Effects of eccentric exercise on microcirculation and microvascular oxygen pressures in rat spinotrapezius muscle

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Kano, Yutaka, Danielle J. Padilla, Brad J. Behnke, K. Sue Hageman, Timothy I. Musch, and David C. Poole. Effects of eccentric exercise on microcirculation and microvascular oxygen pressures in rat spinotrapezius muscle. J Appl Physiol 99: 1516–1522, 2005. First published June 30, 2005; doi:10.1152/japplphysiol.00069.2005.—A single bout of eccentric exercise results in muscle damage, but it is not known whether this is correlated with microcirculatory dysfunction. We tested the following hypotheses in the spinotrapezius muscle of rats either 1 (DH-1; n = 6) or 3 (DH-3; n = 6) days after a downhill run to exhaustion (90–120 min; −14° grade): 1) in resting muscle, capillary hemodynamics would be impaired, and 2) at the onset of subsequent acute concentric contractions, the decrease of microvascular O2 pressure (PmvO2), which reflects the dynamic balance between O2 delivery and utilization, would be accelerated compared with control (Con, n = 6) rats. In contrast to Con muscles, intravital microscopy observations revealed the presence of sarcomere disarrangements in DH-1 and DH-3 and increased capillary diameter in DH-3 (Con: 5.2 ± 0.1; DH-1: 5.1 ± 0.1; DH-3: 5.6 ± 0.1 μm; both P < 0.05 vs. DH-3). At rest, there was a significant reduction in the percentage of capillaries that sustained continuous red blood cell (RBC) flux in both DH running groups (Con: 90.0 ± 2.1; DH-1: 66.4 ± 5.2; DH-3: 72.9 ± 4.1%, both P < 0.05 vs. Con). Capillary tube hematocrit was elevated in DH-1 but reduced in DH-3 (Con: 22 ± 2; DH-1: 28 ± 1; DH-3: 16 ± 1%; all P < 0.05). Although capillary RBC flux did not differ between groups (P > 0.05), RBC velocity was lower in DH-1 compared with Con (Con: 324 ± 43; DH-1: 212 ± 30; DH-3: 266 ± 45 μm/s; P < 0.05 DH-1 vs. Con). Baseline PmvO2 before contractions was not different between groups (P > 0.05), but the time constant of the exponential fall to contracting PmvO2 values was accelerated in the DH running groups (Con: 14.7 ± 1.4; DH-1: 8.9 ± 1.4; DH-3: 8.7 ± 1.4 s, both P < 0.05 vs. Con). These findings are consistent with the presence of substantial microvascular dysfunction after downhill eccentric running, which slows the exercise hyperemic response at the onset of contractions and reduces the PmvO2 available to drive blood-muscle O2 delivery. Novel eccentric exercise that generates high intramuscular forces induces profound muscle damage and dysfunction. For example, after a single bout of eccentric exercise, serum creatine kinase activity becomes elevated (9; for review, see Ref. 48), proteolytic enzymes are increased (8, 44), and a substantial inflammatory response is manifested (15). Moreover, a select population of damaged myocytes demonstrates mononuclear cell infiltration, and there is the presence of multiple central nuclei in these fibers (23). Depending on the time elapsed after the exercise bout (i.e., 1–7 days), either an initial fiber swelling or subsequent fiber degeneration may be observed (23).

Whereas myocyte degenerative changes will impact the ability of the muscle(s) to produce high forces, the capacity for repetitive contractions such as those powering locomotory exercise will likely be affected by any impairment in the ability to deliver O2 and energetic substrates to the muscle fibers. Specifically, the probability that eccentric exercise will damage the microcirculation and therefore the ability to deliver and exchange O2 and substrates must be recognized. Such damage may be incurred directly during the eccentric exercise or secondary to myocyte swelling and/or altered intramuscular pressures. Indeed, Kano and colleagues (23) have demonstrated that eccentric exercise results in a disruption in the capillary geometry in the red and white gastrocnemius 1–7 days after electrically induced eccentric contractions. However, to date it remains unknown whether microcirculatory function and the capacity to deliver and distribute O2 within the capillary bed are impaired after eccentric exercise.

Our laboratory has demonstrated recently that downhill running represents a physiological paradigm that recruits the spinotrapezius muscle as evidenced by the presence of an exercise hyperemic response (22). This muscle stabilizes the scapula, and downhill running consequently subjects the spinotrapezius to eccentric activity. Therefore, the purpose of the present investigation was to utilize downhill running to explore the functional microvascular and O2 delivery (QO2) and O2 utilization (VO2) [i.e., ratio between QO2 and VO2 (QO2/VO2)] sequelae to eccentric exercise. A combination of intravital microscopy and phosphorescence quenching techniques were utilized to address the following hypotheses. Within the spinotrapezius of naive rats, at 1 and 3 days after exhaustive downhill exercise, we hypothesized that 1) the proportion of capillaries sustaining red blood cell (RBC) flux would be decreased and capillary hemodynamics would be impaired, and 2) at the onset of acute concentric contractions initiated at 1 and 3 days after the eccentric exercise, the kinetics of the fall in microvascular oxygen pressure (PmvO2), which reflects the dynamic balance between QO2 and VO2, would be accelerated. Specifically, if microcirculatory impairments act to reduce QO2, a more rapid fall in PmvO2 would be expected at the onset of contractions. Our results substantiated these hypotheses and indicated that eccentric exercise impairs the capacity to deliver...
and distribute O₂ within muscle 1–3 days postexercise. This scenario would be expected to impact negatively Vo₂ kinetics and therefore the oxidative contribution to muscle energetics, particularly during subsequent bouts of exercise.

**METHODS**

**Animal selection and care.** Eighteen female Sprague-Dawley rats (body mass 264 ± 4 g) were used in this study. Rats were maintained on a 12:12-h light-dark cycle and received food and water ad libitum. All experiments were conducted under the guidelines established by the National Institutes of Health and were approved by Kansas State University’s Institutional Animal Care and Use Committee. Rats were randomly assigned to one of two groups: control (Con; n = 6) or downhill running (n = 12).

**Experimental protocol.** Rats in the downhill running group underwent a period of familiarization (1–2 wk) to running on a motor-driven treadmill that entailed exercising for 5–10 min/day at a speed of 20–30 m/min (0% grade). After acclimatization to the treadmill, each rat performed an exercise protocol designed to actively recruit the spinotrapezius muscle (22). To ensure that each rat was fatigued by the protocol, all animals ran individually on a −14°C decline for 90 min (5-min bouts with 2 min of rest between successive bouts). The running speed was maintained at 40 m/min until the rat could no longer keep pace with the treadmill. At this time, the speed was decreased to 20 m/min for the subsequent exercise bouts. Each rat performed approximately nine exercise bouts at 40 m/min and nine bouts at 20 m/min. Subsequently, each rat ran continuously until it was unable to maintain pace with the treadmill despite humane encouragement. This final run constituted an average of 9 min of downhill running at 20 m/min. Thus each rat ran for a total duration of 99 min on average. Presence of fatigue was confirmed by exhibition of extreme lethargy in righting themselves when placed in the supine position. The downhill running group was further divided into two subgroups in which examination of the effects of downhill running was performed at 1 and 3 days postrunning [DH-1 (n = 6) and DH-3 (n = 6), respectively]. After the designated period of time, each rat underwent two procedures: phosphorescence quenching for determination of PmvO₂ at the onset of contractions and examination of the microcirculation within the spinotrapezius muscle. Heart rate and mean arterial pressure were monitored continuously throughout both data-acquisition periods. Total duration of the experiments did not exceed 3 h. Control rats were not exercised because it has been demonstrated previously that the classical protocol of inclined running does not recruit the spinotrapezius muscle as determined by the lack of increase of blood flow measured using radiolabeled microspheres (22, 35).

**Surgical preparation for phosphorescence quenching.** Before the surgical procedures, the animals were anesthetized with pentobarbital sodium (50 mg/kg ip to effect and supplemented as necessary). The rat was placed on a heating pad (38°C) to maintain body temperature. To monitor arterial blood pressure and heart rate (model 200, Digitimer, BPA, Louisville, KY), the left carotid artery was cannulated (polyethylene-50, Intra-Medic polyethylen tubing, Clay Adams Brands; Sparks, MD). This cannula also allowed for infusion of the phosphorescent probe [palladium meso-tetra(4-carboxyphenyl) porphyrin/1517MICROCIRCULATION AND OXYGEN PRESSURES

The spinotrapezius muscle was a postural muscle that lies in the middorsal region of the rat; it originates from the lower thoracic and upper lumbar region and inserts on the spine of the scapula. The right spinotrapezius muscle was exposed by a U-shaped skin incision to provide access for electrical stimulation and measurement of PmvO₂. After the overlying skin was reflected and fascia was removed, the muscle surface was superfused with Krebs-Henseleit solution equilibrated with 5% CO₂-95% N₂ at 38°C and adjusted to pH 7.4. For the induction of indirect bipolar muscle contractions in the spinotrapezius, stainless steel electrodes were attached to the muscle proximal to the motor point (cathode) and across the caudal extremity (anode) close to the spinal attachment.

**PmvO₂ measurements.** The phosphor R2 was infused via the arterial cannula ~15 min before each experiment. The experiments were conducted in a darkened room to prevent contamination from ambient light. After a 10- to 15 min stabilization period after the surgery, twitch muscle contractions (1 Hz, 3–5 V, 2-ms pulse duration) were elicited for 3 min using a Grass S88 stimulator (Quincy, MA). This contraction profile provides a blood flow response consistent with moderate-intensity exercise (4). PmvO₂ was determined at 2-s intervals at rest and after the rest-to-stimulus transition for 3 min. After the 3-min stimulation period, PmvO₂ was measured for 3–5 min into recovery before undergoing surgical exteriorization of the contralateral (left) spinotrapezius muscle for intravital observation.

**The theoretical basis for phosphorescence quenching has been detailed previously (3, 4, 16). Briefly, the Stern-Volmer relationship (43) describes quantitatively the O₂ dependence of the phosphorescent probe. R2 is a nontoxic dendrimer (31) that binds completely to albumin at 38°C and pH 7.4, with a quenching constant of 409 mmHg/s and lifetime of decay in the absence of O₂ of 601 μs (33, 36). In addition to binding with albumin, the net negative charge of R2 also facilitates restriction of the compound to the vascular space (38).

PmvO₂ reflects the PO₂ within the capillary bed, which constitutes the principal intramuscular vascular space. To determine PmvO₂, a PMOD 1000 frequency domain phosphorometer (Oxygen Enterprises, Philadelphia, PA) was utilized. The common end of the bifurcated light guide was placed ~2–3 mm above the medial region of the spinotrapezius (i.e., superficial to dorsal surface), and blood was sampled within the microvasculature up to ~500 μm deep within a circular region ~2 mm in diameter. The phosphorometer employs a sinusoidal modulation of the excitation light (524 nm) at frequencies between 100 Hz and 20 kHz, which allows for phosphorescence lifetime measurements from 10 μs to ~2.5 ms. In the single-frequency mode, 10 scans (100 ms) were used to acquire the resultant lifetime of the phosphorescence (700 nm) and were repeated every 2 s (for review, see Ref. 51). To obtain the phosphorescence lifetime, the logarithm of the intensity values was taken at each time point and the linearized decay was fit to a straight line by the least squares method (7).

**Modeling of PmvO₂ profiles.** Curve fitting was accomplished by use of KaleidaGraph software (Synergy Software, Reading, PA) and was performed on the PmvO₂ data by using a one-component exponential model by means of the following equation:

\[
PmvO₂(t) = PmvO₂_{\text{baseline}} - \Delta PmvO₂[1-e^{-t/TD}] + \text{residuals}
\]

where \( PmvO₂_{\text{baseline}} \) is PmvO₂ at time \( t \), \( \Delta PmvO₂ \) designates the decrease of PmvO₂ from resting baseline to steady state during contractions, TD is the time delay, and \( \tau \) is the time constant.

Goodness of model fit was determined via three criteria: 1) the coefficient of determination (i.e., \( R^2 \)), 2) the sum of the squared residuals term (i.e., \( \chi^2 \)), and 3) visual inspection of the model.

**Intravital microscopy studies of the microcirculation.** After the phosphorescence quenching procedures were completed, the left spinotrapezius was exteriorized as described previously (18, 25–28, 39) to examine the microcirculation. This procedure takes ~45 min (range 40–50 min) and does not perturb the microvasculature or blood flow at rest or during 1-Hz contractions (2). Fascial removal and disturbance were minimized to avoid any associated muscle damage (34). All exposed tissue as well as the dorsal surface of the spinotrapezius was superfused with Krebs-Henseleit solution at 38°C (see Surgical preparation for phosphorescence quenching) while the muscle was sutured (6.0 silk, Ethicon, Somerville, NJ) at five equidistant points around the perimeter to a thin-wire horseshoe-shaped manifold (39). The muscle was then protected with Saran Wrap (Dow Brands, Indianapolis, IN).

The rat was placed on a circulation-heated Lucite platform, and the spinotrapezius was observed by use of an intravital microscope
Table 1. MAP and HR data throughout the phosphorescence quenching and intravital microscopy procedures

<table>
<thead>
<tr>
<th></th>
<th>CON (n = 6)</th>
<th>DH-1 (n = 6)</th>
<th>DH-3 (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>109 ± 3</td>
<td>98 ± 6</td>
<td>113 ± 6</td>
</tr>
<tr>
<td>Phosphorescence quenching</td>
<td>116 ± 2</td>
<td>107 ± 2</td>
<td>115 ± 5</td>
</tr>
<tr>
<td>Intravital microscopy</td>
<td>372 ± 15</td>
<td>395 ± 23</td>
<td>379 ± 25</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>335 ± 12</td>
<td>361 ± 19</td>
<td>370 ± 35</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats; MAP, mean arterial pressure; HR, heart rate; CON, control; DH-1 and DH-3, rats 1 and 3 days, respectively, after a novel bout of exhaustive downhill running. No significant differences between animals or between procedures were detected (P > 0.05).

RESULTS

Animal data. Body weights did not differ between Con and the downhill running groups (Con: 270 ± 8; DH-1: 261 ± 8; DH-3: 259 ± 8 g; P > 0.05), and the weight of the spinotrapezius muscle was not altered by downhill running (Con: 0.46 ± 0.01; DH-1: 0.45 ± 0.02; DH-3: 0.43 ± 0.03 g wet wt; P > 0.05). Mean arterial pressure and heart rate remained constant throughout each experimental protocol and did not differ between the protocols or between groups (Table 1; P > 0.05).

Microcirculation measurements. Fiber width and sarcomere length did not differ significantly between Con, DH-1, and DH-3 (P > 0.05; Table 2). However, sarcomere disruptions were observed in DH-1 and DH-3 (but not Con). A bands were either out of register or smeared (percentage of screen area with affected sarcomeres, DH-1, 32.2 ± 5.9, DH-3, 40.7 ± 5.6, Fig. 1). Collapsed or obstructed capillaries were not apparent in either DH-1 or DH-3. However, there was a significant reduction in the percentage of capillaries that sustained continuous RBC flux in both downhill groups (Con: 90.0 ± 2.1, DH-1: 66.4 ± 5.2, DH-3: 72.9 ± 4.1%, both P < 0.01 vs. Con; Fig. 2). The percentage of capillaries with blood cell velocity (V_RBC) was determined in all capillaries that were continuously RBC perfused by following the RBC path length over several frames (~5–10 capillaries/area) and for the maximum capillary length over which the RBC remained in crisp focus. Red blood cell flux (F_RBC) was measured by counting the number of cells in a capillary passing an arbitrary point over not less than 5 frames per measurement. For each capillary in which hemodynamic data were gathered, capillary tube hematocrit (Hct_cap) was calculated as

\[ \text{Hct} = \frac{V_{\text{RBC}} \times r}{V_{\text{RBC}} + r} \]

where V_RBC is RBC volume, which was taken to be 61 μm^3 (1), and capillaries were approximated as circular in cross section.

Statistics. Values are expressed as means ± SE where the group mean is that of the individual muscles rather than the individual measurements across muscles. Group differences were determined by a one-way analysis of variance and a Student-Newman-Keuls post hoc test. Where a directional a priori hypothesis was tested (i.e., RBC velocity and flux and time constant of PmvRBC, fall), a one-tailed test was utilized. Statistical significance was established at P < 0.05.

Table 2. Morphological and RBC capillary hemodynamic data for rat spinotrapezius muscle in CON, DH-1, and DH-3 rats

<table>
<thead>
<tr>
<th></th>
<th>CON (n = 6)</th>
<th>DH-1 (n = 6)</th>
<th>DH-3 (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphological data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capillary diameter, μm</td>
<td>5.2 ± 0.1</td>
<td>5.1 ± 0.1</td>
<td>5.6 ± 0.1*</td>
</tr>
<tr>
<td>Fiber width, μm</td>
<td>59.5 ± 4.5</td>
<td>65.1 ± 4.5</td>
<td>61.6 ± 2.8</td>
</tr>
<tr>
<td>Sarcomere length, μm</td>
<td>2.4 ± 0.1</td>
<td>2.3 ± 0.1</td>
<td>2.7 ± 0.1</td>
</tr>
<tr>
<td>RBC hemodynamic data</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flux, RBCs per min</td>
<td>28 ± 5</td>
<td>22 ± 3</td>
<td>20 ± 4</td>
</tr>
<tr>
<td>Velocity, μm/s</td>
<td>324 ± 43</td>
<td>212 ± 30*</td>
<td>266 ± 45</td>
</tr>
<tr>
<td>Capillary tube hematocrit, %</td>
<td>22 ± 2</td>
<td>28 ± 1*</td>
<td>16 ± 1*†</td>
</tr>
<tr>
<td>Countercurrent flow, %</td>
<td>23 ± 3</td>
<td>25 ± 2</td>
<td>28 ± 2</td>
</tr>
<tr>
<td>Total lineal capillary density, capillaries/mm</td>
<td>21 ± 1</td>
<td>24 ± 2</td>
<td>21 ± 1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats; RBC, red blood cells. *P < 0.05 vs. CON, †P < 0.05, different from DH-1.
countercurrent flow was not different between groups \((P > 0.05); \text{Table } 2\). In DH-3, but not DH-1, mean capillary diameter was increased significantly compared with Con and DH-1 \((P < 0.05); \text{Table } 2\). Capillary tube hematocrit differed between groups and was highest in DH-1 and lowest in DH-3 \((P < 0.05\text{ vs. Con}; \text{Table } 2\). Although capillary RBC flux did not differ between groups \((P > 0.05); \text{Table } 2\), RBC velocity was lower \((P < 0.05)\) in DH-1, but not DH-3 \((P > 0.05)\), compared with Con \(\text{Fig. } 3\).

Microvascular \(P_{mvO_2}\) response. The dynamic \(P_{mvO_2}\) profiles in response to electrical stimulation are shown in \(\text{Fig. } 4\) for representative Con, DH-1, and DH-3 rats. No differences were found in mean values for baseline \(P_{mvO_2}\), TD, or \(\Delta P_{mvO_2}\) values between Con, DH-1, and DH-3 \((P > 0.05); \text{Table } 3\).

However, the time constant was faster in the DH-1 and DH-3 than Con \((\text{Con}: 14.7 \pm 1.4, \text{DH-1}: 8.9 \pm 1.4, \text{DH-3}: 8.7 \pm 1.4 \text{ s}, \text{both } P < 0.05 \text{ vs. Con}), \text{but DH-1 and DH-3 were not different from one another } (P > 0.05); \text{Table } 3, \text{Fig. } 4\).

DISCUSSION

This investigation is the first to demonstrate that downhill running, which forces eccentric contractions within the rat spinotrapezius, impairs both muscle microcirculatory flow and also the balance between \(Q_{O_2}\) and \(V_{O_2}\) at the onset of contractions as evidenced by the accelerated fall of \(P_{mvO_2}\). One consequence of this lowered \(O_2\) pressure head during the first 20–40 s of muscle contractions will be an impaired blood–myocyte \(O_2\) diffusion. These findings complement and may help explain the extensive and prolonged structural damage \((23, 47)\) and impaired muscle function \((9)\) that follow a single bout of eccentric exercise.

Microcirculation. The control structural data obtained herein are in close agreement with values obtained previously for this spinotrapezius preparation in rats with body masses of 250–
Baseline, ΔPo₂mv, and model parameters of Po₂mv response during electrical stimulation (1 Hz; 3–5 V) of the spinotrapezius muscle of CON, DH-1, DH-3 rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CON (n = 6)</th>
<th>DH-1 (n = 6)</th>
<th>DH-3 (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline Po₂mv, mmHg</td>
<td>27.9 ± 1.5</td>
<td>25.1 ± 1.8</td>
<td>28.9 ± 2.1</td>
</tr>
<tr>
<td>ΔPo₂mv, mmHg</td>
<td>11.3 ± 1.4</td>
<td>11.7 ± 1.1</td>
<td>10.1 ± 1.9</td>
</tr>
<tr>
<td>Time delay, s</td>
<td>11.3 ± 1.1</td>
<td>13.0 ± 1.0</td>
<td>11.7 ± 1.4</td>
</tr>
<tr>
<td>Time constant, s</td>
<td>14.7 ± 1.4</td>
<td>8.9 ± 1.4²</td>
<td>8.7 ± 1.4³</td>
</tr>
</tbody>
</table>

Values are means ± SE, n, no. of rats. Values for each variable are taken from the fitted model. See methods for details. Po₂mv, microvascular Po₂; ΔPo₂mv; decrease in Po₂mv from resting baseline to steady state during contractions. *Significantly different from CON (*P < 0.05).

300 g (25–28, 39, 40). Specifically, when corrected to a sarcomere length of 2.7 μm, mean fiber diameter was 56 μm, which falls within the reported range of 50–57 μm for rats of a similar body mass. Moreover, literature values for mean capillary diameter are 5.4–6.2 μm compared with 5.2 μm herein. Also, the appearance of enlarged capillary diameters at 3 days after downhill running is consistent with several previous studies that have examined damaged muscle (23, 37, 42).

The mechanism for this effect is not known. However, there are inextensible collagenous fibers that mechanically tether the capillaries to adjacent fibers, and it is possible that alterations in fiber geometry related to the 10–20% increase in fiber diameter (when corrected for sarcomere length differences among muscles) may have forced the increase observed in capillary diameter.

With respect to microcirculatory function, there is a body of previous literature that indicates that a substantial proportion of capillaries in resting skeletal muscle of healthy animals contain stationary RBCs (10, 11, 19, 29). Indeed, the notion that initially non-RBC-flowing capillaries are recruited at exercise onset has been invoked to explain the increase of muscle O₂ diffusion capacity at exercise onset. In marked contrast, in vivo studies using dye injection in the rat at rest have found that essentially all muscle capillaries sustain some plasma flow irrespective of muscle fiber type (24). In healthy spinotrapezius muscles observed at a resting sarcomere length of ~2.7 μm, our laboratory demonstrated continuous RBC flow in 80–96% of visible capillaries (25–28, 39, 40), and this range encompasses the present findings (~90%). However, chronic disease conditions such as heart failure (25, 40) and Type 1 diabetes (28) increase the percentage of non-RBC-flowing capillaries to 30–50%. The present investigation indicates that a sizeable increase (27–34%) in non-RBC-flowing capillaries is also found 1–3 days after a single exhaustive bout of eccentric exercise.

The observation that capillary tube hematocrit was increased in DH-1 but reduced at DH-3 is intriguing. The elegant experiments of Duling and colleagues (e.g., Ref. 50) indicate that the capillary endothelial glyocalyx layer is intrinsic in setting a differential flow of plasma and RBCs in the capillary that lowers tube hematocrit below that present systemically. It is possible, therefore, that muscle damage consequent to eccentric exercise impacted the endothelial glyocalyx layer and thus its effect on capillary hemodynamics. By DH-3 any such effect was gone, and the reduction in capillary tube hematocrit below control values might be expected on the basis of the hypoporemic conditions extant within these capillaries.

Po₂mv. Within the healthy spinotrapezius muscle at the onset of 1-Hz contractions, Po₂mv does not fall immediately but rather evidences a modest delay (i.e., TD) where Po₂mv remains close to baseline before decreasing exponentially to the steady-state contracting value (4). Coupled with capillary hemodynamic measurements across the transition to contractions, it is evident that this behavior reflects an almost instantaneous increase in RBC flux (i.e., QO₂; Ref. 27) that is matched temporally and quantitatively with increases of V₀2 (3). The subsequent fall in Po₂mv results from a relatively greater increase of V₀2 than QO₂. The mean TD and τ for the control muscles in the present investigation (11 and 15 s, respectively) fit closely with values reported previously for the healthy spinotrapezius (i.e., TD, 11–14 s; τ, 16–19 s, Refs. 3, 5, 13).

One particularly striking finding from the present investigation was the accelerated fall (i.e., ~40% faster) in Po₂mv in both 1- and 3-day posteccentric exercise groups (i.e., τ, < 9 s for both, Table 3). In this respect, the sequelae of downhill running resembles the Po₂mv response of rats suffering from moderate chronic heart failure (13) with both conditions being characterized by capillary hemodynamic impairments that include a significant increase in the proportion of capillaries that do not support continuous RBC flow. Therefore, it is logical to consider that the impaired microcirculatory hemodynamics might be responsible for the more rapid fall of Po₂mv found in the present investigation (see Mechanistic insights into myocyte O₂ delivery below).

Mechanistic insights into myocyte O₂ delivery. The elegant modeling of Federspiel and Popel (14) and Groebbe and Thews (20) suggests that the capacity of the capillary bed for blood-myocyte O₂ diffusion is determined principally by the number of RBCs lying adjacent to that myocyte at any given time. Thus, within RBC-flowing capillaries, RBC flux and hematocrit, as well as the available length of capillaries, are important determinants of perfusive and diffusive O₂ conductance. Capillaries that do not flow may contain stationary RBCs. However, with zero perfusive O₂ flux, RBC Po₂ will equilibrate fairly rapidly with intramyocyte Po₂, at which time blood-myocyte O₂ transport will cease and such capillaries will not supply O₂. It is possible but unlikely that these nonflowing capillaries did not contain the R2 phosphorescent probe. What is more likely is that the perfusive and diffusive characteristics of the tissue were impaired, resulting in a mismatching of QO₂ and V₀2 (or at least O₂ demand). Thus, although there was a population of capillaries in which Po₂mv may have reached near equilibration with the low intramyocyte Po₂, it is quite feasible that there may have been other capillaries either with impaired O₂ diffusion characteristics or that abutted myocytes with a low O₂ requirement. This latter effect may account for the absence of lowered Po₂mv at rest or during the contracting steady state in DH-1 and DH-3 rats. As mentioned above, it is pertinent that the spinotrapezius microcirculatory dysfunction found herein after eccentric exercise (i.e., reduced percentage of capillaries not supporting RBC flow, decreased RBC velocity and flux) resembles that observed in experimental heart failure of a moderate severity (25, 40). Moreover, under both of these conditions the Po₂mv profile evidences a faster fall (shorter time constant) (13). At the onset of contractions in the spinotrapezius capillaries of rats in heart failure, this Po₂mv profile was associated with an extremely sluggish increase of
RBC flux and velocity and the absence of RBC flow in those vessels not flowing at rest (40). It is tempting to speculate, therefore, that in the present investigation following eccentric exercise, similar perfusion deficits to those found in heart failure may have produced the altered PmvO₂ profile. The effect of this is to decrease PmvO₂ transiently below values seen in control muscle, which would lower the driving pressure for O₂ diffusion from capillary to myocyte across the critical transition period at the onset of contractions (i.e., 20–40 s, Fig. 4).

In healthy individuals, several lines of evidence (for review, see Ref. 17) indicate that neither pulmonary nor muscle VO₂ kinetics at exercise onset are limited by QO₂ per se. Rather, some intramuscular enzymatic process(es), some of which are modulated by endogenous nitric oxide (21), are thought to set the speed of VO₂ kinetics and therefore determine the size of the O₂ deficit and the resulting intracellular perturbation (e.g., hydrogen ions and phosphocreatine among others) upon initiating muscle contractions. In contrast, within disease states such as severe chronic heart failure, muscle perfusive O₂ conductance may be impaired to an extent that mandates a PmvO₂ fall below levels found in healthy muscle (13). From consideration of Fick’s law of diffusion, a lowered pressure driving O₂ from blood into the myocyte is expected to constrain the diffusive flux of O₂, thereby contributing to a slowing of VO₂ kinetics (6, 41, 45). The present results suggest that one consequence of the microcirculatory dysfunction demonstrated after eccentric exercise may be slowed VO₂ kinetics. In turn, these slowed VO₂ kinetics would be associated with reduced contractile function and impaired exercise tolerance.

Model considerations. Downhill running has been employed effectively as a model for eccentrically induced damage in humans (46) and rats (30). We have recently demonstrated in the rat that downhill running recruits the spinotrapezius, a scapular-stabilizing muscle, as indicated from the threefold increase in blood flow above that found at rest (22). Such eccentric contractions reduce muscle force-producing capacity (9) while disrupting the extracellular matrix (47), elevating proteolytic enzyme activity (44), and causing profound ultrastructural damage to the myocytes (23). In addition, levels of heat shock proteins HSP27 and HSP70 as well as serum creatine kinase may become elevated (46, 49).

The primary focus of the present investigation was to undertake a novel evaluation of the effect of downhill running on the microcirculation and the balance of QO₂/VO₂ during contractions rather than quantify myocyte damage per se. However, intravital light microscopy did reveal the presence of damaged myocytes at 1 and 3 days after downhill running (Fig. 1). Specifically, in contrast to myocytes in control (nonexercised) spinotrapezius muscles, after downhill running a population of fibers evidenced severe sarcomeric disruptions. These disruptions presented as a loss of register of the sarcomeres, and, in the extreme, sarcomeres across the myocyte thickness displayed a smeared appearance that extended for 10–40 μm or more along the length of the myocyte and averaged 32 and 41% of the fiber area at DH-1 and DH-3, respectively. Such damaged fibers were often, but not always, abutted by nonflowing capillaries (Fig. 1). An important question for future investigations is whether there is a direct relationship between nonflowing capillaries and myocyte damage and, if so, the mechanistic basis for such a relationship. With respect to supporting oxidative function during contractions and supplying nutritive flow to resting muscle, the presence of nonflowing capillaries will compromise performance and possibly recovery from injury. However, it is also possible that by reducing capillary flow adjacent to damaged myocytes, the opportunity for generation and/or delivery of reactive O₂ species that may incur further damage is reduced. It will be valuable for future studies to explore the role of reactive O₂ species in microvascular dysfunction after eccentric exercise.

In conclusion, the present investigation has demonstrated the presence of impaired capillary hemodynamics and a compromised QO₂/VO₂ matching 1 and 3 days after novel eccentric exercise. Furthermore, these findings suggest that the spinotrapezius muscle presents a viable model for studying microvascular and myocyte injury consequent to eccentric contractions. Apart from being a highly accepted model for microvascular studies, it is pertinent that the spinotrapezius has a fiber composition (12) and oxidative capacity (32) that resemble closely that of the human quadriceps. Thus elucidation of the mechanisms responsible for eccentric muscle damage in this muscle may be applicable to prevention of postexercise damage and debilitation in humans.

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