Respiratory muscle blood flows during physiological and chemical hyperpnea in the rat

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Departments of Kinesiology and Anatomy and Physiology, Kansas State University, Manhattan, Kansas 66506–5602; and Department of Physiology, Kirksville College of Osteopathic Medicine, Kirksville, Missouri 63501

Poole, David C., William L. Sexton, Bradley J. Behnke, Christine S. Ferguson, K. Sue Hageman, and Timothy I. Musch. Respiratory muscle blood flows during physiological and chemical hyperpnea in the rat. J. Appl. Physiol. 88: 186–194, 2000.—Whether the diaphragm retains a vasodilator reserve at maximal exercise is controversial. To address this issue, we measured respiratory and hindlimb muscle blood flows and vascular conductances using radiolabeled microspheres in rats running at their maximal attainable treadmill speed (96 ± 5 m/min; range 71–116 m/min) and at rest while breathing either room air or 10% O2–8% CO2 (balance N2). All hindlimb and respiratory muscle blood flows measured increased during exercise (P < 0.01), whereas increases in blood flow while breathing 10% O2–8% CO2 were restricted to the diaphragm only. During exercise, muscle blood flow increased up to 18-fold above rest values, with the greatest mass specific flows (in ml·min⁻¹·100 g⁻¹) found in the vastus intermedius (680 ± 44), red vastus lateralis (536 ± 18), red gastrocnemius (565 ± 47), and red tibialis anterior (602 ± 44). During exercise, blood flow was higher (P < 0.01) in the costal diaphragm (395 ± 31 ml·min⁻¹·100 g⁻¹) than in the crural diaphragm (286 ± 17 ml·min⁻¹·100 g⁻¹). During hypoxia+hypercapnia, blood flows in both the costal and crural diaphragms (550 ± 70 and 423 ± 53 ml·min⁻¹·100 g⁻¹, respectively) were elevated (P < 0.05) above those found during maximal exercise. These data demonstrate that there is a substantial functional vasodilator reserve in the rat diaphragm at maximal exercise and that hypoxia + hypercapnia-induced hyperpnea is necessary to elevate diaphragm blood flow to a level commensurate with its high oxidative capacity.

costal diaphragm; crural diaphragm; vasodilator reserve; maximal exercise; hypoxia; hypercapnia

THE MAMMALIAN DIAPHRAGM IS generally regarded as the principal muscle of inspiration. The diaphragm's structural and functional capacities for oxygen delivery (i.e., flow capacity), exchange (i.e., capillary bed), and utilization (i.e., mitochondrial volume, oxidative enzyme capacity) greatly exceed those of most skeletal muscles (6, 8, 10, 15, 22–24, 29, 31). However, our understanding of the importance of the diaphragm to support exercise ventilation and performance is limited. Specifically, measurements of extremely high diaphragm blood flows during exercise in the dog (25) and pony (22–24), as well as the rat (27), suggest an important role for the diaphragm. In contrast, the exercise capacity and maximal oxygen uptake (VO₂max) of rats is unaffected by unilateral diaphragm paralysis and is only reduced ~20% after bilateral diaphragm paralysis (12).

In the resting animal [dog (4), rat (36)] and during moderate-intensity exercise [rat (36)], when diaphragm blood flows are relatively low, a regional heterogeneity of blood flow exists within the costal diaphragm and between the costal and crural diaphragm regions. Also, a markedly higher blood flow has been reported in the costal vs. the crural diaphragm in horses at maximal or near maximal running speeds (24). To date, it is not known whether this regional heterogeneity of blood flow persists in the rat diaphragm at maximal running speeds that are calculated to require an oxygen uptake in excess of the VO₂max. Moreover, at these speeds, the distribution of blood flow among the major respiratory muscles, i.e., diaphragm, intercostals, abdominal muscles, and scalenus muscles, is unknown. Such data would provide an indication of the relative importance of each muscle or group of muscles in supporting the ventilatory effort commensurate with very high exercise-induced gas exchange demands.

The purpose of the present investigation was threefold, specifically in animals running at their maximal attainable speed (i.e., a speed calculated to require ≥150% VO₂max) and during chemically induced hyperpnea (hypoxia+hypercapnia) at rest: 1) to assess the relationship between blood flow (and vascular conductance) in the diaphragm and in the major locomotory skeletal muscles; 2) to determine the distribution of blood flow and vascular conductance among the diaphragm and other respiratory muscles (intercostals, abdominals, scalenus); and 3) to determine the regional distribution of blood flow and vascular conductance within the diaphragm (ventral, medial, and dorsal costal and crural). One point of particular importance is whether the diaphragm's vascular bed is maximally recruited during very intense exercise. Consistent with its high oxidative capacity, diaphragm blood flow would be expected to achieve values similar to those found in the most oxidative skeletal muscles or portions thereof (i.e., red vastus lateralis, vastus intermedius, red gastrocnemius, red tibialis anterior; Ref. 8). However, to

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date, measurements of whole diaphragm blood flow in the rat running at or above speeds yielding \( V_{O2\max} \) are often substantially lower than those reported for the skeletal muscles listed above (2). Pertinent to the issue of whether maximal exercise fully recruits the diaphragm's vascular capacity, vasodilator (adenosine) experiments in the healthy pony (22) and in ponies with laryngeal hemiplegia (23) suggest that there is little or no vasodilator reserve remaining in the diaphragm at maximal exercise. However, similar experiments in the pig with the use of dipyridamole (20) and application of inspiratory resistance in humans (13) indicate that maximal exercise, at least in these species, does not recruit the full vascular capacity of the diaphragm and/or other respiratory muscles.

Based on the above evidence, the following hypotheses were tested: compared with maximal exercise, diaphragm blood flow and vascular conductance are greater during chemical stimulation than exercise. Thus chemical stimulation will unveil the presence of a blood flow and oxygen delivery reserve in this muscle at maximal exercise. If this is demonstrated to be the case, we speculate that, during hypoxic hypercapnic gas breathing, diaphragm blood flow will rise to a level more commensurate with the high oxidative capacity of the diaphragm. Moreover, because pulmonary mechanics and respiratory muscle activation during hyperpnea at rest and exercise hyperpnea may be very different (1, 18), chemically induced ventilatory stimulation is likely to alter the distribution of blood flow within the diaphragm and among the respiratory muscles from that evoked by exercise hyperpnea.

**METHODS**

Female Sprague-Dawley rats were initially familiarized with running on a motor-driven treadmill for a period of 2 wk. During these sessions, the rats exercised between 5 and 10 min/day at an initial speed of 20 m/min and a 5% grade. After acclimatization, each rat was tested for the maximal speed at which it would run freely on the treadmill for 1.5–2.5 min. We defined this speed as “maximal exercise” for each animal, and, in each instance, it exceeded substantially that speed reported to yield \( V_{O2\max} \) in the rat (e.g., Ref. 37). This treadmill speed was recorded for each individual animal and used in the final exercise protocol in which blood flow to the tissues was determined during exercise.

**Instrumentation.** The morning of each experiment, the rat was anesthetized with halothane, and polyethylene catheters (PE-10 connected to PE-50) were placed into the right carotid artery and caudal (tail) artery. The right carotid artery catheter was advanced toward the heart and secured in a position just inside the aortic arch. This was accomplished by advancing the catheter toward the left ventricle while the arterial pressure waveform was being monitored. When the catheter reached the aortic valve, the pressure waveform became distorted. The catheter was retracted 2–3 mm and secured in place. The caudal artery catheter was advanced toward the bifurcation of the descending aorta and secured in place. Both catheters were tunneled subcutaneously to the midscapular region of the rat and exteriorized through a puncture wound made in the skin. After closure of the incisions, the anesthesia was removed and the rats were given a minimum of 2 h to recover. This period of recovery was chosen because previous studies by Flaim et al. (11) have shown that cardiac or circulatory dynamics, regional blood flow, arterial blood gases, and acid-base status resume normal values and remain unchanged during a 1- to 6-h recovery period following halothane anesthesia.

**Exercising measurements.** After the recovery period, the rat was placed into a channel on the treadmill. The right carotid catheter was connected to a pressure transducer, and the caudal artery catheter was connected to a 1-ml glass syringe placed in a Harvard withdrawal pump (model 907, Harvard Apparatus, South Natick, MA). Exercise was initiated with a 3-min warm-up at a speed of 28 m/min up a 5% grade. Treadmill speed was progressively increased over the next 30 s to the previously established maximal speed for that animal. When the designated speed was reached, the rat was required to exercise steadily for another 30 s before blood withdrawal from the tail artery catheter (microspheres reference sample) was initiated at a rate of 0.25 ml/min. At the same time, arterial blood pressure and heart rate (HR) were recorded from the carotid artery catheter. After 30 s of blood withdrawal (4.0 min of total exercise time and \( \geq 1.0 \) min of exercise at the designated maximal speed), the carotid artery catheter was disconnected from the pressure transducer, and 6–7 \( \times \) 105 (0.10 ml) radioactive microspheres were slowly infused into the aortic arch of the running animal. Briefly, the microspheres (146Sc, 88Sr, 113Sn) used in the present study were 15–3 \( \mu \)m in diameter as specified by the manufacturer (New England Nuclear Research Products, E.I. DuPont de Nemours, Boston, MA). These isotopes were infused in random order under the three experimental conditions in which blood flows were measured (i.e., exercise, postrecovery (i.e., rest), and gas breathing). The microspheres were suspended in normal saline containing 0.01% Tween 80 with a specific activity ranging from 7 to 15 mCi/g. Before each infusion, the microspheres were thoroughly mixed and agitated by sonication to prevent clumping. The microspheres were injected into the ascending aorta in a volume of \( \sim 0.10 \) ml over 5–10 s. Blood withdrawal from the caudal artery catheter was maintained for 30 s after the microsphere injection to ensure that all microspheres had been cleared from the withdrawal catheter. In addition, 0.2 ml of arterial blood was taken from the carotid artery catheter after the injection of the microspheres for the determination of arterial blood gases (CIBA- Corning 238 PH/blood gas analyzer, Medfield, MA) and acid-base status, including blood lactate concentration (YSI 2300 glucose and lactate analyzer, Yellow Springs Instruments, Yellow Springs, OH). As rapidly as possible after exercise was terminated, the rectal temperature of the rat was measured with a thermistor (Yellow Springs Instruments) to correct the blood gases and pH for differences in temperature between the animal and measurement electrodes (28). The animal was then allowed a minimum of 60 min to recover.

**Postrecovery measurements.** After the recovery period, the rectal temperature of the animal was measured and recorded. The rat was then placed into a Plexiglas chamber (4.8 liters), and both the carotid and caudal artery catheters were exteriorized through a rubber diaphragm. While the animal sat quietly in the chamber, room air was drawn through the chamber at \( \sim 3 \) l/min for a minimum of 15 min. After demonstration that the animal had reached a reasonable resting steady state as indicated by HR and blood pressure measurements, a second infusion of radioactive microspheres was performed for the determination of tissue blood flows at rest, and another arterial blood sample was taken from the carotid artery catheter for the determination of arterial blood gases, acid-base status, and blood lactate concentration. During these procedures and the hypoxia+hypercapnia, the effluent gas from the chamber was monitored continuously by
Hypoxic–hypercapnic gas-breathing measurements. After the second blood flow determination, the influent gas was switched from room air to a 10% O2–8% CO2 (balance N2) gas mixture. This hypoxic and hypercapnic gas mixture reached equilibration within the chamber in ~1 min and was maintained for an additional ~10 min before a third microspheres infusion and blood sampling procedure was performed. The procedure for the infusion of microspheres was identical to that used under the previous two room-air conditions. The work of Cragg and Drysdale (7) has established that, in the lightly anesthetized rat, this gas mixture evokes a substantial ventilatory response that develops within 2 min and is sustained for at least 10 min after the transition. From the relationships between arterial blood gases and ventilation published by Cragg and Drysdale and the arterial blood gases measured in this investigation (Table 1), a total average minute ventilation of ~390 ml/min is estimated.

For each animal, the ordering of the three conditions was the same (i.e., exercise, rest, hypoxia + hypercapnia). The rationale behind this strategy was to avoid any potential for blood loss to impair exercise performance and tissue blood flows.

Tissue sampling and blood flow determination. Immediately after completion of the gas-breathing measurements, each rat was killed with an overdose of pentobarbital sodium administered via the right carotid artery catheter (>50 mg/kg body wt), and the placement of each catheter was verified by anatomic dissection. Selected organs of the abdominal region and muscles of the right and left hindlimbs of each rat were collected. In addition, the diaphragm, intercostal (between the 4th and 5th ribs), and scalenus muscles were collected. The tissues were blotted, weighed, and placed immediately into counting vials.

Tissue blood flows were determined by using the radionuclide-tagged microspheres technique that has been adapted for use in the exercising rat, as described originally by Armstrong and Laughlin (2) and modified for use in our laboratories (26). The radioactivity of the tissues was determined on a Packard Cobra II Auto-Gamma Spectrometer (Packard Instrument, Downers Grove, IL) set to record the peak energy activity of each isotope for 2 min. The radioactivity of the tissues was then analyzed by computer, taking into account the cross-talk fraction between the different isotopes. Blood flow to selected splanchic organs, kidneys, and muscles was calculated by the reference sample method as described by Ishise et al. (17) and is expressed in milliliters per minute per 100 g of tissue. Adequate mixing of the microspheres was verified for each injection by demonstrating <15% difference in blood flows to the right and left kidney and/or selected right and left hindquarter muscles.

Tissue blood flows were determined for the following abdominal organs: right and left kidney, liver, spleen, and stomach. Blood flow to the diaphragm was measured in both the costal (ventral, medial, and dorsal regions) and crural regions after the removal of the central tendon. Blood flows were also measured for right and left soleus, plantaris, the deep red portion of the lateral head of the gastrocnemius, the superficial white portion of the medial head of the gastrocnemius, the mixed portion (i.e., remainder) of the gastrocnemius, the deep red portion of the tibialis anterior, the superficial white portion of the tibialis anterior, and the extensor digitorum longus muscles. Blood flows were also determined for the internal and external intercostal muscles as a group (between the 4th and 5th ribs), the abdominal muscles as a group, and the scalenus muscles.

Statistical analysis. All statistical analyses were performed by using SigmaStat 2.0 (Jandel Scientific, San Rafael, CA). Data for rest, maximal exercise, and hypoxia + hypercapnia were compared by using a one-way ANOVA for repeated measures. When statistically significant differences were indicated, pairwise comparisons were made by using the Student-Newman-Keuls post hoc test. Correlations between blood flow and citrate synthase data were performed by using general linear regression. The statistical significance of differences among experimental treatments was based on P < 0.05. Data are expressed as means ± SE.

RESULTS

Of the 10 rats that started the conditioning program, one animal died during instrumentation and one animal appeared lethargic after instrumentation and was unable to run faster than 65 m/min compared with a previous best of 103 m/min. Consequently, the results from these animals were discarded, and data for the remaining eight rats are presented. At the end of the conditioning period, the rats weighed 254 ± 6 g, and, after instrumentation, they achieved a running speed of 96 ± 5 m/min (range 71–116 m/min).

Cardiovascular responses. During the maximum speed run, HR increased from 425 ± 18 beats/min at rest to 543 ± 12 beats/min, and mean arterial blood pressure increased from 127 ± 4 mmHg at rest to 145 ± 3 mmHg (both P < 0.05). During gas breathing, HR decreased from its resting value to 353 ± 20 beats/min (P < 0.05), and mean arterial blood pressure increased to 146 ± 4 mmHg (P < 0.05), which was not significantly different from that found during exercise.

Blood-gas and metabolic responses. These responses are presented in Table 1. Briefly, as expected, the run induced a significant arterial acidosis reflecting the elevation of blood lactate. Arterial Pco2 decreased during exercise, indicative of a marked hyperventilation. During hypoxia + hypercapnia, a pronounced arterial hypoxemia and hypercapnia were evident, with pH falling to values similar to those seen during exercise but in the absence of a lactic acidosis.

Hindlimb muscle blood flows. Blood flows to selected hindlimb muscles or muscle regions representative of the spectrum of rat skeletal muscle fiber types are presented in Fig. 1A and Table 2. During exercise, blood flow increased up to 18-fold, with the greatest absolute values found in those red muscles or muscle regions in which highly oxidative fibers predominate (2, 8) (i.e.,
muscle regions composed predominantly of glycolytic fibers (i.e., vastus lateralis, 80 ± 15; rectus femoris, 156 ± 24; gastrocnemius, 108 ± 13; tibialis anterior, 170 ± 25 ml·min⁻¹·100 g⁻¹). Blood flow to the soleus muscle during exercise (342 ± 21 ml·min⁻¹·100 g⁻¹) was intermediate with respect to the values presented above for oxidative and glycolytic muscle.

Breathing the hypoxic+hypercapnic gas did not significantly alter blood flow from the values found at rest in any hindlimb muscle examined (Fig. 1A and Table 2).

Visceral organ blood flows. As expected, blood flow to the kidneys, spleen, and stomach was significantly reduced during exercise when compared with values in the resting animal (Fig. 1B). Hypoxic+hypercapnic gas breathing significantly reduced splenic blood flow from that found at rest; however, it remained higher than that found during exercise. The gas-breathing condition did not significantly alter kidney, liver, or stomach blood flow from values observed at rest.

Respiratory muscle blood flow. Treadmill running elicited significant increases in blood flow in all respiratory muscles examined (Fig. 2). Within these muscles, blood flow to the whole diaphragm (360 ± 26 ml·min⁻¹·100 g⁻¹) was substantially higher than that found in the intercostal (68 ± 6 ml·min⁻¹·100 g⁻¹), scalenus (152 ± 36 ml·min⁻¹·100 g⁻¹), or abdominal (148 ± 21 ml·min⁻¹·100 g⁻¹) muscles. Breathing hypoxic+hypercapnic gas at rest elevated diaphragm blood flow a further 41% (P < 0.05) above that value seen during exercise to 508 ± 62 ml·min⁻¹·100 g⁻¹. However, intercostal, scalenus, and abdominal muscle blood flows breathing 10% O₂-8% CO₂ were not different from rest breathing room air.

Respiratory muscle weights. In the subset of four rats from which each respiratory muscle was fully dissected, the weights of these muscles were as follows (in g): abdominals, 12.50 ± 0.50; intercostals (right and left), 5.87 ± 0.50; and scalenus (right and left), 1.00 ± 0.06.

vastus intermedius, 680 ± 44; vastus lateralis, 536 ± 18; gastrocnemius, 565 ± 47; and tibialis anterior, 602 ± 44 ml·min⁻¹·100 g⁻¹). In contrast, the lowest blood flows were found in those white muscles or muscle regions composed predominantly of glycolytic fibers.

Table 2. Hindlimb skeletal muscle blood flows

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Rest</th>
<th>Maximal Exercise</th>
<th>Hypoxia+Hypercapnia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vastus intermedius</td>
<td>42 ± 11</td>
<td>680 ± 44*</td>
<td>30 ± 9</td>
</tr>
<tr>
<td>Vastus medialis</td>
<td>15 ± 3</td>
<td>286 ± 35*</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>Vastus lateralis, red</td>
<td>42 ± 10</td>
<td>536 ± 18*</td>
<td>19 ± 4</td>
</tr>
<tr>
<td>Vastus lateralis, mixed</td>
<td>21 ± 4</td>
<td>298 ± 17*</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>Vastus lateralis, white</td>
<td>15 ± 2</td>
<td>80 ± 15*</td>
<td>10 ± 1</td>
</tr>
<tr>
<td>Rectus femoris, red</td>
<td>26 ± 10</td>
<td>395 ± 13*</td>
<td>14 ± 5</td>
</tr>
<tr>
<td>Rectus femoris, white</td>
<td>23 ± 3</td>
<td>156 ± 24*</td>
<td>16 ± 2</td>
</tr>
<tr>
<td>Tibialis anterior, red</td>
<td>45 ± 10</td>
<td>602 ± 44*</td>
<td>24 ± 13</td>
</tr>
<tr>
<td>Tibialis anterior, white</td>
<td>23 ± 2</td>
<td>170 ± 25*</td>
<td>19 ± 5</td>
</tr>
</tbody>
</table>

Values are means ± SE in ml·min⁻¹·100 g⁻¹. *Significant difference from both rest and hypoxia + hypercapnia, P ≤ 0.05. There was no difference between rest and hypoxia + hypercapnia.
costal diaphragm was significantly higher than that in hypercapnia, blood flow to each region of the vessels and the respiratory muscles are presented in Tables 3-5. From rest to maximal exercise, P < 0.05. *Significant difference from rest and maximal exercise, P < 0.05.

Regional diaphragm blood flows. At rest, there was no regional heterogeneity of blood flow within the diaphragm (Fig. 3). However, during exercise and hypoxia + hypercapnia, blood flow to each region of the costal diaphragm was significantly higher than that in the crural diaphragm. In comparison with exercise, blood flow during hypoxia + hypercapnia was significantly higher within the medial and dorsal costal regions and the crural diaphragm (Fig. 3).

Vascular conductances. Vascular conductances for the hindlimb muscles, visceral organs, and the respiratory muscles are presented in Tables 3-5. From rest to exercise, hindlimb muscle vascular conductance increased between approximately fivefold (vastus lateralis, white) and 17-fold (vastus medialis), whereas, in response to hypoxia + hypercapnia it was unchanged (Table 3). During exercise, vascular conductance decreased significantly in the kidneys, spleen, and stomach to 36, 3, and 19% respectively, of its value at rest (Table 4). Hypoxia + hypercapnia increased vascular conductance to a significantly greater extent than found during exercise in the whole diaphragm and each diaphragm region examined, with the exception of the ventral costal region (Table 5).

**DISCUSSION**

Despite the presence of exercise-induced blood flows in excess of 500 ml·min⁻¹·100 g⁻¹ in several hindlimb skeletal muscles, diaphragm blood flow (and vascular conductance) remained substantially less than that evoked by breathing a hypoxic + hypercapnic inspirate at rest. Thus these data demonstrate that the rat running at its maximal speed, as defined herein, does not utilize the full blood flow capacity or vascular conductance available within the diaphragm. With both exercise and hypoxia + hypercapnia, blood flow and vascular conductance were higher in the costal than in the crural diaphragm. On the basis of blood flow and vascular conductance, the pattern of respiratory muscle recruitment differed substantially between the exercise and the chemically induced hyperpnea. In addition to increasing diaphragm blood flow, treadmill exercise increased blood flow to the intercostal, scalenus, and abdominal muscles 6- to 10-fold above that found at rest. In marked contrast, the blood flow increase during hypoxia + hypercapnia was restricted...

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**Table 3. Hindlimb skeletal muscle vascular conductances**

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Rest</th>
<th>Maximal Exercise</th>
<th>Hypoxia + Hypercapnia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vastus intermedius</td>
<td>0.323 ± 0.084</td>
<td>4.750 ± 0.377*</td>
<td>0.204 ± 0.059</td>
</tr>
<tr>
<td>Vastus medialis</td>
<td>0.119 ± 0.024</td>
<td>2.001 ± 0.270*</td>
<td>0.069 ± 0.008</td>
</tr>
<tr>
<td>Vastus lateralis, red</td>
<td>0.320 ± 0.066</td>
<td>3.722 ± 0.151*</td>
<td>0.127 ± 0.026</td>
</tr>
<tr>
<td>Vastus lateralis, mixed</td>
<td>0.166 ± 0.025</td>
<td>2.066 ± 0.135*</td>
<td>0.085 ± 0.013</td>
</tr>
<tr>
<td>Vastus lateralis, white</td>
<td>0.119 ± 0.016</td>
<td>0.551 ± 0.103*</td>
<td>0.072 ± 0.009</td>
</tr>
<tr>
<td>Rectus femoris, red</td>
<td>0.220 ± 0.078</td>
<td>2.728 ± 0.058*</td>
<td>0.099 ± 0.036</td>
</tr>
<tr>
<td>Rectus femoris, white</td>
<td>0.187 ± 0.029</td>
<td>1.083 ± 0.172*</td>
<td>0.111 ± 0.015</td>
</tr>
<tr>
<td>Soleus</td>
<td>0.774 ± 0.170</td>
<td>2.383 ± 0.174*</td>
<td>0.552 ± 0.146</td>
</tr>
<tr>
<td>Plantaris</td>
<td>0.157 ± 0.029</td>
<td>2.010 ± 0.168*</td>
<td>0.185 ± 0.078</td>
</tr>
<tr>
<td>Gastronemius, red</td>
<td>0.300 ± 0.044</td>
<td>3.923 ± 0.354*</td>
<td>0.343 ± 0.092</td>
</tr>
<tr>
<td>Gastronemius, mixed</td>
<td>0.174 ± 0.023</td>
<td>1.895 ± 0.130*</td>
<td>0.130 ± 0.028</td>
</tr>
<tr>
<td>Tibialis anterior, red</td>
<td>0.349 ± 0.072</td>
<td>4.199 ± 0.348*</td>
<td>0.165 ± 0.094</td>
</tr>
<tr>
<td>Tibialis anterior, white</td>
<td>0.180 ± 0.013</td>
<td>1.195 ± 0.189*</td>
<td>0.132 ± 0.037</td>
</tr>
<tr>
<td>Extensor digitorum</td>
<td>0.127 ± 0.013</td>
<td>1.628 ± 0.216*</td>
<td>0.120 ± 0.056</td>
</tr>
</tbody>
</table>

Values are means ± SE in ml·min⁻¹·mmHg⁻¹·100 g⁻¹. *Significant difference from both rest and hypoxia + hypercapnia, P < 0.05. There was no difference between rest and hypoxia + hypercapnia.

**Table 4. Visceral organ vascular conductances**

<table>
<thead>
<tr>
<th>Organ</th>
<th>Rest</th>
<th>Maximal Exercise</th>
<th>Hypoxia + Hypercapnia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidneys</td>
<td>4.645 ± 0.459</td>
<td>1.661 ± 0.562*</td>
<td>3.049 ± 0.315†</td>
</tr>
<tr>
<td>Liver</td>
<td>0.306 ± 0.124</td>
<td>0.118 ± 0.031</td>
<td>0.211 ± 0.064</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.904 ± 0.621</td>
<td>0.091 ± 0.049*</td>
<td>0.934 ± 0.079†</td>
</tr>
<tr>
<td>Stomach</td>
<td>1.313 ± 0.128</td>
<td>0.246 ± 0.046*</td>
<td>1.106 ± 0.166</td>
</tr>
</tbody>
</table>

Values are means ± SE in ml·min⁻¹·mmHg⁻¹·100 g⁻¹. *Significant difference from rest, P ≤ 0.05. †Significant difference from maximal exercise, P ≤ 0.05.
to the diaphragm, whereas flow to the other respiratory muscles was not different from that observed at rest.

Cardiovascular responses to hypoxia + hypercapnia. The cardiovascular responses to hypoxia + hypercapnia are substantially different from those to hypoxia alone. Specifically, Hirakawa et al. (14) have demonstrated that inspired hypoxia + hypercapnia (10% + 7%) evoked an elevated mean arterial pressure concomitant with a bradycardia of similar magnitude to that reported herein. That investigation demonstrated that hypoxia + hypercapnia caused sympathetic and parasympathetic activation mediated via peripheral chemoreceptor stimulation, which shifted the mean arterial pressure-renal sympathetic nerve activity relationship toward the right and increased the maximum gain of the baroreflex response.

Diaphragm vasodilator reserve at maximal exercise. The literature is equivocal regarding whether the diaphragm retains a vasodilator reserve at maximal exercise. For example, Manohar (22, 23) failed to elicit an increase in diaphragm blood flow after adenosine infusion into the pulmonary artery of ponies during maximal treadmill running. However, mean aortic pressure was reduced significantly by this procedure, whereas diaphragm blood flow was not. This suggests that adenosine may have elevated vascular conductance within the diaphragm. No isopressure control was performed in those studies, which raises the possibility that there may have been some vascular effect of adenosine that was masked by the systemic hypotension. Supinski and colleagues (40) addressed this issue using nitroprusside infusions during fatiguing electrical stimulation of diaphragm strips in the anesthetized dog. Nitroprusside did not elevate diaphragm blood flow significantly even when phrenic arterial pressure was constrained so that it did not fall below control values. It is pertinent that electrical stimulation typically induces muscle blood flows and vascular conductances that are well below those obtained during spontaneous diaphragmatic contractions in the dog (cf. Ref. 31).

In marked contrast to the above-mentioned study, the following studies support the presence of a substantial vasodilator reserve in the diaphragm at maximal exercise. Laughlin et al. (20) demonstrated a 49% increase in diaphragm vascular conductance after dipyridamole infusion in miniature swine running at \( V_{\text{O}}_{2\max} \). In humans exercising at \( V_{\text{O}}_{2\max} \), Harms et al. (13) demonstrated that application of an inspiratory resistance attenuated the increase in blood flow to the working limbs, suggesting an elevated conductance within the inspiratory muscles. However, it was not possible to apportion this increased conductance and blood flow among the different inspiratory muscles. Moreover, Reid and Johnson (31) were required to apply additional stressors in combination with respiratory muscle loading (i.e., hypoxia or a vasodilator cocktail) to induce what they considered to be a maximally vasodilated condition within the diaphragm of the anesthetized dog.

The present investigation demonstrates clearly the presence of a vasodilator reserve in the diaphragm of the rat at maximal running speeds. Figure 4 illustrates that, for the hindlimb muscles sampled in this investigation, oxidative capacity (citrate synthase activity) is correlated highly with blood flow at maximal exercise. However, the diaphragm blood flow at maximal exercise falls well below the 95% confidence interval of this relationship and considerably below that of other muscles that possess a similarly high oxidative capacity. It was only during hypoxia + hypercapnia, when diaphragm blood flow is elevated further (i.e., from 360 to 508 ml·min\(^{-1}\)·100 g\(^{-1}\)), that the diaphragm blood flow achieved a level consistent with its oxidative capacity. Whereas the present investigation was not designed to determine the maximal blood flow or vascular conductance of which the rat diaphragm is capable, it is notable that hypoxia + hypercapnia increased diaphragm vascular conductance far in excess of that reported in other species [e.g., swine (20), dog (31), and pony (22–24)] during spontaneous breathing. In the present investigation, it was not possible to measure diaphragm venous pressures, and thus the conductance values presented ignore changes in venous pressure that might enhance or reduce the pressure differential across the muscle vasculature. Whereas the magnitude of any differences in venous pressure between conditions is expected to be extremely modest,
this remains a source of imprecision in the ability to quantify exactly the effects of exercise and hypoxic/hypercapnic gas breathing on respiratory and hindlimb muscle vascular conductance per se.

Control of diaphragm perfusion. There are several putative explanations for the submaximal diaphragm blood flows and vascular conductances found during maximal exercise. These include the following. 1) Diaphragm energetic demands do not require additional blood flow. 2) There is a diaphragmatic vasoconstriction arising either reflexogenically from stimulation of group III or IV afferent nerve endings or humorally via norepinephrine spillover from the same muscles (34). There is evidence in the canine that the cardiovascular reflex elicited via these thin-fiber afferents (primarily group IV) in response to capsaicin infusion into resting hindlimb muscle (gastrocnemius) might be more powerful than that from the resting diaphragm (16). The mechanistic basis for this effect may relate to a greater density or total number of thin-fiber afferent nerve endings being present in hindlimb muscles vs. the diaphragm, at least in the canine gastrocnemius. Whether this means that, in some instances, blood flow to the diaphragm or other respiratory muscles will be compromised to enhance perfusion of the limb muscles has not been established; in fact, experiments in humans (13) suggest the opposite. Because of the necessity for maintaining arterial blood pressure, exercising locomotor muscles (at least in humans) do not vasodilate maximally during large-muscle-mass exercise (cf. Refs. 19 and 32). Should diaphragmatic vasodilation also be constrained by the same processes, a submaximal (and possibly suboptimal) perfusion would be expected. This mechanism has been proposed as an explanation for diaphragmatic fatigue documented in humans after intense exercise (3) and is consistent with the observation that diaphragm fatigue can be constrained or even reversed by elevating perfusion (39).

3) There may be mechanical factors extraneous to the vasculature that impede diaphragmatic blood flow during exercise (5). It is known that thoracoabdominal pressure swings influence diaphragm blood flow, and it is also possible that the efficacy of the "muscle pump" within the diaphragm may be different during exercise vs. hyperpnea at rest. The present investigation does not permit discrimination among these factors.

Distribution of respiratory muscle blood flow. From rest to exercise, the total blood flow to the diaphragm, abdominal, intercostals, and scalenus muscles increased 7.8-fold from 3.4 to 26.2 ml/min (Table 6). In comparison, hypoxia+hypercapnia increased respiratory muscle blood flow only 1.9-fold to 6.5 ml/min. Thus the spectacular increase in diaphragm blood flow in hypoxia+hypercapnia was not accompanied by elevations in blood flow to the other respiratory muscles examined. As seen in Table 6, the greatest relative increases in blood flow above resting values were seen in the abdominal and scalenus muscles during exercise.

As mentioned in the introduction, in humans it is apparent that pulmonary mechanics and respiratory muscle activation are very different during the hyperpnea of exercise vs. voluntary hyperpnea at rest (1, 18). At a given pulmonary ventilation, the work of breathing for voluntary hyperpnea may increase 15–40% per breath above that required for exercise hyperpnea (18). In the present investigation, it was remarkable that the hypoxia+hypercapnia-induced hyperpnea increased blood flow and vascular conductance in the diaphragm but had no effect on blood flow or vascular conductance in the other inspiratory muscles or in the expiratory muscles examined. The work of Smith et al. (38) demonstrates that, depending on the stimulus, selective canine inspiratory and expiratory muscles may be recruited independently. Moreover, during hypoxia, canine diaphragm electrical activity remains elevated over time, whereas that of the expiratory muscles decreases to or below control values. This is very different from hypocapnia per se, in which augmented inspiratory and expiratory muscle activity is sustained.

Uncoupling of inspiratory and expiratory muscle activity has been attributed to either hypoxic depression of the brain stem neurons or medullary hypocapnia, both of which may depress expiratory neurons (35, review Ref. 33). In the conscious goat, the observation that, during hyperpnea induced by selective carotid body stimulation, pronounced central nervous system hypocapnia does not affect inspiratory or expiratory muscle activity suggests that it is central nervous system hypocapnia rather than hypoxia that inhibits expiratory muscle activity. However, in the present investigation, the lack of increased expiratory and select inspiratory muscle blood flow cannot be attributed to hypocapnia. Whether this reflects a difference between the rat and higher species or some facet of the hypoxic+hypercapnic conditions examined herein remains to be resolved.

Diaphragm blood flow heterogeneity. On the basis of functional (9) and metabolic (30, review Ref. 29) distinctions, the costal and crural diaphragms have been considered as two separate muscles. Specifically, the costal and crural diaphragms can contract independently of one another and exert very different mechanical effects on the ventilatory system (9). Moreover, Powers et al. (29, 30) reported that oxidative capacity is higher in the costal than in the crural diaphragm and

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Rest</th>
<th>Exercise</th>
<th>Hypoxia+Hypercapnia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diaphragm</td>
<td>0.85</td>
<td>2.23</td>
<td>3.18</td>
</tr>
<tr>
<td>Intercostals</td>
<td>0.60</td>
<td>4.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Scalenus</td>
<td>0.16</td>
<td>1.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Abdominals</td>
<td>1.75</td>
<td>18.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Total</td>
<td>3.36</td>
<td>26.23</td>
<td>6.48</td>
</tr>
</tbody>
</table>

Values are in ml/min.
that the costal diaphragm adapts to exercise training by increasing its oxidative capacity, whereas the crural does not. In ponies at near-maximal running speeds, Manohar (24) found that costal diaphragm blood flow was ~60% higher than in the crural diaphragm. In the present investigation, blood flow was 20–30% higher in the costal diaphragm compared with the crural diaphragm under both exercising and hypoxya+ hypercapnia conditions.

We and others have reported previously that, at rest [4, 36] and during moderate-intensity exercise (36), there is a heterogeneity of blood flow within the costal diaphragm. Consistent with the notion that the medial and dorsal costal costal regions contributes proportionally more to the inspiratory effort (i.e., greater absolute shortening) than does the ventral costal region, mass-specific blood flow was higher in the medial and dorsal costal regions. It is pertinent that in many species [dog (41); horse, human, rat (D. C. Poole, R. N. Petrisko, and M. R. Fedde, unpublished observations)] the medial costal region is thicker (i.e., more myofibrils in parallel) than other costal regions, which means that the absolute blood flow of this region will be considerably higher than that of the ventral and dorsal regions. In the present investigation, this blood flow heterogeneity within the costal diaphragm was proportionally less marked than that seen previously (36). One possible explanation might be that, even in the “resting” condition, and certainly during hyperpnea, diaphragm blood flow was higher than that previously measured during moderate exercise (36). As noted by Armstrong and Laughlin (2), the high diaphragm blood flow in the resting animal may reflect an anticipatory respiratory muscle recruitment indicative of the preexercise condition rather than true “rest.”

Hindlimb muscle and visceral organ blood flow.

The blood flow profile within and among the hindlimb muscles and visceral organs at rest and during exercise coheres closely with that demonstrated previously in rats engaged in high-speed running [i.e., ≥60 mm/min (2, 21, 27)]. However, the exercise-induced mass-specific blood flows within the vastus intermedius and the red portions of the vastus lateralis, gastrocnemius, and tibialis anterior are the highest skeletal muscle blood flows of which we are aware. In fact, the vastus intermedius flow of 680 ml·min⁻¹·100 g⁻¹ exceeds that predicted for a theoretical muscle composed solely of fast oxidative glycolytic fibers (2). These remarkable blood flows were likely achieved, in part, because we chose to study young, relatively small rats (body weight 254 ± 6 g) and required them to run for only ~2 min at their top speed. It is pertinent that some of the highest human muscle blood flows reported to date were obtained in the leg extensor muscles by means of a rapid ramping protocol (32).

In conclusion, at running speeds of 96 ± 5 m/min that generated hindlimb skeletal muscle blood flows well in excess of 500 ml·min⁻¹·100 g⁻¹, diaphragm blood flow (360 ± 26 ml·min⁻¹·100 g⁻¹) remained well below that level predicted from its oxidative capacity (citrate synthase activity). Hyperpnea induced by hypoxya+ hypercapnia elevated diaphragm blood flow a further ~40% to 508 ± 62 ml·min⁻¹·100 g⁻¹, thereby revealing that, at maximal exercise, the rat retains a substantial functional vasodilator reserve within the diaphragm. This vasodilator reserve is present in both the costal and crural diaphragm during maximal exercise and may be important to preserve diaphragm function under conditions of extraordinary metabolic demands.

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