Effects of emphysema on diaphragm blood flow during exercise

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Sexton, William L., and David C. Poole. Effects of emphysema on diaphragm blood flow during exercise. J. Appl. Physiol. 84(3): 971–979, 1998.—Chronic hyperinflation of the lung in emphysema displaces the diaphragm caudally, thereby placing it in a mechanically disadvantageous position and contributing to the increased work of breathing. We tested the hypothesis that total and regional diaphragm blood flows are increased in emphysema, presumably reflecting an increased diaphragm energetic demand. Male Syrian Golden hamsters were randomly divided into emphysema (E; intratracheal elastase 25 units/100 g body wt) and control (C; saline) groups, and experiments were performed 16–20 wk later. The regional distribution of blood flow within the diaphragm was determined by using radiolabeled microspheres in hamsters at rest and during treadmill exercise (walking at 20 feet/min, 20% grade). Consistent with pronounced emphysema, lung volume per unit body weight was greater in E hamsters (C, 59.3 ± 1.8; E, 64.5 ± 5.0 ml/kg; P < 0.001) and arterial P\textsubscript{O}\textsubscript{2} (C, 93 ± 3; E, 69 ± 4 Torr; P < 0.001). At rest, total diaphragm blood flow was not different between C and E hamsters (C, 47 ± 4; E, 59 ± 2 Torr; P < 0.001) and during exercise (C, 93 ± 3; E, 69 ± 4 Torr; P < 0.001). At rest, total diaphragm blood flow was greater in E hamsters (C, 58 ± 7; E, 90 ± 14 ml·min\textsuperscript{-1}·100 g\textsuperscript{-1}; P = 0.03), as a consequence of significantly higher blood flows in the medial and ventral costal regions and crural diaphragm. During exercise in both C and E hamsters, blood flows increased most in the dorsal and ventral costal regions and in the crural diaphragm. In both C and E hamsters, blood flow at rest was lower in the ventral costal region of the diaphragm than in the dorsal and medial costal regions and the crural diaphragm. In addition, exercise-induced increases in intercostal (P < 0.005) and abdominal (P < 0.05) muscle blood flows were greater in E hamsters. The finding that diaphragm blood flow was greater in E hamsters during exercise supports the contention that emphysema increases the energetic requirements of the diaphragm.

Regional blood flow; diaphragm energetics; radiolabeled microspheres

To achieve and sustain contractile function, skeletal muscle blood flow must deliver O\textsubscript{2} and energetic substrates at a level necessary to meet metabolic demands. In emphysema, chronic hyperinflation of the lung displaces the diaphragm caudally, where it assumes a mechanically disadvantageous position. This condition is thought to increase the energetic requirements of the diaphragm, which is the primary muscle of inspiration in both humans (6) and animals (35). Diaphragm blood flow is presumed to be increased in emphysema concomitant with these augmented requirements, but, to our knowledge, this has never been determined. Moreover, should blood flow not increase in proportion to the increase in metabolic demand, respiratory muscle fatigue and failure could result.

There are pronounced regional differences in diaphragm thickness, in vivo fiber length, and the degree of shortening within the costal diaphragm of dogs during passive lung inflation, suggesting that the potential to generate force and cause ventral displacement is not uniform throughout the diaphragm (24, 25, 42). Consistent with these observations, Brancatisano et al. (4) found that the intraregional distribution of blood flow within the costal diaphragm of spontaneously breathing, anesthetized dogs is also nonhomogeneous. Specifically, they found that blood flow was greatest in the medial region of the costal diaphragm and lowest in the most dorsal and most ventral regions of the costal diaphragm. Thus those regions with the greatest blood flow corresponded to those regions with the greatest shortening. Recently, it has been demonstrated that the regional distribution of blood flow within the diaphragm of resting and exercising rats is similarly heterogeneous (34). Whether emphysema alters the regional distribution of diaphragm blood flow has not been determined. Such information provides valuable insights into the altered metabolic and mechanical requirements that emphysema imposes on different diaphragm regions.

At a given perfusion pressure, diaphragm blood flow is dependent on a complex interaction between mechanical, humoral, and neural factors that determine the prevailing vascular resistance (Ref. 39; for review, see Ref. 38). Emphysema does not alter mean arterial pressure at rest or during exercise (23). Thus any differences in diaphragm blood flow in emphysema must result from changes in vascular resistance. With respect to mechanical considerations (i.e., the influence of vascular compression on diaphragm blood flow), muscle fiber hypertrophy and increased demand for greater contractile force in emphysema may be of importance. Specifically, acute increases in muscle tension raise intramuscular pressure and may impede blood flow (3, 28), an effect that may be greater in the diaphragm in emphysema (35) and increase further with elevated ventilatory demands. In addition, thicker muscles develop higher intramuscular pressures (28), which will increase the resistance to flow during contraction. Thus emphysema-induced muscle fiber hypertrophy increases diaphragm thickness and may exacerbate contraction-induced increases in vascular resistance. Furthermore, a lower (i.e., more negative) intrapleural pressure will facilitate diaphragm blood flow, whereas increased abdominal pressure will compromise diaphragm...
blood flow (5). In emphysema, intrapleural pressure has been shown to be less negative, and it is likely that the balance of these pressures is altered in emphysema (27) and may influence diaphragm blood flow.

The purpose of this investigation was to determine whether diaphragm blood flow and its regional distribution are altered in pulmonary emphysema. We hypothesized that, as a consequence of increased energetic requirements in the respiratory muscles of emphysematous animals, total diaphragm blood flow and accessory respiratory muscle blood flows at rest and during exercise would be greater. Furthermore, the greatest increase in flow would be found in the medial and dorsal costal regions of the diaphragm, which typically exhibit the highest flows in healthy, conscious animals (34).

METHODS

Experimental Animals

A total of 77 male Syrian Golden hamsters (Sasco, Omaha, NE), initially weighing 120 ± 1 g, were used in these studies. All animals were housed in individual cages (8 × 10 × 10 in.) on wood shavings bedding under controlled temperature (23°C) and light (12:12-h light-dark cycle). They were allowed free access to water and commercial rodent chow.

Induction of Emphysema

Ten days after arrival, hamsters were randomly assigned to either a control (C; n = 30) or emphysema (E; n = 47) group. Emphysema was produced with intratracheal instillation of elastase as described in detail previously (7, 8, 14, 18, 23, 30, 36). Briefly, all animals were anesthetized with halothane (1.8% halothane in 95% O2-5% CO2). After the ventral aspect of the neck was shaved and cleansed with Betadine and alcohol, a small midline incision was made and the trachea was exposed. E hamsters received a single intratracheal instillation of porcine pancreatic elastase in sterile saline (25 units/100 g body wt; E-0127, Sigma Chemical, St. Louis, MO) by using a 27-gauge needle. C hamsters received an equivalent volume (0.4 ml) of sterile saline. After the intratracheal instillation of either elastase or saline, the animals were systematically rotated at 45° head up for 2–3 min to ensure that the instillate was evenly distributed throughout the lungs. The neck incision was closed, and antibiotic ointment was applied. All hamsters were then placed supine at ~45° in a chamber gassed with 1.8% halothane and 95% O2-5% CO2 for 30–40 min to maintain anesthesia and to facilitate effective distribution of the instillate throughout the lungs. Animals were returned to the Animal Care Facility after recovery. They were checked twice daily, and antibiotic ointment was applied to the incision site for the first 5 days postsurgery. Although emphysema in this model is known to be fully manifest within 3 wk after elastase instillation (36), the purpose of this study was to determine the influence of long-term lung hyperinflation on the diaphragm. Thus the experiments were conducted 16–20 wk after elastase treatment to allow adequate time for adaptation within the diaphragm (7–9, 22).

Treadmill Exercise

Ten days before the start of the experiments, all hamsters were familiarized with exercise on a motorized treadmill. Treadmill grade was set at 20%, and speed was increased progressively over the 10-day familiarization period until the animals could perform 10 min of exercise at 20 ft/min. This intensity constituted only a brisk walk and was not overly stressful for C hamsters; however, it represented the upper limit of what the E hamsters would tolerate during the exercise testing. Given that body mass, apparent locomotory coordination, and arterial Pco2 (Pa,CO2) were not altered with emphysema, we believe that this exercise protocol will have induced similar CO2 clearance requirements in C and E hamsters. Thus any differences in respiratory muscle metabolic requirements and blood flow will occur in response to lung pathology (increased ventilation-perfusion mismatch) and altered mechanical efficiency of the respiratory muscles. This rationale is consistent with previous studies involving exercise and emphysema in hamsters (7, 23).

Surgical Procedures

Animals were fasted overnight before the experiments. The morning of the experiment, the animals were anesthetized with halothane (1.8% halothane in 95% O2-5% CO2). Catheters were implanted into the ascending aorta via the right carotid artery and into the left iliac artery via the left femoral artery. The polyvinyl chloride catheters consisted of a smaller catheter for vessel cannulation (0.5 mm OD, 0.2 mm ID; Dural Plastics and Engineering, Auburn, NSW, Australia) melded with cyclohexanol into a larger catheter (1.02 mm OD, 0.64 mm ID; MRE-040, Braintree Scientific, Braintree, MA). Care was taken to place the tip of the carotid artery catheter well into the ascending aorta (5–8 mm) to ensure adequate mixing of the infused microspheres. The tip of the iliac artery catheter was placed to within 3–6 mm of the bifurcation of the abdominal aorta to permit good withdrawal of reference microsphere samples. Both catheters were tunneled subcutaneously to the midscapular region where they exited and were sutured in place. Catheter positions were confirmed after each experiment. Those animals with incorrectly placed catheters were excluded from the analysis, and their data are not presented. Animals typically recovered consciousness and were moving around their cages within ~10 min after removal from anesthesia and were often eating within 15–30 min. The experiments were performed 4–5 h after completion of the surgery.

Experimental Setup

Extension catheters (30 mm of MRE-033; 0.84 mm OD, 0.36 mm ID; Braintree Scientific) connected the aortic catheter to a pressure transducer (model P23DC, Statham, Hato Rey, Puerto Rico) and the iliac artery catheter to a withdrawal pump (model 901, Harvard Apparatus, Millis, MA). Mean arterial pressure and heart rate (cardiotachograph) were monitored from the aortic catheter by using a polygraph (model 7E, Grass Instruments, Quincy, MA).

Blood Flow Measurements

Tissue blood flows were measured by using 15 µm-diameter radiolabeled microspheres (113Sn, 85Sr, or 46Sc NEN Research Products, Boston, MA) as described previously (21, 34). The microspheres were suspended in saline and 0.01% Tween 80 and were sonicated and mixed vigorously with a magnetic stirrer and stir bar contained within each vial of microspheres before use. For each blood flow measurement, the extension of the aortic catheter was clamped and detached from the pressure transducer and attached to a 1-ml syringe containing 0.1 ml of microsphere suspension (~150,000 microspheres). Blood was withdrawn from the aorta into the syringe (~0.2 ml) and mixed with the microspheres. The contents of the syringe (~0.3 ml) were infused into the ascending aorta over ~20–25 s, followed by slow flush with 0.2 ml of warm saline. To obtain the reference microsphere sample (from the iliac artery catheter), the withdrawal pump was started (0.22 ml/min into a 1-ml disposable syringe
containing 0.2 ml saline to eliminate any air space) ~10 s before the infusion of microspheres and was continued for 60 s after completion of microsphere infusion. In preliminary trials, we determined that this withdrawal time was sufficient to clear all microspheres from the reference catheter. Total withdrawal lasted ~90–120 s (total withdrawal volume of 0.33–0.44 ml). The entire reference syringe and its contents were placed into a counting tube. Infusion of microspheres and saline flush, combined with withdrawal of the reference microsphere sample, typically had no influence on either arterial pressure or heart rate.

**Experimental Protocol**

After connection of the animal’s aortic and iliac artery catheters to the pressure transducer and withdrawal pump, respectively, the hamster was placed into a Plexiglas treadmill lane that was covered with a towel to minimize visual stimuli. Each animal was left undisturbed on the treadmill for 10–20 min until heart rate and blood pressure had decreased to a stable level, indicative of a quiet, resting state. Microspheres were infused for determination of blood flow in the resting state. An arterial blood sample (0.2 ml) was taken from the aorta for measurement of blood gases (model 238, CIBA/Corning). The treadmill was started and speed was increased over the first 30 s to 20 feet/min with treadmill grade set at 20%. After 4 min of walking exercise and achievement of stable mean arterial pressure and heart rate, a second microsphere infusion was performed to measure exercise blood flows and an arterial sample (0.2 ml) was taken for determination of exercise blood gases. The treadmill was stopped immediately after the exercise measurements. Several minutes later, the animal received an intra-abdominal bolus (0.3 ml) of pentobarbital sodium (50 mg/ml; Abbott Laboratories, North Chicago, IL). Once deeply anesthetized, the animals were killed with an intra-arterial bolus (0.5 ml) of pentobarbital sodium (50 mg/ml; Abbott Laboratories). The diaphragm was carefully removed from the costal margin by using a 60-ml syringe connected in parallel with a Validyne 35-cmH2O pressure transducer (Validyne, Northridge, CA). After several repeated inflation-deflation cycles, three to five measurements were made from RV to TLC. If the sequential measurements agreed within 0.25 ml, the average of these measurements was taken as VC. The lungs were then carefully excised, and the trachea was tied off (0 cmH2O airway pressure). Lung volume was determined as the volume of saline displaced by complete immersion of the lungs in saline as described previously (30).

**Respiratory System Vital Capacity (VC) and Lung Volume**

The severity of the emphysematous state was determined at the end of each experiment. With the trachea cannulated, passive VC was defined as the change in lung volume from an airway pressure of ~30 to ~30 cmH₂O measured in vivo as described previously (7, 30). In rodents, these airway pressures define residual volume (RV) and total lung capacity (TLC), respectively (19). Briefly, the change in respiratory system volume (in ml) between RV and TLC was determined by using a 60-ml syringe connected in parallel with a Validyne MP 45-26 cmH₂O pressure transducer (Validyne, Northridge, CA). After several repeated inflation-deflation cycles, three to five measurements were made from RV to TLC. If the sequential measurements agreed within 0.25 ml, the average of these measurements was taken as VC. The lungs were then carefully excised, and the trachea was tied off (0 cmH₂O airway pressure). Lung volume was determined as the volume of saline displaced by complete immersion of the lungs in saline as described previously (30).

**Tissue Samples**

Tissue samples collected after the animals were killed for determination of blood flow included the following: diaphragm, intercostal muscle samples (~0.2 g, internal and external together), abdominal muscle (~0.6-g sample of abdominal wall excluding rectus abdominus), skeletal muscles from the right hindlimb (soleus, plantaris, gastrocnemius, and quadriceps), left and right kidneys, spleen, and stomach. The diaphragm was carefully removed from the costal margin and the crural origin and was divided into costal and crural portions. The costal diaphragm was further divided into dorsal, medial, and ventral regions (34) as shown in Fig. 1.
Student-Newman-Keuls post hoc test. Correlations among data were performed by using general linear regression methods and Pearson product moment. The statistical significance of differences within and among experimental groups was based on \( P \leq 0.05 \).

RESULTS

Experimental Animals

Of the 77 animals used in this study, 1 C and 11 E hamsters died before the start of experiments, most within the first 7 days after elastase instillation, for a mortality rate of 3% in C (1 of 30) and 23% in E hamsters (11 of 47). We determined a priori to include only animals in which both the resting and exercise blood flow data were obtained. Of the remaining sixty-five animals, acceptable data were obtained from 17 C and 16 E hamsters. Reasons for exclusion of animals from the final data set include bad catheter placement and/or inadequate microsphere distribution (5 C and 8 E hamsters), lack of or poor recovery from surgery (2 C and 6 E hamsters), refusal to walk on the treadmill after surgery (1 C and 6 E hamsters), no withdrawal from either the aortic or the femoral artery catheters (1 C and 2 E hamsters), and trial experiments (3 C and 1 E hamsters). Analysis was performed by using data from 17 C and 16 E hamsters.

Body weights of C and E hamsters were not different (Table 1). There were no differences in total diaphragm weight (C hamsters, 562 ± 25 mg; E hamsters, 567 ± 22 mg), or in regional diaphragm weights (data not shown) between C and E hamsters.

Passive VC in E hamsters was 40% greater than in C, and the passive VC-to-body weight ratio was 34% greater in E hamsters (Table 1). Passive vital capacity was positively correlated with body weight in C hamsters \( (n = 17; \ y = 4.31 + 0.0303x; \ r = 0.65, \ P = 0.005) \). However, passive vital capacity in E hamsters was consistently greater than in C hamsters at any given body weight, and there was no correlation between VC and body weight \( (r = 0.04) \). Exsised lung volume, as determined by fluid displacement (measured in grams), was increased 117% in E hamsters, and lung volume-to-body weight ratio was 115% greater in E hamsters (Table 1). Passive VC and lung displacement volume values were highly correlated \( (n = 32, \ r = 0.724, \ P < 0.001) \).

Hematocrit Data and Arterial Blood Gases and pH

Hematocrit was not altered in E hamsters (Table 1). Heart rate and mean arterial pressure were similar in

Table 1. Animal data

<table>
<thead>
<tr>
<th>Animal data</th>
<th>Control</th>
<th>Emphysema</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>152 ± 6</td>
<td>154 ± 6</td>
</tr>
<tr>
<td>Passive vital capacity, ml</td>
<td>8.9 ± 0.3</td>
<td>12.8 ± 0.5 *</td>
</tr>
<tr>
<td>Vital capacity/body weight, ml/kg</td>
<td>59.3 ± 1.8</td>
<td>84.5 ± 5.1 *</td>
</tr>
<tr>
<td>Lung displacement, g</td>
<td>1.45 ± 0.13</td>
<td>3.23 ± 0.39 *</td>
</tr>
<tr>
<td>Lung displacement/body weight, g/kg</td>
<td>9.5 ± 0.7</td>
<td>22.1 ± 3.7 *</td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>55 ± 1</td>
<td>55 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE for 17 control and 16 emphysematous hamsters. * \( P \leq 0.05 \) compared with control group.

Table 2. Hemodynamics, blood gas, and pH data

<table>
<thead>
<tr>
<th></th>
<th>Rest</th>
<th>Exercise</th>
<th>Rest</th>
<th>Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>108 ± 3</td>
<td>122 ± 4 *</td>
<td>115 ± 5</td>
<td>132 ± 4 *</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>365 ± 9</td>
<td>456 ± 10 *</td>
<td>369 ± 10</td>
<td>450 ± 7 *</td>
</tr>
<tr>
<td>( P_{O_2} ), Torr</td>
<td>74 ± 4</td>
<td>93 ± 4 *</td>
<td>59 ± 3</td>
<td>69 ± 4</td>
</tr>
<tr>
<td>( P_{O_2} ), Torr</td>
<td>41 ± 1</td>
<td>33 ± 2 **</td>
<td>42 ± 2</td>
<td>34 ± 1 *</td>
</tr>
<tr>
<td>( P_{CO_2} ), Torr</td>
<td>7.36 ± 0.01</td>
<td>7.41 ± 0.01</td>
<td>7.39 ± 0.02</td>
<td>7.44 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE. Nos. in parentheses, no. of observations per group. MAP, mean arterial pressure; HR, heart rate; \( P_{O_2} \), arterial \( P_{CO_2} \), arterial pH, \( P < 0.05 \) compared with rest value. \( P \leq 0.05 \) compared with control group.

Diaphragm Blood Flow

Total diaphragm blood flow measured at rest on the treadmill was not different between C and E hamsters \( (P = 0.178; \ Fig. 3) \). With treadmill walking, total diaphragm blood flow increased an average of only 24% in C hamsters compared with an increase of 137% in E hamsters \( (P = 0.034; \ Fig. 3) \). Diaphragm blood flow in C and E hamsters \( (n = 32) \) during exercise was directly correlated with passive VC \( (r = 0.48, \ P = 0.005) \) and passive VC-to-body weight ratio \( (r = 0.66, \ P < 0.001) \). Diaphragm blood flow during exercise was inversely correlated with \( P_{O_2} \) \( (r = -0.45, \ P = 0.028) \).
Intercostal and Abdominal Muscle Blood Flows

Resting blood flows in the intercostal and abdominal muscles were similar to those observed in the nonpos- tural hindlimb muscles (e.g., plantaris, gastrocnemius, quadriceps; Fig. 3, Table 3). Blood flow to the intercos- tal muscles increased significantly ($P < 0.002$) from rest to exercise in both groups; however, the exercise- induced increase in blood flow to the intercostal muscles was greater ($P = 0.009$) in E than C hamsters (Fig. 3). Abdominal muscle blood flow was unchanged between rest and exercise in C hamsters. However, abdominal muscle flow increased ($P < 0.044$) with exercise in E hamsters (Fig. 3), although the difference in exercise flows for C and E hamsters was not significant ($P = 0.242$).

Regional Distribution of Blood Flow Within the Diaphragm

Blood flow within the diaphragm of both C ($P < 0.001$) and E ($P < 0.001$) hamsters was not uniformly distributed either at rest or during exercise (Fig. 4). Blood flows were consistently lowest in the ventral costal diaphragm (Fig. 4). The regional heterogeneity of

Table 3. Hindlimb skeletal muscle and organ blood flows

<table>
<thead>
<tr>
<th>Hindlimb skeletal muscles</th>
<th>Control Rest</th>
<th>Control Exercise</th>
<th>Emphysema Rest</th>
<th>Emphysema Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solus</td>
<td>39 ± 9</td>
<td>238 ± 23*</td>
<td>36 ± 8</td>
<td>237 ± 22*</td>
</tr>
<tr>
<td>Plantaris</td>
<td>11 ± 2</td>
<td>139 ± 7*</td>
<td>7 ± 1</td>
<td>126 ± 21*</td>
</tr>
<tr>
<td>Gastrocnemius</td>
<td>17 ± 2</td>
<td>114 ± 13*</td>
<td>12 ± 2</td>
<td>103 ± 10*</td>
</tr>
<tr>
<td>Quadriceps</td>
<td>17 ± 3</td>
<td>97 ± 11*</td>
<td>18 ± 3</td>
<td>106 ± 10*</td>
</tr>
<tr>
<td>Abdominal organs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>628 ± 57</td>
<td>380 ± 32*</td>
<td>546 ± 56</td>
<td>387 ± 52*</td>
</tr>
<tr>
<td>Spleen</td>
<td>206 ± 31</td>
<td>79 ± 15*</td>
<td>193 ± 42</td>
<td>79 ± 17*</td>
</tr>
<tr>
<td>Stomach</td>
<td>137 ± 16</td>
<td>66 ± 10*</td>
<td>124 ± 21</td>
<td>67 ± 10*</td>
</tr>
</tbody>
</table>

Values are means ± SE given in ml·min⁻¹·100·g⁻¹ for 17 control and 16 emphysematous hamsters. There were no differences between control and emphysematous hamsters at rest or during exercise. $*P < 0.05$ compared with rest.

O₂ Delivery to the Diaphragm During Exercise

Hemoglobin concentration was estimated as one-third of the arterial hematocrit (Table 1), and, as
Regional Diaphragm Oxidative Capacity

Diaphragm samples for determination of citrate synthase activity were collected from 22 C and 30 E hamsters. These results are presented in Table 4. Citrate synthase activity was greatest in the crural diaphragm of both C (P < 0.001) and E hamsters (P < 0.001). Citrate synthase activity was uniform among the dorsal, medial, and ventral costal diaphragm regions in C hamsters. However, medial costal citrate synthase activity was greater than dorsal and ventral costal activity in E hamsters (Table 4). There was no difference in either total or regional citrate synthase activity between C and E hamsters. Citrate synthase data for those animals in which blood flow data are presented herein (i.e., 17 C and 16 E hamsters) are identical to those presented for the larger cohort of animals presented in Table 4.

Skeletal Muscle Blood Flows

The overall pattern of blood flow distribution within and among the hindlimb skeletal muscles at rest and during treadmill exercise was similar to that described previously for the rat (20, 34). Flows were greatest in the more axial, more oxidative muscles (e.g., soleus) than in the more glycolytic muscles (e.g., plantaris and gastrocnemius) (Table 3). Hindlimb skeletal muscle blood flows were identical in C and E hamsters at rest and during exercise.

Organ Blood Flows

Blood flows to representative organs (e.g., kidneys, spleen, and stomach) in C and E hamsters were not different at rest or during treadmill exercise (Table 3). Blood flows to the left and right kidneys in C and E hamsters were highly correlated (C, r = 0.93; E, r = 0.95), indicative of good left-to-right distribution of microspheres within the circulation. Blood flow to the kidneys, spleen, and stomach were significantly reduced during treadmill exercise in all animals.

DISCUSSION

The principal original findings of this investigation are as follows. 1) During exercise, emphysema increases diaphragm blood flow in proportion to the degree of lung pathology (i.e., greater excised lung volume-to-body weight ratios) and arterial hypoxemia. 2) There is regional heterogeneity of blood flow within the diaphragm of both C and E hamsters at rest and during exercise, with the greatest flow in the crural diaphragm and the lowest flow in the ventral region of the costal diaphragm. 3) Emphysema increases blood flow to the intercostal and abdominal muscles during exercise, probably reflecting a different pattern of muscle recruitment in the face of an increased work of breathing and a reduced relative contribution of the diaphragm to the total work of breathing.

Emphysematous State

The hamster is considered to represent the closest histopathological model for human panacinar emphysema (7, 22, 35). The magnitude of the increase in lung volume (displacement weight) and reduced PaO2 in the E hamsters in this investigation (Tables 1 and 2) are comparable to those published previously (22, 23). The 5-mo duration of emphysema was selected because lung compliance achieves maximal levels within 3–12 wk after elastase instillation (35), and all lung volumes are elevated within 4–6 mo (7, 8, 22). Also, it has been demonstrated that structural and functional diaphragmatic adaptations become evident within this period (7–9, 22). The values obtained for PaO2 and excised lung volume are consistent with the majority of animals being in a state of moderate emphysema, with

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**Table 4. Diaphragm citrate synthase activity**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Emphysema</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crural diaphragm</td>
<td>68.0 ± 2.0</td>
<td>70.9 ± 1.9</td>
</tr>
<tr>
<td>Costal diaphragm</td>
<td>62.4 ± 2.0*</td>
<td>62.9 ± 1.4*</td>
</tr>
<tr>
<td>Dorsal region</td>
<td>62.1 ± 2.3</td>
<td>60.4 ± 1.4t</td>
</tr>
<tr>
<td>Medial region</td>
<td>63.8 ± 2.1</td>
<td>65.3 ± 1.6</td>
</tr>
<tr>
<td>Ventral region</td>
<td>60.0 ± 2.1</td>
<td>61.8 ± 1.9t</td>
</tr>
</tbody>
</table>

Values are means ± SE given in µmol·min⁻¹·g wet wt⁻¹ for 22 control and 30 emphysematous hamsters. *P < 0.05 between crural and costal diaphragm. IP = 0.05 compared with the medial costal diaphragm in emphysema group.
relatively few animals tending toward the severe condition. It is possible that a more uniform group of severely emphysematous animals would have exacerbated the increase in exercising diaphragm blood flow demonstrated herein.

It must be acknowledged that there are profound structural and functional differences between hamsters and humans that likely have a bearing on the interpretation of the present data. Paramount among these is the high compliance of the hamster rib cage in comparison with that of the human. This feature may allow the hamster to more effectively utilize intercostal muscle function when the diaphragm becomes mechanically compromised in emphysema. In addition, the quadrupedal gait and horizontal body position of the hamster will reduce the effect of gravity per se on the geometric configuration of the diaphragm.

Diaphragm Total and Regional Blood Flow

Total diaphragm blood flow. Skeletal muscle blood flow and \( O_2 \) consumption increase as a linear function of muscle work (29). In addition, maximal \( O_2 \) consumption in skeletal muscle is linearly correlated with \( \text{O}_2 \) delivery (15). Manohar (26) observed that diaphragm blood flow in ponies increased as a direct function of exercise intensity. Thus these studies support the notion that blood flow is highly correlated with and indicative of muscle work. Although it might be argued that the increased blood flow found in the diaphragm of E hamsters is simply a compensatory response to the arterial hypoxemia, this is only partially true. We found that in emphysema the total \( O_2 \) delivery to the diaphragm during exercise was actually increased 55% relative to C hamsters (Fig. 5). Furthermore, because \( \text{PA}_{\text{a}}O_2 \) was not different between C and E hamsters, and because we do not expect that pulmonary gas-exchange requirements differed substantially during the exercise protocol (i.e., both C and E animals were matched for body weight), it appears that the diaphragm in emphysema has higher energetic requirements to achieve the same effective alveolar ventilation. Thus the greater diaphragm blood flow in exercising E hamsters suggests that the diaphragm of emphysematous hamsters is working harder than that of the healthy hamster.

One surprising aspect of the present findings was that, despite the significant increases over C hamsters, diaphragm blood flows during exercise in E hamsters were far lower than those observed in less-oxidative and less-vascular hindlimb muscles (present results; 20, 35). It is pertinent that in the healthy pony, costal and crural diaphragm blood flows at near-maximal exercise were two- to threefold those observed in this study (26). Notwithstanding the species difference, it would be most surprising if the diaphragm blood flows of \( \sim 100 \, \text{ml} \cdot \text{min}^{-1} \cdot 100 \, \text{g}^{-1} \) measured in the E hamster diaphragm in this investigation were maximal. At present, it is not clear whether this indicates that a substantial blood flow reserve exists in the diaphragm of E hamsters or, alternatively, whether emphysema reduces the blood flow capacity of the diaphragm due to either mechanical (e.g., altered tissue pressures) or regulatory effects. Resolution of this question is crucial in determining whether blood flow and \( O_2 \) delivery play a role in respiratory muscle fatigue and failure in emphysema.

Regional diaphragm blood flows. The mammalian (e.g., dog, rat, hamster) costal diaphragm exhibits regional heterogeneity with respect to muscle fiber(s) length and thickness (4, 24, 42). Furthermore, Margulies et al. (25) found regional differences in costal diaphragm shortening in the dog. Collectively, these observations, coupled with the greater blood flows in the medial and dorsal regions of the costal diaphragm in hamsters (Fig. 4) and rats (34), suggest that these regions are of primary importance in driving inspiratory action. In all three species studied to date (i.e., dog, rat, and hamster), blood flow is consistently lowest in the ventral region of the costal diaphragm. However, in contrast to rats and dogs in which flows are greatest in the medial and dorsal regions of the costal diaphragm, blood flows in the hamster diaphragm were actually greatest in the cranial portion. The significance of this difference in diaphragm regional flow patterns among species is unclear.

Emphysema did not significantly alter either the magnitude or the heterogeneous pattern of diaphragm blood flow at rest (Fig. 4). However, both total and regional diaphragm blood flows were greater in E hamsters during treadmill exercise. Furthermore, blood flow to the intercostal and abdominal muscles of E hamsters was also enhanced during exercise compared with control animals, probably reflecting the greater recruitment of these respiratory muscles to compensate for a proportional decrease in the diaphragm’s effective contribution to the ventilatory effort. Whereas the magnitude of the increased blood flow to the intercostal and abdominal muscles appears to be relatively modest, it is important to recognize that their total mass is substantially greater than that of the diaphragm. In hamsters weighing \( \sim 150 \, \text{g} \), the intercostal and abdominal muscles weigh \( \sim 2.0 \) and \( 3.4 \, \text{g} \), respectively (unpublished data). This compares with an average diaphragm weight of \( \sim 0.5 \, \text{g} \). Consequently, total absolute blood flow (i.e., \( \text{ml} \cdot \text{min} \)) to the intercostal and abdominal muscles together rose by more than threefold that seen in the diaphragm of exercising E hamsters.

A recent investigation in healthy humans demonstrated that, during maximal cycle ergometer exercise, altered respiratory muscle energetic demands (and presumably blood flow) cause reciprocal changes in peak leg blood flow (13). Thus, under these conditions, augmented inspiratory work reduced leg muscle blood flow. In the present investigation, it is possible that the elevated respiratory muscle blood flows in the E hamsters may have either reduced or limited increases in limb blood flow during exercise. Impaired muscle energetics have been reported in chronic obstructive pulmonary disease (COPD) patients (10, 11), and a suboptimal muscle blood flow and \( O_2 \) delivery during exercise might contribute to this response. Although it must be acknowledged that the exercise muscle blood flows were not different between C and E hamsters in this
study, it may be that COPD increases the muscle blood flow requirement at a given work rate (32). Thus it is conceivable that leg muscle blood flow may actually have been suboptimal in the E animals. An intriguing observation was that blood flow to the kidneys, spleen, and stomach in E hamsters was not reduced below values found in C hamsters during exercise. This suggests that exercising sympathetic stimulation was not enhanced by emphysema, at least at the exercise level achieved by these animals.

If maximal flow capacity is uniform throughout the diaphragm, it follows that those regions with the highest flows will be closer to their maximal circulatory capacity. This would effectively reduce their flow reserve and may render these regions more susceptible to flow-limited fatigue and, ultimately, failure. This could be especially true in disease states such as emphysema that compromise diaphragm function. The greater blood flows observed in the diaphragm (particularly in the medial costal and crural regions) and other respiratory muscles of E hamsters during exercise are consistent with the increased ventilatory work associated with emphysema. The effect of emphysema on maximal vascular conductance of the diaphragm is not known. However, if it is not substantially increased, E animals will use a greater percentage of their total diaphragm (and other respiratory muscles) blood flow capacity during exercise to achieve the same effective alveolar ventilation (i.e., as reflected in similar PaCO₂). This would reduce their flow reserve and place them at greater risk of diaphragm fatigue and/or failure.

The oxidative capacity of the diaphragm, as estimated by citrate synthase activity, is greater than that found in limb skeletal muscles (31, 34), presumably reflecting the greater chronic metabolic activity of the diaphragm. Powers et al. (31) reported that citrate synthase activity of the rat costal diaphragm was greater than that of the crural diaphragm; however, Sexton and Poole (34) were unable to corroborate that finding. Similarly, Farkas and Roussos (9) found no difference between crural and costal diaphragm oxidative capacity in either C or E hamsters. The results of the present study revealed that citrate synthase activity of the crural diaphragm was greater than that of the costal diaphragm in both C and E hamsters. The results of the present study revealed that citrate synthase activity of the crural diaphragm was greater than that of the costal diaphragm in both C and E hamsters (Table 4). Furthermore, in both C and E hamsters, there is a direct correlation between the regional distribution of blood flow within the diaphragm and citrate synthase activity, where both blood flow (Fig. 4) and citrate synthase activity are greatest in the crural diaphragm and lowest in the ventral costal diaphragm. Increased diaphragm activity, as with exercise training in rats (31) or emphysema in hamsters (9), has been reported to increase diaphragm citrate synthase activity. However, no such adaptations were observed in E hamsters in the present study. We did, however, find that, relative to the dorsal and ventral costal dia- phragm, citrate synthase activity in the medial costal diaphragm of E hamsters was increased and may reflect a regional adaptation within the medial costal diaphragm of E hamsters. Compared with the data of Farkas and Roussos (9), our citrate synthase values for C hamsters are greater, whereas the values for E hamsters are more consistent. Thus the lack of an emphysema-induced increase in diaphragm oxidative capacity in this study might be attributable to the fact that oxidative capacity in C hamsters was greater than observed previously (9).

Emphysema alters diaphragm structure and function dramatically. For example, in emphysema muscle fibers of the diaphragm hypertrophy (18, 40, 41) and shorten (8, 40). These adaptations are accompanied by increased capillarity (22, 41) and may be associated with increased oxidative enzyme activities in type I and type II fibers and enhanced fatigue resistance in vitro (7, 9, 22). Although emphysema presents the diaphragm with a unique spectrum of pathophysiological conditions, these adaptive responses described above are not unlike those seen in hindlimb skeletal muscles under conditions of altered mechanical or metabolic stress. Specifically, muscle fiber hypertrophy results from augmented muscle tension development (for review see Ref. 33), and muscle shortening occurs via loss of sarcomeres in series when muscles are immobilized in a shortened condition (for review see Ref. 12). Increased energetic demands, for example, exercise training, augment hindlimb muscle capillarity (for review see Ref. 33), and oxidative enzyme activity in hindlimb skeletal muscle (16) and diaphragm (31). These observations support the notion that in emphysema, the chronically shortened diaphragm is subjected to increased demands for tension production and also sustained elevations of oxidative function. In this regard, the present investigation adds to these observations by demonstrating for the first time that diaphragm blood flow (and presumably energetic demands) are elevated in emphysematic hamsters.

Conclusions

Emphysema increases blood flow to the diaphragm, intercostal, and abdominal muscles during exercise. However, the pattern of flow distribution within the diaphragm (i.e., lowest in ventral costal and greatest in the crural) is unchanged. Despite the high oxidative capacity and vascularity of the diaphragm, which increase with emphysema, diaphragm blood flows in E animals performing treadmill exercise are far below those found in hindlimb skeletal muscles. It remains to be determined whether this reflects the presence of a blood flow limitation or simply that the respiratory muscle O₂ demands do not require any additional flow.

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REFERENCES


