Effect of eccentric exercise-induced muscle damage on the dynamics of muscle oxygenation and pulmonary oxygen uptake

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Davies RC, Eston RG, Poole DC, Rowlands AV, DiMenna F, Wilkerson DP, Twist C, Jones AM. Effect of eccentric exercise-induced muscle damage on the dynamics of muscle oxygenation and pulmonary oxygen uptake. J Appl Physiol 105: 1413–1421, 2008. First published August 14, 2008; doi:10.1152/japplphysiol.09743.2008.—Unaccustomed eccentric exercise has a profound impact on muscle structure and function. However, it is not known whether associated microvascular dysfunction disrupts the matching of O₂ delivery (Q˙O₂) to O₂ utilization (V˙O₂). Near-infrared spectroscopy (NIRS) was used to test the hypothesis that eccentric exercise-induced muscle damage would elevate the muscle Q˙O₂:V˙O₂ ratio during severe-intensity exercise while preserving the speed of the V˙O₂ kinetics at exercise onset. Nine physically active men completed “step” tests to severe-intensity exercise from an unloaded baseline on a cycle ergometer before (Pre) and 48 h after (Post) eccentric exercise (100 squats with a load corresponding to 70% of body mass). NIRS and breath-by-breath pulmonary V˙O₂ kinetics were measured continuously during the exercise tests and subsequently modeled using standard nonlinear regression techniques. There were no changes in phase II pulmonary V˙O₂ kinetics following the onset of exercise (time constant: Pre, 25 ± 4 s; Post, 24 ± 2 s; amplitude: Pre, 2.36 ± 0.23 l/min; Post, 2.37 ± 0.23 l/min; all P > 0.05). However, the primary phase (Pre, 14 ± 3 s; Post, 19 ± 3 s) and overall (Pre, 16 ± 4 s; Post, 21 ± 4 s) mean response time of the [HHb] signal was significantly slower following eccentric exercise (P < 0.05). The slower [HHb] kinetics observed following eccentric exercise is consistent with an increased Q˙O₂:V˙O₂ ratio during transitions to severe-intensity exercise. We propose that unchanging primary phase V˙O₂ kinetics are associated with an elevated Q˙O₂:V˙O₂ ratio that preserves blood-myocyte O₂ flux.

near-infrared spectroscopy; oxygen uptake kinetics; muscle oxygen delivery; muscle oxygen utilization; delayed-onset muscle soreness

UNACCUSTOMED eccentric exercise has a profound impact on muscle structure and function. Following such exercise, myocytes demonstrate ultrastructural changes, including sarcomere disruption described as “popping” (48), “Z-band streaming” (26, 62), or “smearing” (37), and damage to t-tubules, sarcoplasmic reticulum, and sarcotubular lattice (25). This disruption leads to increased influx of extracellular Ca²⁺ into the sarcoplasm, leading to enhanced proteolytic enzyme activity (57) and an accompanying inflammatory response (24). In addition, intramyocyte contents such as creatine kinase and myoglobin are released into the bloodstream (66). These degenerative changes are associated with delayed-onset muscle soreness (DOMS) and a reduction in maximal force-generating capacity (12, 17, 18).

As myocyte degeneration is known to lead to decrements in maximal force production, any associated damage to the microcirculation could potentially have an adverse affect on submaximal locomotory activity, such as running or cycling. Activities that require repetitive low-force contractions rely on effective vascular function that ensures an adequate blood and O₂ supply to muscle. Accordingly, Kano et al. (37) have reported substantial microvascular dysfunction in rat spinotrapezius muscle following unaccustomed eccentric exercise (downhill running). Specifically, these authors reported an increase in the proportion of capillaries that did not support red blood cell (RBC) flux and an increase in mean capillary diameter in resting muscle. Furthermore, an accelerated fall in microvascular oxygen pressure was observed at the onset of electrically stimulated contractions. Microcirculatory dysfunction such as this could conceivably lead to impaired delivery and distribution of O₂ within the capillary bed. Similarly, the matching of O₂ delivery (Q˙O₂) and O₂ utilization (V˙O₂) at the onset of exercise might be disturbed, thereby compromising blood-muscle O₂ flux and, if sufficiently severe, slow the kinetic adaptation of V˙O₂ at exercise onset (37). Although the compelling weight of evidence supports the premise that V˙O₂ kinetics in healthy individuals are not limited by O₂ delivery, per se, in disease conditions such as chronic heart failure (58) and Type II diabetes (49) where vascular function and capillary hemodynamics are impaired, V˙O₂ kinetics are slowed (53). It is possible that microcirculatory dysfunction brought about by previous eccentric exercise could result in the V˙O₂ kinetics of healthy individuals becoming slower due to a muscle O₂ delivery limitation. That is, the muscle damage could cause individuals to cross the so-called “tipping point” beyond which reductions in muscle O₂ availability begin to measurably lengthen the time constant describing the phase II V˙O₂ response (50).

Near-infrared spectroscopy (NIRS) facilitates the assessment of muscle (hemoglobin + myoglobin) oxygenation and can thus be utilized to determine the dynamic balance between Q˙O₂ and V˙O₂ following the onset of exercise. In particular, the deoxyhemoglobin ([HHb]) NIRS signal can be used to noninvasively estimate O₂ extraction in the skeletal muscle microcirculation. Thus the NIRS-derived [HHb] signal would be expected to demonstrate a slower...
kinetic response at the onset of exercise if, as anticipated, eccentric exercise does compromise blood-muscle O₂ flux.

One recent investigation by Schneider et al. (60) reported no change in VO₂ kinetics at the onset of heavy-intensity exercise 48 and 72 h following bench-stepping exercise designed to incur damage. An explanation for this apparent paradox, i.e., normal VO₂ kinetics in the face of severe muscle damage and impaired microvascular hemodynamics, may be found in the work of Laaksonen et al. (43), who reported that muscle damage increased muscle blood flow during exercise. If microvascular function and therefore the ability to match Q˙O₂:VO₂ effectively is compromised following eccentrically exercised muscle, it is possible that an elevation in the Q˙O₂:V˙O₂ induced muscle damage would elevate the muscle Q˙O₂:V˙O₂ ratio (as indicated by alterations in the [HHb] kinetics) during severe intensity exercise and thus preserve the speed of the VO₂ kinetics at exercise onset.

METHODS

Participants

Nine healthy men (mean ± SD: age 22.7 ± 2.8 yr; height 1.83 ± 0.06 m; mass 76.7 ± 7.0 kg), asymptomatic of illness and preexisting injury, volunteered to participate in the study. All were physically active but were not highly trained and had not undertaken any resistance training of the lower limbs for at least 6 mo before assessment. Participants provided written informed consent to participate in the research, which was approved by the Ethics Committee of the School of Sport and Health Sciences at the University of Exeter and conformed to the Declaration of Helsinki. The participants were requested not to take any anti-inflammatory drugs for the duration of the study and to refrain from heavy exercise for 24 h before each visit.

Procedures

All testing was performed on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). Participants were instructed to report to the laboratory at the same time of day (±1 h) on five separate occasions within a period of 2–3 wk (Fig. 1). On the first visit to the laboratory, seat height and handlebar positions were individually adjusted for comfort, and the adjustments were recorded and replicated in subsequent tests. The height and mass (SECA, Hamburg, Germany) of each participant were also recorded.

Participants then completed an incremental exercise test to volitional exhaustion to determine maximal oxygen uptake (VO₂max) and gas exchange threshold (GET) and to establish future work intensities. This entailed cycling at a self-selected pedal rate (between 70 and 90 rpm) for 4 min at 0 W after which a warm-up phase at moderate intensity (~80% GET) was applied for a further 6 min. After 2 min of passive rest, the subjects pedaled for 4 min of baseline cycling at 0 W after which the severe WR was abruptly applied for 6 min. This test was repeated after a rest period of 2 h. In the second test, the severe exercise bout was continued until the subject volitionally terminated the test at exhaustion.

Eccentric Exercise

To provoke muscle damage, participants performed 100 (smith) squats as 10 sets of 10 repetitions. The load on the bar was calculated to correspond to 70% of each participant’s body mass. Before commencement, participants were instructed in correct and safe lifting technique. During the movement the bar was positioned on the participant’s shoulders, and feet were positioned under the bar, with the back straight and legs fully extended (knee = 180°). The descent phase involved eccentric action of the knee extensors to lower the bar to a knee angle of just past 90°. The lifting phase involved concentric action to return the bar to the starting position. A similar protocol has previously been used to induce eccentric muscle damage (10, 11).

Markers of muscle damage.

Markers of muscle damage. Plasma creatine kinase (CK) activity, perceived muscle soreness, and isokinetic peak torque were measured in the order listed, immediately before and at 24 and 48 h after the muscle-damaging protocol.

 Plasma CK activity was assessed from fingertip capillary samples. The sample was centrifuged at 4,000 rpm (2,000 g) for 5 min, and two 20-μl samples of plasma were then added to 1 ml of a composition of reagents supplied by Randox (CK-NAC 110, Randox Laboratories, Crumlin, Co. Antrim, UK). Following 1 min incubation at 37°C and during continued incubation, absorbance at 340 nm was recorded by spectrophotometry (Jenway 6310 spectrophotometer, Jenway, Essex, UK) at 0, 1, 2, and 3 min. CK values were calculated using the formula CK (U/l) = 8,095 × Δabsorbance 340 nm/min. The mean CK value of the two samples was calculated and used for subsequent analysis. Normal serum values of 24–195 U/l are reported for men using this method (63).

Subjects assessed the soreness of their knee extensors using a blank 0–10 visual analog scale (VAS). The VAS consisted of a 10-cm line labeled from left (no soreness) to right (worst soreness ever). After squattting to 90° knee flexion with hands on hips, subjects were asked to place a mark on the VAS to indicate their level of soreness. Perceived pain was then quantified by measuring the distance to the mark on the line to the nearest 0.1 cm. This procedure has been used as described in previous studies (46, 59, 65).

Following familiarization sessions, isokinetic peak torque was measured using a Biodex B-2000 isokinetic dynamometer (Biodex, Shirley, NY), which was calibrated before each data collection session in accordance with the manufacturer’s guidelines. Participants completed a standardized warm-up of 2 min cycling at 50 W on the cycle ergometer followed by static stretching exercises of the knee extensor/ flexor muscle groups. The highest value of five maximal voluntary contractions (MVCs) at 0.52 rad (30°/s) was recorded. A rest period of 30 s was allowed between contractions. Visual feedback, displaying real-time force, was used to encourage maximal effort. 
Exercise test measures. Pulmonary gas exchange and ventilation were measured breath-by-breath throughout all tests with participants wearing a nose clip and breathing through a low-dead space, low-resistance mouthpiece via an online gas analysis system (Cortex MetaMax 3B, Biophysik, Leipzig, Germany). The system was calibrated before every test in accordance with manufacturer’s guidelines against known concentrations of cylinder gases (15% oxygen, 5% carbon dioxide) and a 3-liter calibration syringe (Hans Rudolph, Kansas City, MO). The VO₂ data gathered from the pre- and post-eccentric exercise tests were subsequently modeled to provide estimates of the VO₂ kinetic parameters (see Modeling of VO₂ and [HHb] Data).

Heart rate (HR), blood lactate concentration ([La]), and ratings of perceived exertion (RPE) (9) were recorded at 2 min and 4 min and at the end of exercise. HR and [La] measures were also recorded during baseline cycling. HR was monitored using a wireless chest strap telemetry system (Polar Electro T31, Kempele, Finland) and measured continuously via a link to the Cortex gas analysis system. Finger tip blood samples were collected and analyzed for [La] using a YSI 2300 STAT plus analyzer (Yellow Springs, OH). Participants were familiarized with Borg’s 6–20 RPE Scale and provided with instructions on how to employ the scale (9). Participants were encouraged to focus on their overall perception of exertion when reporting their RPE.

NIRS. Oxygenation profiles of the right vastus lateralis muscle were recorded in all exercise tests using a continuous-wave near-infrared spectrometer (NIRS; Hamamatsu NIO 300, Hamamatsu Photonics KK, Japan). The system monitored concentration changes in oxyhemoglobin (HbO₂) and deoxyhemoglobin (HHb), which were calculated from the light attenuation change by utilizing the modified Beer-Lambert law. The HHb concentration ([HHb]) signal obtained from the NIRS was regarded as being relatively insensitive to blood volume changes during exercise and thus reflected the balance between the delivery and utilization of oxygen (22).

Pulsed light was emitted at 1-s intervals from the emmission probe at four different wavelengths (775, 810, 850, and 910 nm) and was detected, as a function of distance, using a three-segment photodiode detection probe that received NIRS signals at 2 Hz. The probes were housed in the black silicone holder provided. The interoptode spacing between emitter and receiver was 4 cm, and the penetration depth was approximately one-half of the distance between the emitter and the receiver, i.e., 2 cm. Before placement on the right vastus lateralis, the site was shaved and cleaned using an alcohol swab. The NIRO300 system was then calibrated and the probe holder secured by means of a double-sided adhesive sheet ~12 cm above the lateral epicondyle of the left leg, with the location marked using an indelible marker pen to enable reproduction of the probe positions in subsequent tests (48 h). The thigh with attached probe holder was then wrapped in a dark-colored, elastic bandage to further secure the probes and to eliminate ambient light that might contaminate the NIRS signal.

The NIRS data gathered represented relative concentration changes in the hemoglobin chromophores and were, therefore, not representative of absolute tissue O₂ values. As [HHb] was measured as a change from baseline values, the probe gain was zero-set before testing with the subject at rest in a seated position. Differences in the thickness of the overlying adipose tissue may influence the amplitude of the NIRS signal. However, the same subjects were employed pre- and post-eccentric exercise, and the probe positions were rigorously maintained for each subject in each test; thus no correction for intersite adiposity was necessary. Following exercise testing the data were downloaded, and the resulting text files were stored for subsequent analysis.

Modeling of VO₂ and [HHb] Data
The breath-by-breath data from each exercise test were filtered manually to remove outlying breaths, defined as breaths ± 3 SD from the adjacent five breaths. The data for each individual were then interpolated to provide 1-s values, and the two data sets from each of the pre- and post-eccentric exercise tests were time-aligned and averaged. The first 20 s of data after the onset of exercise (the phase I response) was deleted, and a biexponential model was used to analyze the VO₂ responses to severe exercise, as described by the following equation:

\[ \text{VO}_2(t) = \text{VO}_2\text{baseline} + A_p \left[ 1 - e^{-\tfrac{t}{T_{dp}}} \right] + A_s \left[ 1 - e^{-\tfrac{t}{T_{ds}}} \right] \]

where \( t \) is time; \( \text{VO}_2\text{baseline} \) is baseline VO₂; \( A_p \) and \( A_s \) are the primary and slow component amplitudes, respectively; \( T_{dp} \) and \( T_{ds} \) are the primary and slow component time delays, respectively; and \( \tau_p \) and \( \tau_s \) are the time constants of the primary and slow components, respectively. The parameters of the model were determined by using a nonlinear least squares algorithm. In the equations above, \( \text{VO}_2(t) \) represents the absolute \( \text{VO}_2 \) at a given time \( t \), and \( \text{VO}_2\text{baseline} \) represents the average \( \text{VO}_2 \) through the baseline cycling period. Because the time to exhaustion was not identical in the first and second bouts of severe exercise, we fitted the data \( t \) to the end of exercise in both bouts (MRTimead) and 2) to the same point in time (given by the time to exhaustion in the shortest bout) (MRTend). The primary component “gain” (i.e., \( A_p/\Delta WR \)) was calculated from the projected asymptotic \( \text{VO}_2 \). In addition, the “actual” gain attained at the end of exercise was calculated.

To provide information on the effect of eccentric muscle-damaging exercise on the dynamics of muscle oxygenation, we also modeled the \( \Delta [\text{HHb}] \) response to severe exercise. The NIRS-derived [HHb] data were time-aligned and averaged to provide a single response for each subject pre- and post-eccentric exercise. The time delay before an increase in [HHb] after exercise onset was determined as the first point greater than 1 SD above the mean of the baseline (19). [HHb] data were then fitted with a biexponential model similar to that described by the equation above, with the exception that the fitting point greater than 1 SD above the mean of the baseline (19). [HHb] data were then fitted with a biexponential model similar to that described by the equation above, with the exception that the fitting window started at the onset of exercise (i.e., at \( t = 0 \)). Subsequently, [HHb] data were fitted with a monoexponential model from the onset of exercise to the time point representing the interface of the primary and slow component to determine the rate of adaptation of muscle deoxygenation during the primary phase (MRT₁). In addition, the [HHb] dynamics for the entire response were modeled with a similar monoexponential function (MRT₂).

Statistical Analysis
Changes in the markers of muscle damage (peak torque, soreness, and CK activity) were analyzed using a series of one-way repeated measures (RM) ANOVA. All data were checked for assumptions of normality. As the CK activity data were found not to be normally distributed, the values were log-transformed before statistical analysis (65). Following transformation CK activity data were normally distributed. Changes in HR, RPE, [La], and ventilation were analyzed using separate two-way RM ANOVAs (test × time). Assumptions of sphericity were evaluated using Mauchly’s test. Where sphericity was violated (\( P < 0.05 \)), the Greenhouse-Geisser (GG) correction factor was applied. Post hoc Tukey tests modified for repeated measures (61) were run to determine where significant differences occurred. Paired t-tests were used to determine significant differences in time to exhaustion and the VO₂ and [HHb] kinetic responses to severe intensity exercise before and after eccentric exercise. All data were analyzed using the statistical software package SPSS for Windows (version 13). Statistical significance was set at 0.05.
RESULTS

Markers of Muscle Damage

The eccentric exercise was effective in provoking significant changes in all markers of muscle damage. Table 1 shows changes in isokinetic peak torque, perceived muscle soreness, and plasma CK activity before and at 24 h and 48 h post-eccentric exercise. Isokinetic peak torque (30°/s) decreased by 21% at 24 h post-eccentric exercise and remained depressed at 48 h ($F_{2,16} = 21.85, P < 0.001$). Significant soreness was reported 24 h after eccentric exercise with the highest values at 48 h ($F_{2,16} = 80.50, P < 0.001$). Plasma CK activity increased after eccentric exercise, with the highest activity observed at 24 h ($F_{2,16} = 17.15, P < 0.001$). Changes in markers of muscle damage were detected in all subjects, although considerable intersubject variability was observed. Peak decrements in isokinetic peak torque (30°/s) ranged from 12 to 44%, and peak increases in soreness and plasma CK activity ranged from 53 to 95% and 146 to 1,176%, respectively.

Response to Severe-Intensity Exercise

Table 2 shows the $\dot{V}_O_2$ responses to severe intensity exercise. There were no changes in the phase II $\dot{V}_O_2$ kinetics following eccentric exercise, nor was there a change in the slow component time delay (TD) ($P > 0.05$). However, the amplitude of the slow component was significantly reduced ($t_8 = 3.84, P < 0.05$), and the overall mean response time (MRT) was significantly faster ($t_8 = 4.01, P < 0.05$) following eccentric exercise. A significantly shorter time to exhaustion was observed in the second bout of severe-intensity exercise [pre-eccentric exercise, 7 min:24 s ($\pm 2$ min:41 s); 48 h post-eccentric exercise, 6 min:14 s ($\pm 2$ min:46 s)] ($t_8 = 2.58, P < 0.05$). The $\dot{V}_O_2$ response of a representative subject is illustrated in Fig. 2.

There was a significant increase in RPE values reported during severe-intensity exercise following eccentric exercise ($F_{1,8} = 6.7, P < 0.05$). However, there were no significant differences in the blood lactate or HR responses before and after eccentric exercise (Table 3) ($P > 0.05$). In addition, there was a significant increase in the ventilatory equivalent for $O_2$ ($V\dot{E}/V\dot{O}_2$) following eccentric exercise [pre-eccentric exercise, 29.3 ($\pm 3.5$); 48 h post-eccentric exercise, 32.6 ($\pm 5.2$)] ($F_{1,8} = 7.45, P < 0.05$). There was also a significant interaction of time $\times$ test ($F_{0.72} = 3.15, P < 0.05$) on $V\dot{E}/V\dot{O}_2$. Post hoc Tukey tests indicated that $V\dot{E}/V\dot{O}_2$ was significantly greater post-eccentric exercise for the last 70% of exercise with mean values rising from 25.3 to 36.0 (pre-eccentric exercise) and from 26.7 to 39.9 (48 h post-eccentric exercise) ($P < 0.05$).

Table 1. Changes in markers of muscle damage values before and at 24 h and 48 h after eccentric exercise

<table>
<thead>
<tr>
<th>Measured Variable</th>
<th>Pre</th>
<th>24 h Post</th>
<th>48 h Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak torque (30°/s), Nm</td>
<td>287±39</td>
<td>227±44*</td>
<td>228±60*</td>
</tr>
<tr>
<td>Soreness, visual analog scale 0–10</td>
<td>0.6±0.5</td>
<td>6.4±1.8*</td>
<td>7.1±1.5*</td>
</tr>
<tr>
<td>CK activity, U/l</td>
<td>172±123</td>
<td>740±666*</td>
<td>373±208</td>
</tr>
</tbody>
</table>

Values are means ± SD before (Pre) and at 24 h and 48 h after eccentric exercise (Post). Soreness, visual analog scale 0–10. CK, creatine kinase. *Significantly different ($P < 0.05$) from Pre value.

Table 2. Pulmonary $O_2$ uptake responses to severe-intensity exercise before and after eccentric muscle-damaging exercise

<table>
<thead>
<tr>
<th>Primary component</th>
<th>Pre</th>
<th>48 h Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>$TD_p$, s</td>
<td>12±4</td>
<td>12±2</td>
</tr>
<tr>
<td>$\tau_s$</td>
<td>25±4</td>
<td>24±3</td>
</tr>
<tr>
<td>$A_p$, $\dot{O}_2$, l/min</td>
<td>2.36±0.23</td>
<td>2.37±0.23</td>
</tr>
<tr>
<td>Gain, ml·min$^{-1}$·W$^{-1}$</td>
<td>9.02±0.45</td>
<td>9.04±0.52</td>
</tr>
<tr>
<td>Slow component</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$TD_s$, s</td>
<td>102±19</td>
<td>107±29</td>
</tr>
<tr>
<td>$A_{2end}$ $\dot{O}_2$, l/min</td>
<td>0.63±0.33</td>
<td>0.37±0.17*</td>
</tr>
<tr>
<td>$A_{2ref}$ $\dot{O}_2$, l/min</td>
<td>0.67±0.33</td>
<td>0.38±0.17*</td>
</tr>
<tr>
<td>Overall response</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$MRT_{ref}$</td>
<td>61±15</td>
<td>51±9*</td>
</tr>
<tr>
<td>$MRT_{end}$</td>
<td>64±15</td>
<td>51±9*</td>
</tr>
<tr>
<td>Peak $\dot{V}_O_2$, l/min</td>
<td>3.89±0.44</td>
<td>3.70±0.36</td>
</tr>
</tbody>
</table>

Values are means ± SD. $TD_p$, $\tau_s$, $A_p$, and gain are the time delay, time constant, amplitude, and increase in $\dot{V}_O_2$ per unit increase in work rate for phase II kinetics, respectively. $TD_s$, $A_{2ref}$, and $A_{2end}$ are the time delay, amplitude to 6 min, and amplitude to end of exercise for the slow component, respectively. $MRT_{end}$ and $MRT_{ref}$ are the mean response times fitted to the end of exercise in both bouts and to the same point in time (given by the time to exhaustion in the shortest bout), respectively. Peak $\dot{V}_O_2$ tended to be lower post-eccentric exercise, but the difference was not statistically significant. *Significant difference ($P < 0.05$) from Pre value.

The results of the kinetic response of [HHb] to severe intensity exercise pre- and 48 h post-eccentric exercise are shown in Table 4. Most importantly, with respect to our experimental hypothesis, both the [HHb] MRT$T_1$ and MRT$T_{end}$ were significantly slower following eccentric exercise ($P < 0.05$). There was no significant correlation between any of the markers of muscle damage and indexes of muscle oxygenation in the post-condition ($P > 0.05$). The [HHb] response of a representative subject is illustrated in Fig. 3A. The altered $Q\dot{O}_2$:$\dot{V}_O_2$ balance is most pronounced during the initial response following the onset of severe intensity exercise (Fig. 3B), with the greatest mean difference observed over the first 5–20 s (Fig. 3C). There were no significant differences in the pre- and post-conditions between the amplitude of the response in either the primary phase or slow component ($P > 0.05$). Similarly there was no difference between the total hemoglobin responses in the two conditions (Fig. 3D).

Fig. 2. A representative subject’s $\dot{V}_O_2$ uptake ($\dot{V}_O_2$) response to severe cycle exercise pre (○) and 48 h post (●)-eccentric exercise. The vertical line represents the transition from unloaded to loaded cycling.
DISCUSSION

The principal original finding of this investigation is that eccentric, muscle-damaging exercise results in a slowing of muscle [HHb] kinetics without altering pulmonary VO$_2$ kinetics during high-intensity cycle exercise in humans. We interpret the slower [HHb] kinetics, in the face of unchanged pulmonary VO$_2$, to be consequent to a local elevation of the Q$_{O2}$:VO$_2$ ratio. The observation that the [HHb] kinetics were over 30% slower 48 h after the performance of eccentric exercise suggests that the matching of Q$_{O2}$ and V$_{O2}$ was profoundly altered as a consequence of the intervention.

The dynamic balance between O$_2$ delivery and O$_2$ utilization has been keenly debated with regard to possible limitations to muscle VO$_2$ kinetics (50). During transitions to exercise intensities below GET, the compelling weight of evidence supports the premise that metabolic inertia is the principal limitation to muscle O$_2$ uptake (2, 28, 31). However, for transitions to the premise that metabolic inertia is the principal limitation to severities below GET, the compelling weight of evidence supports exercise suggesting that the matching of Q$_{O2}$ and V$_{O2}$ was over 30% slower 48 h after the performance of eccentric exercise. Specifically, an increase in the diameter of muscle of subjects interrogated herein. Kano et al. (37) reported increase in the energy demand of contraction, might account for the faster oxygen saturation reported by Ahmadi et al. (1).

The experimental protocol used in the present study differs fundamentally from that employed by Kano et al. (37). These authors electrically induced twitch muscle contractions (1 Hz, 2–5 V, 2-ms pulse duration) in rat spinotrapezius muscle, whereas in our study, human subjects performed dynamic high-intensity cycle-exercise transitions. While taking these differences into consideration, it is important to note that rat spinotrapezius muscle does provide a highly acceptable, comparative model for the analysis of human microvascular and myocyte damage as it exhibits a fiber composition (20) and oxidative capacity (44) that closely resemble that of the human quadriceps. Thus the disruption observed in rat microvasculature subsequent to eccentric exercise would, most likely, also be present in the damaged muscle of subjects interrogated herein. Kano et al. (37) reported disrupted capillary geometry and substantial microvascular dysfunction following eccentric exercise. Specifically, an increase in the capillary luminal area of damaged muscle was reported, which resulted in a decreased microvascular P$_{O2}$ (Pmv$_{O2}$) at the onset of electrically stimulated contractions. In addition, a 27–34% increase in non-RBC-flowing capillaries was found 1–3 days after a single bout of eccentric exercise.

Such structural and functional alterations to the microvasculature might act to alter the matching of Q$_{O2}$ to VO$_2$ both spatially and temporally with respect to tissue energetic requirements. Specifically, the lower [HHb] observed at any given VO$_2$ across the transition post-eccentric exercise suggests that achievement of the requisite blood-myocyte O$_2$ flux might demand a higher microvascular O$_2$ driving pressure. Blood-muscle O$_2$ flux is primarily determined by the number of erythrocytes lying adjacent to active myocytes at any given time (21, 32). A decrease in the proportion of capillaries supporting RBC flow would lead to a reduction in blood-muscle O$_2$ flux. Similarly, an increase in the diameter of free-flowing capillaries would increase the carrier-free diffusion distance and lead to a reduction in O$_2$ diffusing capacity. Fick’s law of diffusion states that:

Table 3. RPE, HR, and [La] during severe-intensity exercise pre- and 48 h post-eccentric exercise

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>48 h Post</th>
<th>Pre</th>
<th>48 h Post</th>
<th>Pre</th>
<th>48 h Post</th>
<th>Pre</th>
<th>48 h Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPE*</td>
<td>NA</td>
<td>NA</td>
<td>15 ± 1</td>
<td>16 ± 1</td>
<td>17 ± 1</td>
<td>18 ± 2</td>
<td>19 ± 1</td>
<td>19 ± 1</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>83 ± 9</td>
<td>86 ± 6</td>
<td>159 ± 8</td>
<td>163 ± 11</td>
<td>174 ± 11</td>
<td>176 ± 11</td>
<td>184 ± 9</td>
<td>181 ± 11</td>
</tr>
<tr>
<td>[La], mmol/l</td>
<td>0.9 ± 0.5</td>
<td>1.0 ± 0.4</td>
<td>3.5 ± 1.0</td>
<td>3.8 ± 1.0</td>
<td>6.9 ± 3.1</td>
<td>6.8 ± 1.8</td>
<td>8.4 ± 1.4</td>
<td>8.7 ± 1.8</td>
</tr>
</tbody>
</table>

Values are means ± SD. RPE, ratings of perceived exertion based on Borg’s 6–20 scale (9); HR, heart rate; [La], blood lactate concentration; NA, not applicable. *Significant main effect for time (P < 0.05).

Table 4. [HHb] response to severe-intensity constant-load exercise pre- and 48 h post-eccentric exercise

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>48 h Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRT$_1$, s</td>
<td>14 ± 3</td>
<td>19 ± 3*</td>
</tr>
<tr>
<td>Primary amplitude, %</td>
<td>91 ± 8</td>
<td>88 ± 8</td>
</tr>
<tr>
<td>Primary amplitude, AU</td>
<td>390 ± 102</td>
<td>297 ± 72</td>
</tr>
<tr>
<td>MRT$_2$, s</td>
<td>16 ± 4</td>
<td>21 ± 4*</td>
</tr>
<tr>
<td>SC amplitude, %</td>
<td>9 ± 8</td>
<td>12 ± 8</td>
</tr>
<tr>
<td>SC amplitude, AU</td>
<td>33 ± 25</td>
<td>41 ± 25</td>
</tr>
</tbody>
</table>

Values are means ± SD. [HHb], deoxyhemoglobin concentration; MRT$_1$, and MRT$_2$, are the mean response time of the primary phase and the overall response, respectively; SC, slow component; AU, arbitrary units. *48 h Post value significantly slower than Pre value (P < 0.05).
where VO_{2m} is muscle VO_{2} and DO_{2} is the diffusing capacity for O_{2}. Such reductions to DO_{2} would be expected to negatively impact VO_{2} particularly in the presence of concomitant alterations to PmvO_{2}, similar to those reported by Kano et al. (37). The O_{2} pressure gradient from blood to myocyte that drives O_{2} diffusion is determined principally by alterations of PmvO_{2} (51, 52). Thus the accelerated fall of PmvO_{2} observed by Kano and colleagues during the first 20–40 s of electrically stimulated muscle contractions would be expected to have a profound influence on the muscle O_{2} diffusing capacity and result in a slowing of VO_{2} kinetics (5, 6, 8). However, the PmvO_{2} herein (as judged by the [HHb] response) appeared to be elevated during the dynamic rest-exercise transitions post-eccentric exercise, and the VO_{2} kinetic response remained unchanged.

A given VO_{2} is achieved through the interaction of O_{2} delivery (QO_{2}) and O_{2} diffusing properties (7). Additionally, changes in pulmonary VO_{2} across the rest-exercise transition are known to directly reflect leg VO_{2} during cycling exercise (54) and demonstrate a good approximation of the muscle VO_{2} kinetics (31). Therefore, the elevated QO_{2}:VO_{2} ratio evidenced by the slower [HHb] kinetic response (i.e., lower [HHb] at a given VO_{2} across the exercise transition) must, in this instance, be due to compensatory changes to O_{2} delivery. Pertinent to this issue, increased blood flow (and therefore increased O_{2} delivery) has been observed by Laaksonen et al. (43), who found that blood flow to the exercising quadriceps femoris was elevated by 25% after a prior bout of exhaustive eccentric exercise. Importantly, they also reported that VO_{2} remained unchanged and suggested that the altered QO_{2}:VO_{2} balance observed may have been due to impaired oxygen extraction. Thus, following eccentric muscle-damaging exercise, adaptive, compensatory mechanisms may act to elevate PmvO_{2} across the rest-exercise transition, preserving blood-myocyte O_{2} flux as indicated by the unchanged phase II VO_{2} kinetics.

The higher ratings of perceived exertion reported for a given exercise intensity (70%\%\%\%) following eccentric exercise may be due, in part, to the enhanced ventilatory response that accompanies the increased muscle soreness. It has been proposed that an altered sense of effort may be part of a central protective mechanism whereby neural inhibition serves to reduce force generation to prevent further injury (42, 47, 57). Additionally, Hotta et al. (35) suggested that changes in neural factors contribute not only to alterations in force generation but also to an enhanced ventilatory response. Group III and IV afferent fibers located in and around the blood vessels of exercising muscle are involved in modulating the ventilatory response. Distension of these blood vessels provokes a discharge from the afferent fibers that leads to an increase in ventilation (33, 34). Thus neural monitoring of peripheral
vascular events might account, in part, for the enhanced $V_{\text{E}}/V_{\text{O}_2}$ observed in this study if there were alterations to the microvasculature as a result of eccentric exercise. Pertinent to this issue, in studies employing male Wistar rats, Kano et al. (38) have reported changes in the capillary lumen shape (luminal ellipticity) that increase the luminal cross-sectional area by up to 62% following eccentric exercise. Similar increases in the cross-sectional area of the microvessels in the muscles recruited in the present investigation might serve to augment the ventilatory response via neural modulation.

It has been proposed that, following eccentric exercise, alterations to motor unit recruitment patterns arise to meet the energetic demands of a given WR (17). Reports of elevated blood lactate concentration following eccentric exercise have been attributed to the additional recruitment of type II fibers and a concomitant rise in the rate of glycogenolysis (15, 27). However, Chen et al. (16) reported that neural adaptation to motor unit activation patterns occurred after a single bout of eccentric exercise, such that additional type I motor units were recruited. Additional fiber recruitment has been implicated in the development of the $V_{\text{O}_2}$ slow component in high-intensity exercise (41). Specifically, it has been proposed that the development of a $V_{\text{O}_2}$ slow component may be related, in part, to the recruitment of additional type II fibers (3, 55, 67). The reduction in the amplitude of the $V_{\text{O}_2}$ slow component in high-intensity exercise (41).

Experimental Considerations

As mentioned earlier, our findings contrast with those of Kano et al. (37) due to fundamental differences in the muscle activation processes employed rather than species variation. We studied dynamic exercise transitions to severe intensity (70%Δ) cycle exercise, whereas Kano and colleagues utilized a set rate of electrical stimulation (1 Hz, 3–5 V, 2-ms pulse duration) to induce muscle contractions. Electrical stimulation induces recruitment of all fibers, whereas voluntary exercise recruits specific fibers and fiber types, dependent on exercise intensity and duration (39). Thus the muscle activation and attendant fiber recruitment patterns studied herein present a more ecologically valid model for investigation. As such, this study may provide a more realistic insight into the effects of eccentric muscle-damaging exercise on functional human performance.

NIRS is an established technique for the measurement of muscle oxygenation (e.g., 23, 36). However, the reliability and reproducibility of the NIRS-derived [HHb] signal is dependent on the precise placement of the optodes. In the present study, optode location was marked on each individual subject during the first visit to the laboratory, and placement was carefully reproduced on subsequent visits. Koga et al. (40) have revealed the presence of significant heterogeneity with respect to the dynamics of muscle oxygenation within the quadriceps muscles of healthy subjects following the onset of exercise. These findings are not surprising given that muscle blood flow, motor unit distribution and recruitment, and consequently vascular responses are known to be heterogeneous within and across muscles. However, it is important to recognize that the NIRS data reported herein are representative of changes within the superficial muscle area under interrogation only and as such may not be representative of the entire muscle mass affected by eccentric exercise.

In conclusion, the present investigation suggests that eccentric, muscle-damaging exercise alters the matching of $Q_{\text{O}_2}$ and $V_{\text{O}_2}$ during severe-intensity exercise. Specifically, across the rapid metabolic transition following the onset of exercise, for a given $V_{\text{O}_2}$, the [HHb] signal is reduced. We propose that structural and possibly functional alterations to the microvasculature act to increase the $Q_{\text{O}_2}/V_{\text{O}_2}$ ratio both spatially and temporally with respect to tissue energetic requirements. Accordingly, following eccentric muscle-damaging exercise, compensatory mechanisms act to elevate the microvascular driving pressure for blood-myocyte $O_2$ flux, enabling a preservation of the kinetics of $V_{\text{O}_2}$ across the rest-to-exercise transition.

REFERENCES