HIGHLIGHTED TOPIC | Skeletal and Cardiac Muscle Blood Flow

Downhill running: a model of exercise hyperemia in the rat spinotrapezius muscle

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Kano, Yutaka, Danielle Padilla, K. Sue Hageman, David C. Poole, and Timothy I. Musch. Downhill running: a model of exercise hyperemia in the rat spinotrapezius muscle. J Appl Physiol 97: 1138–1142, 2004. First published May 7, 2004; 10.1152/japplphysiol.00334.2004.—To utilize the rat spinotrapezius muscle as a model to investigate the microcirculatory consequences of exercise training, it is necessary to design an exercise protocol that recruits this muscle. There is evidence that the spinotrapezius is derecruited during standard treadmill exercise protocols performed on the uphill treadmill (i.e., 6° incline). This investigation tested the hypothesis that downhill running would effectively recruit the spinotrapezius muscle as assessed by the presence of an exercise hyperemia response. We used radioactive 15-μm microspheres to determine blood flows in the spinotrapezius and selected hindlimb muscles of female Sprague-Dawley rats at rest and during downhill (i.e., 6° incline; 331 ± 5 g body wt, n = 7) and level (i.e., 0° incline; 320 ± 11 g body wt, n = 5) running at 30 m/min. Both level and downhill exercise increased blood flow to all hindlimb muscles (P < 0.01). However, in marked contrast to the absence of a hyperemic response to level running, blood flow to the spinotrapezius muscle increased from 26 ± 6 ml·min⁻¹·100 g⁻¹ at rest to 69 ± 8 ml·min⁻¹·100 g⁻¹ during downhill running (P < 0.01). These findings indicate that downhill running represents an exercise paradigm that recruits the spinotrapezius muscle and thereby constitutes a tenable physiological model for investigating the adaptations induced by exercise training (i.e., the mechanisms of altered microcirculatory control by transmission light microscopy).

Within the past two years, it has been possible to measure spinotrapezius capillary hemodynamics during muscle contractions (17, 33). This raises the exciting possibility that the spinotrapezius can be utilized to understand the effects of physiological adaptations, for example, to exercise training on capillary hemodynamics and O₂ exchange in contracting muscle. Unfortunately, conventional treadmill running of rats on the slightly inclined treadmill does not appear to recruit this muscle. Specifically, such running paradigms have been shown to reduce spinotrapezius muscle blood flow below resting values (26). Thus any vascular adaptations to that exercise (21) are not likely to result from augmented muscle metabolism or exercise hyperemia per se.

Downhill running forces eccentric muscle activity and substantially alters muscle recruitment profiles (9). Because one primary role of the spinotrapezius is to stabilize the scapula, we hypothesized that running on the declined treadmill would produce a hyperemic response in this muscle. Our findings of nearly a threefold increase in spinotrapezius blood flow above resting indicate that this muscle is recruited for this type of activity. We believe that this provides a unique and valuable muscle intravitral microscopy model for investigating physiological adaptations to exercise training in health and disease.

METHODS

Animal selection and care. Twelve female Sprague-Dawley rats (average body wt of 326 ± 5 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 Kansas State University’s Institutional Animal Care and Use Committee.

All rats were familiarized with running on a motor-driven treadmill. During the period of familiarization (2–3 wk), rats exercised for 5–10 min/day at a speed of 20–30 m/min and 0% grade.

Surgical procedures and experimental protocol. After it was established that all rats were proficient runners, each animal was anesthetized with 5% isoflurane. Rats were maintained on a 2% isoflurane-oxygen mixture, with one catheter (PE-10 connected to PE-50) placed in the ascending aorta via the right carotid artery and another in the caudal (tail) artery, as previously described (27). Both catheters were tunneled subcutaneously to the dorsal aspect of the cervical region and exteriorized through a puncture wound in the skin. After incisions were closed, anesthesia was terminated and the animal was given ~1–2 h to recover. This period of recovery was selected because previous studies by Flaim et al. (11) showed that cardiac or

DIRECT OBSERVATION OF SKELETAL muscle microcirculation by intravitral microscopy is key to understanding microvascular control, capillary hemodynamics, and O₂ exchange. Unfortunately, there are very few muscles anatomically and optically suitable for transmission light microscopy. Of these, the rat spinotrapezius muscle possesses the following singular advantages: 1) it can be exteriorized and transilluminated without disruption of the nervous or primary vascular supplies (2, 12, 28, 40); 2) it comprises a mosaic of the three principal fiber types found in mammalian muscle (8); and 3) the oxidative capacity approximates that found in the untrained human quadriceps (8, 22). Consequently, it is not surprising that intravitral microscopy of the spinotrapezius has been integral to our understanding of muscle microcirculation in health (4, 21, 23, 25, 31, 37, 39) and chronic diseases such as heart failure (15), Type 1 diabetes (18), and hypertension (13).
circulatory dynamics, regional blood flow, arterial blood gases, and acid-base status are stable in the awake, unrestrained rat 1–6 h after halothane anesthesia.

After this recovery period, the final experimental protocol was initiated. Each rat was placed on the treadmill, and, after a period of stable heart rate (HR) and arterial blood pressure (~15 min), the tail artery catheter was connected to a 1-ml plastic syringe that was connected to a Harvard infusion/withdrawal pump (model 907). Exercise was initiated, and the speed of the treadmill was increased progressively during the next 30 s to a speed of 30 m/min. Rats were separated at random into two groups; one that ran on a flat surface and one that ran down a ~14° incline. All rats were required to exercise steadily for another 9 min. After 9.5 min of total exercise time, blood withdrawal from the tail artery catheter was initiated at a rate of 0.25 ml/min. Simultaneously, HR and arterial blood pressure were measured via the carotid artery catheter. After 10 min of total exercise time, the carotid artery catheter was disconnected from the pressure transducer and 0.5–0.6 × 10^5 radioactively labeled microspheres (15 μm diameter; Perkin-Elmer Life and Analytical Sciences, Boston, MA) were injected into the aortic arch to determine blood flow to exercising muscle (46 Sc, 85 Sr, 141 Ce, in random order). Approximately 30 s after the injection, blood withdrawal from the tail artery catheter was stopped, and exercise was terminated. Subsequent blood flow determinations were performed at 60 min after termination of exercise, as rats sat quietly on the treadmill belt. This experimental strategy of measuring blood flow at rest after exercise minimizes the potential for blood loss to affect the exercise response and facilitates resting measurements that do not reflect the preexercise anticipatory response in rats (1).

After the second (60 min) resting blood flow determination, each animal was given an overdose of pentobarbital sodium (>50 mg/kg). The thorax was opened, and placement of the carotid artery catheter into the aortic arch was confirmed by anatomic dissection. The right and left kidney, right and left spinotrapezius muscle, and selected right hindlimb muscles [soleus, plantaris, gastrocnemius, tibialis anterior, and extensor digitorum longus (EDL)] were identified, removed, weighed, and placed immediately into counting vials. The radioactivity of each tissue was determined on a gamma scintillation counter (Packard Auto Gamma spectrometer, model 5230, Downers Grove, IL). By taking into account the cross-talk fraction between isotopes, we determined blood flows to each tissue using the reference sample method (26). Blood flows to the left and right spinotrapezius muscle were averaged, and adequate mixing of the microspheres was verified for each injection by demonstrating a <15% difference between blood flows to the right and left kidneys.

Statistics. Values are expressed as means ± SE. A two-way ANOVA with a repeated measures design was used in combination with a Scheffé’s post hoc test to evaluate significant main and interaction effects. Statistical significance was established at P ≤ 0.05.

RESULTS

Body weight was not significantly different between level and downhill exercise rats. HR during exercise was significantly greater than that measured at rest for both the level running and downhill running groups of rats (Table 1). However, no differences in HR were found between the groups. Mean arterial pressure at rest was similar between the level and downhill exercise groups of rats. Moreover, mean arterial pressure was not significantly different from rest in either group.

Adequate mixing of the microspheres in each animal under both conditions of exercise and at rest was demonstrated when differences in blood flow to the right and left kidney averaged 8.1 ± 1.1% (i.e., blood flow at rest for rats that ran on the level surface = 636 ± 72 ml·min⁻¹·100 g⁻¹ for right kidney vs. = 603 ± 57 ml·min⁻¹·100 g⁻¹ for left kidney). Therefore, none of the rats that participated in the experiments was excluded from the study.

During the resting condition, blood flows to the spinotrapezius and selected hindlimb muscles were not different between the level and downhill exercise groups (Table 2 and Fig. 1). Muscle blood flow to hindlimb muscles increased significantly from rest to exercise (P < 0.001). Moreover, the increase in blood flow to the EDL was significantly greater during downhill running compared with the level condition. Although blood flow to the spinotrapezius muscle did not increase during level running, it increased significantly during downhill running (~266% of resting value; P < 0.001, Fig. 1).

DISCUSSION

The most important original observation from this investigation is that downhill running induces a significant exercise hyperemia in the spinotrapezius muscle. This finding provides a physiological training paradigm for resolving the mechanistic bases for important microcirculatory functional adaptations to acute and chronically increased metabolic demands in a popular and highly relevant intravital model for the study of skeletal muscle.

Absolute increase of muscle blood flow. The magnitude of the increase in spinotrapezius blood flow to 69 ml·min⁻¹·100 g⁻¹ during downhill exercise was certainly modest compared with that found in some of the most oxidative rat skeletal muscles during high-speed uphill running (5% grade, 96 ± 5 m/min; blood flow = 536 ± 18 and 680 ± 44 ml·min⁻¹·100 g⁻¹ in the red portion of the vastus lateralis and the vastus intermedius, respectively; Ref. 29). However, the oxidative capacity of the spinotrapezius is a modest 14.0–15.2 μm·g⁻¹·min⁻¹, which is only approximately one-third of that of the highly oxidative limb muscles (3, 8). From the relationship between citrate synthase activity and exercising flows shown in Ref. 29, the peak spinotrapezius blood flow was estimated as ~174–194 ml·min⁻¹·100 g⁻¹. Thus the blood flow measured during downhill running herein is ~36–40% of peak. For future investigations that might employ this modality to explore the effects of training on the microcirculation, it is pertinent to ask whether this hyperemic response is sufficient to promote structural and functional adaptations. Two compelling arguments suggest that it is. 1) Chronic prazosin (α₁-antagonist) treatment, which doubles capillary red blood cell velocity, induces a profound capillary neogenesis in rat skeletal muscle (7). 2) Blood flow at maximal oxygen consumption during conventional cycle ergometry (cardiac output limitation) is only ~150 ml·min⁻¹·100 g⁻¹, i.e., approximately one-third

<table>
<thead>
<tr>
<th>HR, beats/min</th>
<th>Incline</th>
<th>Rest</th>
<th>Exercise</th>
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<tbody>
<tr>
<td>L</td>
<td>382±14</td>
<td>530±8*</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>413±13</td>
<td>534±10*</td>
<td></td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>133±8</td>
<td>137±5</td>
<td></td>
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<tr>
<td>D</td>
<td>127±3</td>
<td>131±4</td>
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Values are means ± SE. L, level exercise; D, downhill exercise; HR, heart rate; MAP, mean arterial pressure. *Mean different from rest (P < 0.01).
the peak capacity found in humans (19, 32). These observations indicate that extensive training-induced vascular and muscle adaptations (35) are incurred by exercise intensities and modalities that recruit only a modest portion of maximal muscle blood flow (10). Hence, although other downhill running speeds and inclines may potentially elevate spinotrapezius blood flow to a greater extent, the paradigm used herein (30 m/min, ~14°) would be expected to promote training adaptations in response to repeated exercise bouts.

Our laboratory (26) has previously documented that blood flow to the spinotrapezius muscle is reduced from resting values when rats perform treadmill exercise on a level or upward incline. Moreover, these reductions in blood flow are substantial (i.e., ~30–40%) and consistent with the idea that the spinotrapezius muscle is not recruited during level or uphill running (26). In that previous investigation, resting blood flow measurements were performed ~60 min after the first exercise bout (and just before a second exercise bout) in our attempt to minimize any preexercise anticipatory response to exercise. This preexercise anticipatory response to exercise has been shown by Armstrong et al. (1) to produce significant increases in HR and skeletal muscle blood flow in the rat. Therefore, the issue of what may be construed as “true” resting muscle blood flow in the rat remains unclear and potentially controversial.

In the present investigation, a similar strategy was employed in an attempt to minimize any preexercise anticipatory response in the animal. Thus resting hemodynamic and blood flow measurements were performed ~60 min after the exercise bout. Interestingly, we found that blood flow to the spinotrapezius muscle averaged 30 ± 4 ml·min\(^{-1}\)·100 g\(^{-1}\) in the present study, whereas blood flow to the spinotrapezius muscle was 60 ± 6 ml·min\(^{-1}\)·100 g\(^{-1}\) in the previous investigation (26). What may account for these differences in resting blood flow remains unclear at this time, but a number of factors that could be contributing are worthy of attention. First, the resting blood flow measurements made in our previous study (26) were performed just before a second exercise bout. Thus, for whatever reason, these animals may have experienced a preexercise anticipatory response to this second bout of exercise (i.e., resting HR = 455 ± 12 beats/min). This did not appear to be a potentially confounding variable (i.e., resting HR = 400 ± 10 beats/min) in the present study. Second, significant differences existed between the two studies regarding the ages (2–3 mo in the present study vs. 5–6 mo in the previous study), weight (326 ± 5 g in the present study vs. 365 ± 10 g in the previous study), and strain (Sprague-Dawley in the present study vs. Wistar in the previous study) of the rats along with significant differences in exercise protocols (downhill running for 10 min vs. uphill or level running for 5 min) used in each investigation, respectively. Therefore, it would be difficult to equate the conditions of rest found between the two studies, thus also demonstrating the potential problem of comparing resting hemodynamic and blood flows across different studies. However, we believe that these differences in resting spinotrapezius muscle blood flow found between the studies do not detract from the results found under the tightly controlled experimental conditions described for each individual investigation.

**limitations associated with exercise-trained muscles for intravital microscopy.** Muscles with optical characteristics suitable for intravital microscopy have typically been difficult to recruit or possess characteristics that limit the relevance of the observed response to the human population. This is shown, for example, by 1) the swimming protocols used to investigate the cremaster muscle’s response to training (43). In addition, 2) conventional treadmill running on a level or inclined treadmill does not recruit and therefore “train” the spinotrapezius (20, 26). 3) The diaphragm is certainly recruited and trained by treadmill running (30). Unfortunately, diaphragm intravital microscopy is very challenging (16) and has not been attempted during contractions. 4) The EDL muscle preparation developed by Tyml and Budreau (42) could be utilized for training studies. Unfortunately, only the surface vessels can be visualized, and the muscle is composed almost exclusively of fast-twitch muscle fibers (~96% fast twitch; Ref. 8). In contrast, the rat spinotrapezius muscle is 41% type I and 59% type II fibers, approximating that of the human quadriceps (35, 44),

Table 2. Muscle blood flows measured at rest and during level or downhill exercise

<table>
<thead>
<tr>
<th>Muscle Blood Flow, ml · min(^{-1}) · 100 g(^{-1})</th>
<th>Incline</th>
<th>Rest</th>
<th>Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ankle extensors</strong></td>
<td></td>
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<td></td>
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<tr>
<td>Soleus</td>
<td>L</td>
<td>102±16</td>
<td>275±27†</td>
</tr>
<tr>
<td>D</td>
<td>95±14</td>
<td>296±25†</td>
<td></td>
</tr>
<tr>
<td>Plantaris</td>
<td>L</td>
<td>18±6</td>
<td>177±25†</td>
</tr>
<tr>
<td>D</td>
<td>13±2</td>
<td>215±18†</td>
<td></td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>Red</td>
<td>42±10</td>
<td>411±58†</td>
</tr>
<tr>
<td>D</td>
<td>36±7</td>
<td>410±24†</td>
<td></td>
</tr>
<tr>
<td>Middle</td>
<td>L</td>
<td>16±4</td>
<td>146±34†</td>
</tr>
<tr>
<td>D</td>
<td>11±1</td>
<td>126±9†</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>L</td>
<td>11±2</td>
<td>46±12†</td>
</tr>
<tr>
<td>D</td>
<td>11±1</td>
<td>41±6†</td>
<td></td>
</tr>
<tr>
<td><strong>Ankle flexors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tibialis</td>
<td>Red</td>
<td>37±21</td>
<td>370±51†</td>
</tr>
<tr>
<td>D</td>
<td>21±6</td>
<td>465±69†</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>L</td>
<td>20±5</td>
<td>110±23†</td>
</tr>
<tr>
<td>D</td>
<td>15±2</td>
<td>164±30†</td>
<td></td>
</tr>
<tr>
<td>Extensor digitorum longus</td>
<td>L</td>
<td>14±3</td>
<td>74±7†‡</td>
</tr>
<tr>
<td>D</td>
<td>11±2</td>
<td>115±8†</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significantly different from downhill (i.e., interaction, P < 0.01). †Mean different from rest (P < 0.01).

![Fig. 1. Blood flow in the spinotrapezius muscle at rest and during level (0°) and downhill (~14°) treadmill exercise. *P < 0.001 vs. rest. †P < 0.01 vs. level running.](image-url)
as does the oxidative potential [compare Delp and Duan (8) with Leek et al. (22)].

Special considerations for downhill running. The results of this investigation are consistent with those of Delp et al. (9) in that downhill running will produce significant increases in blood flow to the hindlimb muscles of the exercising rat. Moreover, our results are similar to those of Delp et al. in that downhill running produces greater increases in blood flow to the ankle flexor muscles (i.e., EDL) compared with level exercise, although the magnitude of the response found in the present investigation was greater than that reported previously (9). This difference in the magnitude of the blood flow response may be ascribed to both the faster treadmill speed and the longer exercise duration used in the present investigation. However, it does not negate the similarity in skeletal muscle blood flow pattern found between the present investigation and that of Delp et al., and it supports the previous observation that blood flows may be equivalent in several ankle extensor muscles and higher in ankle flexor muscles during eccentrically biased (i.e., downhill) compared with level running (9).

One potential problem with downhill running is that it forces eccentric contractions that can damage skeletal muscle. Specifically, after a single bout of eccentric exercise, there is ultrastructural damage within the myocytes (14), the extracellular matrix is disrupted (38), and proteolytic activity is increased (34). These alterations are accompanied by elevated heat shock proteins (HSP27 and HSP70; Ref. 41) and serum creatine kinase activity as well as reduced muscle force production (6). Thus it may be argued that such exercise would not produce so-called “normal” adaptations to exercise training. What is remarkable and as yet not fully understood is that as little as one single bout of prior eccentric exercise induces muscle adaptations that greatly reduce muscle damage after subsequent bouts (6, 36, 41). Thus, for the purposes of exercise training rats using downhill running, after any initial damage is resolved, it is reasonable to presume that muscles should undergo conventional training adaptations. Whether this is so remains to be demonstrated. It is also pertinent that downhill running may constitute an important exercise modality for persons undergoing rehabilitation of chronic disease (heart failure, emphysema) and elderly populations. Specifically, eccentric exercise elicits powerful muscle contractions at relatively low cardiorespiratory stress compared with concentric exercise (24). Thus it may prove an important paradigm to retain or restore muscle mass in the legs. However, the effect of downhill exercise or exercise training on microvascular flow and oxygen exchange has not been determined.

Conclusions. In humans, skeletal muscle constitutes 30–40% of body mass and is the largest recruitable vascular compartment. Improved vascular function within skeletal muscle (for example, via exercise training) can improve the prognosis and outcome for patients suffering from chronic diseases such as heart failure, Type 1 and Type 2 diabetes, hypertension, hypercholesterolemia, and emphysema (reviewed in Ref. 5). To understand the mechanisms by which exercise and exercise training improve muscle vascular function as well as oxygen and substrate exchange, it is crucial to have a viable model to study muscle in which intravitral microscopy could be used. The present findings suggest that downhill running effectively recruits the spinotrapezius muscle, which is an excellent model for intravitral observation of the microcirculation at rest and during contractions.

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