 Hz) to the second (65 ± 0.1 Hz) heavy exercise bouts with no significant change within a bout.

4. Discussion

The results of this study of repeated bouts of heavy exercise at about 84-88% $\text{VO}_{2\text{peak}}$ supported the hypothesis that LBF and VO_2 would be higher throughout the transition phase of the second bout of exercise. In this case, the LBF and VO_2 in the second bout of exercise remained significantly above values in the first bout of exercise, a finding consistent with observations [Krustrup P, González-Alonso J, Quistorff B and Bangsbo J.(2001)] from repeated bouts of intense exercise that represented an energy equivalent of $\sim 125\%$ $\text{VO}_{2\text{peak}}$ for the knee extensor muscles [Bangsbo J, Krustrup P, González-Alonso J, Boushel R and Saltin B.(2000)]. Elevated LBF and VO_2 at least during the transition phase were anticipated based on the proposal of Gerbino et al. and the subsequent observations of blood flow and muscle VO_2 in a model of repeated bouts of forearm exercise [MacDonald MJ, Naylor HL, Tschakovsky ME and Hughson RL.(2001)]. The elevated heart rate during the second bout of heavy exercise was consistent with the observation of higher cardiac output [Tordi N, Perrey S, Harvey A and Hughson RL.(2003)] that would support the higher LBF. Whole body VO_2 was significantly elevated in the second bout of heavy exercise from the baseline period to the end of exercise and two markers of the rate of the change in VO_2 , the MRT and the $\Delta\text{VO}_{2,6,3}$ indicated faster attainment of the end exercise value. Overall, these results suggest that elevated muscle blood flow allowed VO_2 to be higher, but they do not confirm that VO_2 was higher because of the increased O_2 delivery.

During the first ~ 15 -s after the onset of constant load exercise, there is an initial rapid increase in muscle blood flow that is a function of the increased activity of the muscle pump [Sheriff DD, Rowell LB and Scher AM.(1993)] with some contribution of vasodilation [Shoemaker JK and Hughson RL.(1999); Tschakovsky ME, Shoemaker JK and Hughson RL.(1996)]. Compression of veins within the muscle drives blood toward the heart so that upon muscle re-

laxation the veins are empty and have a relatively low blood pressure. This allows for a greater pressure gradient across the muscle capillary bed from the arterial to venous circulations. In this early phase, there is little matching of blood flow directly to the site of metabolic demand. After approximately 15-s progressive vasodilation occurs to match the blood flow to the metabolic demand [Shoemaker JK and Hughson RL.(1999)] so that muscle blood flow continues to increase for as little as 30-60-s for very light exercise or for several minutes with heavier exercise [Bangsbo J, Gibala MJ, Krustrup P, González-Alonso J and Saltin B. (2002); Krustrup P, González-Alonso J, Quistorff B and Bangsbo J.(2001); Rådegran G and Saltin B.(1998); Shoemaker JK and Hughson RL.(1999); van Beekvelt MCP, Shoemaker JK, Tschakovsky ME, Hopman MTE and Hughson RL.(2001)].

The higher LBF in the second bout of exercise was consistent with the observations for heavy intensity forearm exercise [MacDonald MJ, Naylor HL, Tschakovsky ME and Hughson RL.(2001)] as well as for repeated bouts of intense knee extension exercise [Krustrup P, González-Alonso J, Quistorff B and Bangsbo J.(2001)]. It has been speculated that the residual metabolic acidosis and/or other vasodilators associated with metabolism contribute to the elevated blood flow in the second of two bouts of heavy exercise [Gerbino A, Ward SA and Whipp BJ.(1996); MacDonald MJ, Naylor HL, Tschakovsky ME and Hughson RL.(2001)]. The metabolic by-product does not appear to be elevated temperature alone as Koga et al. have shown that increasing muscle temperature does not accelerate VO_2 kinetics.

There has been considerable debate concerning whether the rapid increase in muscle blood flow at the onset of exercise precedes or follows the increase in muscle oxidative metabolism. Some investigators have suggested that even for heavy to intense exercise the increase in blood flow and the delivery of O_2 precede the availability of other substrates for oxidative metabolism [Bangsbo J, Krustrup P, González-Alonso J, Boushel R and Saltin B.(2000); Burnley M, Doust JH, Ball D and Jones AM.(2002)]. In the first ~ 15 -s of intense exercise, as with moderate exercise [Grassi B, Poole DC, Richardson RS, Knight DR, Erickson BK and Wagner PD.(1996); Hughson RL, Shoemaker JK, Tschakovsky M and Kowalchuk JM.(1996)], flow does exceed O_2 demand as shown by the reduction in O_2 extraction [Bangsbo J, Krustrup P, González-Alonso J, Boushel R and Saltin B.(2000); Krustrup P, González-Alonso J, Quistorff B and Bangsbo J.(2001); MacDonald MJ, Naylor HL, Tschakovsky ME and Hughson RL.(2001); van Beekvelt MCP, Shoemaker JK, Tschakovsky ME, Hopman MTE and Hughson

RL.(2001)]. The mechanism responsible for an early decline in O_2 extraction is probably the rapid increase in muscle blood flow due to the muscle pump action in combination with failure to match O_2 delivery to the active muscle fibers. Within the first minute of exercise O_2 extraction reaches a value that is maintained for the duration of exercise whether for moderate [Grassi B, Poole DC, Richardson RS, Knight DR, Erickson BK and Wagner PD.(1996); Hughson RL, Shoemaker JK, Tschakovsky M and Kowalchuk JM.(1996); MacDonald MJ, Naylor HL, Tschakovsky ME and Hughson RL.(2001); MacDonald MJ, Tarnopolsky MA and Hughson RL.(2000)] or heavy to intense exercise [Bangsbo J, Krstrup P, González-Alonso J, Boushel R and Saltin B.(2000); Krstrup P, González-Alonso J, Quistorff B and Bangsbo J.(2001); van Beekvelt MCP, Shoemaker JK, Tschakovsky ME, Hopman MTE and Hughson RL.(2001)]. As VO_2 is observed to increase beyond this first minute of exercise even for moderate intensities [Grassi B, Poole DC, Richardson RS, Knight DR, Erickson BK and Wagner PD.(1996); Hughson RL, Shoemaker JK, Tschakovsky M and Kowalchuk JM.(1996); MacDonald MJ, Naylor HL, Tschakovsky ME and Hughson RL.(2001); MacDonald MJ, Tarnopolsky MA and Hughson RL.(2000)], the increased utilization of O_2 corresponds with increased delivery [Bangsbo J, Krstrup P, González-Alonso J, Boushel R and Saltin B.(2000); Krstrup P, González-Alonso J, Quistorff B and Bangsbo J.(2001); van Beekvelt MCP, Shoemaker JK, Tschakovsky ME, Hopman MTE and Hughson RL.(2001)]. That is, muscle blood flow increases in proportion to the metabolic signal even during the transient phase of exercise. There is no support for the notion that VO_2 would have been able to increase more rapidly in the second bout of exercise if muscle blood flow had not also been elevated.

Substrate for oxidative metabolism increases progressively at the onset of exercise and it certainly acts as one of the factors that limit the rate of increase in VO_2 [Greenhaff PL, Campbell-O'Sullivan SP, Constantin-Teodosiu D, Poucher SM, Roberts PA and Timmons JA.(2002)]. It has been argued that if the substrate increased more rapidly then VO_2 could increase more rapidly [Timmons JA, Gustafsson T, Sundberg CJ, Jansson E and Greenhaff PL.(1998)]. Recent experiments in which the pyruvate dehydrogenase enzyme was activated by dichloroacetate found during intense exercise that VO_2 did not increase at a faster rate than in the control experiments [Bangsbo J, Gibala MJ, Krstrup P, González-Alonso J and Saltin B. (2002)]. Repeated bouts of exercise at an intensity that was expected to cause fatigue in ~4-min evoked a pattern that was similar to that observed in

the present study of heavy, but submaximal, exercise [Krstrup P, González-Alonso J, Quistorff B and Bangsbo J.(2001)]. That is, muscle VO_2 was higher in the second and third bouts of intense exercise than in the first. These results might be explained by either activation of more muscle fibers in these subsequent exercise bouts [Burnley M, Doust JH, Ball D and Jones AM.(2002)] as the iEMG data of the current experiments would suggest or by more rapid increase in the substrates necessary to drive oxidative metabolism. Rossiter et al. observed a significant acceleration of whole body VO_2 and a trend to increased rate of change in phosphocreatine breakdown with less total breakdown in the second of two bouts of heavy exercise. These results were consistent with accelerated rate of oxidative phosphorylation.

In the current experiments, we found an elevated VO_2 at any time in the second exercise bout, but the rate of increase as described by the time constant (τ_2) even though 16.5% faster on average (**Table 1**) was not significantly different between bouts. There was a significant shortening of MRT in the second bout of exercise as well as a smaller $\Delta VO_{2, 6-3}$. These findings are consistent with other observations of the second of two bouts of heavy exercise that have been separated by 5-6 min [Gerbino A, Ward SA and Whipp BJ.(1996); Kearney MT, Cowley AJ, Stubbs TA and MacDonald IA.(1996)]. Recent experiments [Tordi et al.(2003)] in which two bouts of heavy exercise were separated by three bouts of high intensity all-out sprint cycling did have a significant acceleration of the τ_2 as was found in the study of Rossiter et al. There are several major issues here that can determine whether a significant acceleration of τ_2 is observed. The modeling process assumes that the system under investigation is linear. That is, when the second phase response is analyzed by an exponential component it is assumed that a single time constant can describe the full range between TD_2 to TD_3 (i.e. from 16.0 to 125.9 s in the first bout, see **Table 1**) as if it were described by a single metabolic control factor. The recent conclusion from the study by Rossiter et al. that examined metabolic controllers by magnetic resonance spectroscopy and VO_2 kinetics was that the system is nonlinear [Rossiter HB, Ward SA, Kowalchuk JM, Howe FA, Griffiths JR and Whipp BJ.(2001)] and therefore a single exponential term should not describe the process. Hughson et al. described a computer simulation of the VO_2 response to a step change in work rate in which a nonlinearity in the time constant was introduced but the exponential curve fitting procedure was unable to detect it.

The question about what establishes the rate of in-

crease in muscle metabolism at the onset of exercise does not have a simple answer. There must be an appropriate increase in metabolic substrates at the onset of exercise and in the case of a repeated bout of exercise it is possible that the metabolic demand occurs in a greater number of muscle fibers as the first bout of exercise might have fatigued some fibers. An important consideration is that there are almost certainly interactions between metabolic substrates and the substrate O_2 [Tschakovsky ME and Hughson RL.(1999)] because mitochondrial PO_2 observed during exercise [Richardson.(1995)] is below the critical PO_2 at which compensations in the cellular redox and phosphorylation potentials must be made to sustain the required rate of oxidative metabolism [Arthur PG, Hogan MC, Bebout DE, Wagner PD and Hochachka PW.(1992); Wilson DF, Erecinska M, Drown C and Silver IA.(1979)]. During recovery from moderate intensity exercise, the rate of restoration of phosphocreatine in humans is slowed by breathing a hypoxic gas and accelerated by breathing a hyperoxic gas [Haseler LJ, Hogan MC and Richardson RS.(1999)]. O_2 demand and O_2 supply interact throughout the adaptive phase at the onset of exercise but it appears that in the absence of an increase in O_2 supply that muscle VO_2 could not increase at a more rapid rate [Hughson RL, Tschakovsky ME and Houston ME.(2001); Tschakovsky ME and Hughson RL.(1999)]. Support for this hypothesis has been obtained in heavy leg cycling exercise when O_2 supply was increased by hyperoxia and by the combination of hyperoxia and heavy prior exercise [MacDonald Pederson Hughson.(1997)] as well as when blood flow to the forearm was elevated in the early phase of exercise by increasing muscle perfusion pressure [Perrey S, Tschakovsky ME and Hughson RL.(2001)]. The observation in the current study of elevated VO_2 in the second of two bouts of heavy exercise is also consistent with O_2 supply being a critical factor determining the rate of increase in oxidative metabolism.

The delay in increase in oxidative metabolism at the onset of exercise must be compensated by availability of energy from alternative supply pathways. It is well established that muscle phosphocreatine decreases in an approximately exponential manner at the onset of exercise and that this can be used as a surrogate for the rate of increase in oxidative metabolism [Rossiter HB, Ward SA, Kowalchuk JM, Howe FA, Griffiths JR and Whipp BJ.(2001)]. The significant increase in blood lactate concentration in the first bout of exercise also supports the conclusion that anaerobic glycolysis with lactate accumulation contributes to the net energy production. In the second exercise bout, whether one looks at the slightly faster τ_2 , the significantly faster

MRT or the relatively smaller increase in blood lactate from start to end of exercise than observed in the first bout, all indications are that oxidative metabolism made a greater contribution to the total energy supply.

Muscle activation during the repeated bouts of heavy exercise was monitored by the EMG. The iEMG was normalized to the value at the end of the first minute of the first bout of exercise under the assumption that any fatigue effects would be minimal at this time. By four-minutes of exercise, the iEMG of the vastus lateralis, vastus medialis and the combined leg muscles had increased significantly above the baseline value in both the first and second exercise bouts. This observation is consistent with other studies of iEMG during heavy exercise and support the notion that the increase in VO_2 measured as the so-called VO_2 slow component might be related to the increased recruitment of muscle fibers required as some of the earlier recruited fibers fatigued [Burnley M, Doust JH, Ball D and Jones AM.(2002); Scheuermann BW, Hoetling BD, Noble ML and Barstow TJ.(2001); Shinohara M and Moritani T.(1992); Tordi N, Perrey S, Harvey A and Hughson RL.(2003)]. The iEMG of the external oblique muscle was measured as an indicator of upper body accessory muscle contribution to the exercise. We found no change in the activity of this muscle within or across exercise bouts supporting the notion that the exercise was confined to the leg muscles. The MPF of the EMG signal was slightly, but significantly elevated in the second exercise bout. Altered muscle action potential conduction velocity due to factors including muscle fatigue, elevated temperature or recruitment of different muscle fiber types might contribute to this observation but the variability between studies suggests caution in interpretation of the data [Borroni F, Candau R, Millet GY, Perrey S, Fuchslocher J and Rouillon JD.(2001); Burnley M, Doust JH, Ball D and Jones AM.(2002); Scheuermann BW, Hoetling BD, Noble ML and Barstow TJ.(2001); Shinohara M and Moritani T.(1992); Tordi N, Perrey S, Harvey A and Hughson RL.(2003)].

In conclusion, the current study has shown that muscle blood flow is elevated in the second of two bouts of heavy, but submaximal exercise. The increased delivery of O_2 during the adaptive phase for oxidative metabolism appears to be critical as previous studies have shown that O_2 extraction does not increase above the value attained within the first minute even though VO_2 continues to increase [Bangsbo J, Krstrup P, González-Alonso J, Boushel R and Saltin B.(2000); Hughson RL, Shoemaker JK, Tschakovsky M and Kowalchuk JM.(1996); Krstrup P, González-Alonso J, Quistorff B and Bangsbo J.(2001); van Beekvelt MCP, Shoemaker JK, Tschakovsky ME, Hopman MTE and Hughson RL.(2001)]. Our results are consis-

tent with the original hypothesis of Gerbino et al. and the observations of MacDonald et al. that the first bout of exercise leaves a residual effect that facilitates a more rapid increase in muscle blood flow at the onset of the second exercise bout. Taken together with previous observations showing a strong link between VO_2 and O_2 delivery [Hughson RL, Shoemaker JK, Tschakovsky M and Kowalchuk JM.(1996); Perrey S, Tschakovsky ME and Hughson RL.(2001); Tschakovsky ME and Hughson RL.(1999)] and with the coupling of the rate of phosphocreatine recovery to arterial blood PO_2 , these results support the hypothesis that the rate of increase in oxidative metabolism at the onset of exercise depends on the interaction between O_2 delivery and metabolic substrate supply [Tschakovsky ME and Hughson RL.(1999)].

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