Effect of pulmonary emphysema on diaphragm capillary geometry

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Poole, David C., and Odile Mathieu-Costello. Effect of pulmonary emphysema on diaphragm capillary geometry. J. Appl. Physiol. 82(2): 599–606, 1997.—In emphysema, the diaphragm shortens by losing sarcomeres. We hypothesized that unless capillaries undergo a similar shortening, capillary geometry must be altered. Without quantifying this geometry, capillary length and surface area per fiber volume, which are critical measurements of the structural potential for blood-tissue exchange, cannot be resolved. Five months after intratracheal elastase (E) or saline (control; C) instillation, diaphragms from male Syrian golden hamsters were glutaraldehyde perfusion fixed in situ at reference lung positions (residual volume, functional residual capacity, total lung capacity) to provide diaphragms fixed over a range of sarcomere lengths. Subsequently, diaphragms were processed for electron microscopy and analyzed morphometrically. Emphysema increased lung volume changes from −20 to 25 cmH₂O airway pressure (i.e., passive vital capacity) and excised lung volume (both P < 0.001). In each region of the costal diaphragm (i.e., ventral, medial, dorsal), sarcomere number was reduced (all P < 0.05). Capillary-to-fiber ratio increased (C = 2.2 ± 0.1; E = 2.8 ± 0.1; P < 0.01) and fibers hypertrophied (C = 815 ± 35; E = 987 ± 67 µm²; P < 0.05; both values at 2.5 µm sarcomere length). Capillary geometry was markedly altered by the loss of sarcomeres in series. Specifically, the additional capillary length derived from capillary tortuosity and branching was increased by 183% at 2.5 µm sarcomere length compared with C values (C, 359 ± 43; E, 1,020 ± 158 µm²; P < 0.01). This significantly increased total capillary length (C, 3.115 ± 173; E, 3,851 ± 219 mm² at 2.5 µm, P < 0.05) and surface area (C, 456 ± 13; E, 519 ± 24 cm²; P < 0.05) per fiber volume. Thus emphysema substantially alters diaphragm capillary geometry and augments the capillary length and surface area available for blood-tissue exchange.

In pulmonary emphysema, the energetic requirements of the respiratory muscles are augmented (2), and respiratory muscle fatigue and ultimately failure may occur (13, 28). Sustained skeletal muscle contractile function demands an adequate substrate flux, and by far the most compelling mechanistic bases advanced to explain respiratory muscle failure focus on an O₂ supply-demand imbalance. Consequently, limitations of either respiratory muscle blood flow (Q) and/or blood-tissue O₂ exchange capacity must be considered as potentially important in the etiology of respiratory muscle failure. Recently, it has been determined that the site of the principal resistance for O₂ diffusion into skeletal muscle fibers resides in that short distance between the red blood cell and the immediately subsarcomemal cytoplasmic space (7, 32). Thus muscle O₂ diffusing capacity will be dependent critically on the capillary number and surface area available for exchange per fiber rather than other features such as intramyocyte diffusion distance, for example (17). To date, we are unaware of any measurements of either diaphragm Q or capillary geometry and surface area in the diaphragm in emphysema.

In emphysema, diaphragm muscle fibers shorten (4, 5, 30) so as to reestablish a favorable position on their length-tension relationship. In addition, diaphragm fibers may (11, 12, 31) or may not (6, 26) hypertrophy. The increased respiratory muscle metabolic demands found in emphysema (2) are associated with elevated levels of diaphragm mitochondrial enzymes (6, 12) analogous to those seen after exercise training (21). Furthermore, capillary-to-fiber ratio is increased in the diaphragm of emphysematous animals, which is indicative of capillary proliferation (12, 31). However, this capillary neogenesis is sufficient only to prevent any reduction of capillary density that would otherwise have resulted from hypertrophy of the fiber (12). Thus, unless the three-dimensional geometry of the capillary bed is altered, this adaptation would not be expected to increase the capacity of the diaphragm for oxygen diffusion per unit volume of muscle fiber.

In the healthy diaphragm, acute increases of fiber length in the proximity of functional residual capacity (FRC) reduce bulk Q (29). This is likely attributable to microvascular stretching, which is expected to occur at sarcomere lengths of >2.3 µm (3) and possibly arteriolar occlusion. In support of these observations, diaphragm capillary length per fiber volume increases and capillary diameter decreases as the diaphragm is stretched passively from total lung capacity (TLC) (~2.2 µm) to FRC (~2.8 µm, Ref. 18). It is not known whether emphysema affects the relationship between capillary diameter and sarcomere length found in the healthy animal (19).

The purpose of this investigation was to examine the effect of pulmonary emphysema on diaphragm capillary geometry. Structural alterations of the capillary bed might be important in altering the supply-utilization balance in the diaphragm of emphysematous hamsters in the face of elevated metabolic demands. We hypothesized that the loss of sarcomeres in series and consequent fiber shortening would increase capillary tortuosity at any given sarcomere length and that this would relieve the stretch on the capillary bed at low lung volumes (i.e., long sarcomere lengths).
METHODS

The methods used to create the emphysema condition in these animals and then perfusion fix the diaphragms and measure sarcomere lengths have been published previously (18). In that study, we examined filament length in the same samples by electron microscopy.

Emphysema Model

All procedures were conducted in accordance with the rules and regulations of the University of California, San Diego Animal Subjects Committee. Male Syrian Golden hamsters (7–9 wk old, 125–130 g body wt) were divided into control (C) and emphysema (E) groups at random. Under deep ketamine/xylazine anesthesia (150/7.5 mg/kg im), emphysema was induced by using a single intratracheal instillation of pancreatic porcine elastase (25 IU/100 g body wt, Sigma Chemical, St. Louis, MO) in 0.3 ml of normal saline (10, 27). For this procedure, we surgically exposed the trachea and used a 27-gauge hypodermic needle for instillation of the elastase (12). To ensure a more uniform elastase distribution in the lungs, each hamster was supported in a vertical head-up position and was rotated gently from side to side during instillation. This procedure has been proven to be effective in producing panacinare emphysema with increased lung compliance, elevated lung volumes, and reduced internal surface area (27). C animals were given 0.3 ml/100 g body wt normal saline by using the method described above. The animal studies were conducted 23–24 wk after instillation.

In vivo measurements of vital capacity, defined as the lung volume change from an airway pressure of −20 to 25 cmH2O, which in rodents defines residual volume (RV) and TLC, respectively (4, 19), were used to establish the presence of emphysema. Briefly, the anesthetized hamsters were tracheostomized and airway pressure was set by using a 60-ml syringe connected in parallel with a Validyne MP 45–26 cmH2O pressure transducer (Validyne, Northridge, CA). After a brief period of mechanical hyperventilation to induce apnea, two or more excursions were made from RV to TLC, respectively (4, 19), were used to establish the presence of emphysema. RV and TLC were determined by connecting a Validyne pressure transducer in series with a Validyne pressure amplifier (Validyne, Northridge, CA) on the tracheostomy. Airway pressure was set to either −20 (RV), 0 (FRC), or 25 (TLC) cmH2O immediately before vascular perfusion. Subsequently, by using a pressurized saline reservoir (11.06 g NaCl/l, 350 mosM, 6.25% GA solution in 0.1 M sodium cacodylate hydrochloride (0.3 ml, 30 mg/ml) was infused to ensure a vasodilated preparation. Outflow was facilitated by severing the major vessels to the right forelimb. Airway pressure was set to either −20 (RV), 0 (FRC), or 25 (TLC) cmH2O immediately before vascular perfusion. Subsequently, by using a pressurized saline reservoir (11.06 g NaCl/l, 350 mosM, 20,000 U heparin/ml) at a constant reservoir pressure of −110 mmHg, the animal was perfused with saline until the fluid flowing out of the severed forelimb vessels was clear. Airway pressure was then monitored continuously as the animal was perfusion fixed by using 300–400 ml of glutaraldehyde (GA) fixative (i.e., 6.25% GA solution in 0.1 M sodium cacodylate buffer adjusted to 430 mosM with NaCl; total osmolarity of fixative 1,100 mosM; pH 7.4) for 10 min. The abdominal cavity was opened, and the diaphragm was carefully shaved from all rib cage, vertebral, and organ attachments within 15–20 min of GA perfusion fixation. After photographing and caliper measurements of fiber length (central tendon attachment to distal end of fibers) at three fixed locations in each hemisphere (i.e., midventral, medial, and dorsal costal), a sample (~1 cm × 0.5 cm × entire thickness of diaphragm) was then taken from the left medial costal region. It was cut into ~20 longitudinal strips, immersed in GA fixative, and processed for microscopy as described below.

Tissue Processing and Sectioning

Tissues were prepared for microscopy by using standard techniques, as described previously (15, 18). Sections 1 μm in thickness were cut by using a LKB Ultrrotome III and stained with 0.1% aqueous toluidine blue solution. From each diaphragm, eight blocks were cut to obtain four longitudinal and four transverse sections. Figure 1 shows an example of a portion of one transverse and one longitudinal section. The angles of sectioning used to provide longitudinal and transverse sections were determined as described in detail previously (15). Briefly, each block was positioned in the LKB Ultrrotome III specimen holder as close as possible either transverse or longitudinal to the muscle fiber axis. Consecutive sections were then obtained at different angles with respect to the fiber axis by changing systematically the specimen holder orientation by 1 and 5° for longitudinal and transverse sections, respectively. Sections were defined as transverse when a change of sectioning angle by 5° in either direction produced fiber sections with reduced A-band spacing. For transverse sections, A-band spacing was estimated at up to 10 sites over the whole section. As a consequence of the uniform orientation of fibers in the diaphragm, there was a unique sectioning angle that produced a maximum A-band width over the whole section. For longitudinal sections, sarcomere length was measured by oil-immersion light microscopy at a final magnification of ×1,000. Ten series of 10 consecutive sarcomeres were measured at each sectioning angle at sites selected to provide full coverage of the section. Sections were considered to be longitudinal when a change of sectioning angle in either direction gave fiber sections with greater sarcomere length. The mean sarcomere length presented for each animal represents the average sarcomere length of the four blocks sectioned and was included in the previous report (18). Total sarcomere number was estimated by using the caliper measurement of fiber length at each location and dividing by mean sarcomere length determined in the medial costal diaphragm for each animal.

Morphometric Analysis

Capillary and fiber morphometric measurements were made on diaphragms from six C and six E animals (see Fig. 1). Each section was subsampled systematically to yield as many nonoverlapping micrographs as possible. The number of micrographs ranged from 14 to 22 and 9 to 17 per section for longitudinal and transverse sections, respectively, yielding ~500–800 fibers in each muscle. A 100-point square grid (Mikron Instruments, San Diego, CA) was placed behind the reticle of a standard light microscope, and the sections were counted at a final magnification of ×400. Point counting was used to determine capillary numerical and volume densities. All points were collected, stored, and processed on an Apple computer. The standard error of estimates of capillary measurements was calculated by pooling data from all micrographs from one sample and applying formulas for the
standard error of ratios. The standard error, therefore, is a measurement of the between-micrograph variability at the sampling site analyzed in each preparation and indicates the degree of biological variability present in each sample. The group mean standard error indicates the biological variability between animals.

Capillary length per volume of muscle fiber, $J_v(c,f)$, the degree of capillary anisotropy ($K$), and the relative contribution of the anisotropic components (tortuosity and branching) to capillary length per fiber volume $c(K,0)$ were estimated by using the method developed by Mathieu et al. (14). Briefly, the method is as follows: capillary length per fiber volume $J_v(c,f)$ is related to capillary numerical density in transverse $Q_A(0)$ and longitudinal $Q_A(p/2)$ sections [Eq. (1)] by the equations

$$J_v(c,f) = c(K,0) \cdot Q_A(0)$$

and

$$J_v(c,f) = c(K,p/2) \cdot Q_A(p/2)$$

where $c(K,0)$ and $c(K,p/2)$ are anisotropy coefficients for transverse and longitudinal sections, respectively. Combining Eqs. 1 and 2 gives, after rearrangement

$$Q_A(0)/Q_A(p/2) = c(K,p/2)/c(K,0)$$

The ratio $c(K,p/2)/c(K,0)$ in the Fisher axial distribution model is a uniform and monotonic function of $K$. Thus both $c(K,0)$ and $J_v(c,f)$ can be estimated by the following procedure: capillary numerical density is determined on transverse $Q_A(0)$ and longitudinal $Q_A(p/2)$ sections (4 blocks each). This gives the ratio $R = Q_A(0)/Q_A(p/2)$ [Eq. (3)], which is used to determine $K$ and $c(K,0)$ from a table (or graph) of known coefficients (14). Then, $J_v(c,f)$ is estimated via Eq. 1 or 2. For straight capillaries oriented perfectly with the muscle fibers, $K = \infty$ and $c(K,0) = 1$; for randomly oriented (isotropic) capillaries, $K = 0$ and $c(K,0) = 2$.

As in previous studies (15, 18), all estimates of capillarity were expressed by using the muscle fibers as the reference.
space in each sample. This procedure obviates any bias resulting from nonuniform preservation of the extracellular space. Fiber cross-sectional area was measured by point counting on the same transverse sections used to measure capillarity. Capillary-to-fiber ratio was calculated from capillary and fiber numerical counts on these sections. Mean capillary diameter [d(c)] was measured by image analysis on 100–150 capillaries per sample, from circular profiles only (difference between minimum and maximum diameters <15%). Capillary surface per volume of muscle fiber [S(c,f)] was calculated as

\[ J_{c,f} \cdot \pi \cdot d(c) \]  

(4)

Normalizing for Sarcomere Length

Because many structural features such as fiber cross-sectional area, capillary tortuosity [c(K,0)], capillary density, capillary length per fiber volume, and capillary diameter are altered with muscle shortening, we normalized these data to a sarcomere length of 2.5 µm where appropriate. This sarcomere length is on the plateau of the mammalian muscle sarcomere length-tension relationship (19) and corresponds closely to the mean sarcomere length found in the diaphragms in this investigation. By removing the variability due to differences in sarcomere length between diaphragm samples, the normalization improves the signal-to-noise ratio and allows better detection of structural differences between samples, independent of their sarcomere length at tissue fixation.

Statistical Analysis

Differences between E and C animals were compared by unpaired t-test. Standard least-squares regression techniques were used to investigate correlations between lung volume and estimated sarcomere number, as well as the relationships between \( Q_s(0)/Q_s(\pi/2) \) or K and sarcomere length for the purpose of normalizing capillary values to 2.5-µm sarcomere length. A significance level of \( P < 0.05 \) was accepted. All values are presented as means ± SE.

RESULTS

There was no difference in the final weight of C (152.0 ± 9.0 g) vs. E (152.9 ± 13.6 g) hamsters (\( P > 0.05 \)). Gross inspection of the lungs from E hamsters revealed a substantial increase of air trapping, coalesced alveoli, and distended airspaces. The presence of increased compliance and air trapping was supported by the substantial increase (78%; \( P < 0.001 \)) of passive vital capacity and saline displacement lung volume (128%; \( P < 0.001 \)).

By using the mean values for sarcomere length measured in the medial costal region of each diaphragm (Table 1), the total number of sarcomeres from the central tendon to the costal margin was calculated for the ventral, medial, and dorsal regions of the costal diaphragm (Table 2). Compared with C diaphragms, diaphragms from E hamsters exhibited fewer sarcomeres along the fiber length from central tendon to costal margin. This effect was quantitatively similar in each hemisphere and region of the costal diaphragm and, as expected, the loss of sarcomeres was correlated negatively \( (r = -0.729, P < 0.01) \) with the change in lung volume from airway pressures of -20 to 25 cmH2O (i.e., RV to TLC).

There was clear evidence of capillary neogenesis as demonstrated by the increased capillary-to-fiber ratio \( (C = 2.2 ± 0.1, E = 2.8 ± 0.1; P < 0.01) \) (Table 1). However, this did not result in an increased capillary density, i.e., number per fiber square millimeter \( [Q_s(0)] \): C = 2.756 ± 163, E = 2.831 ± 103 mm\(^{-2} \); \( P > 0.05 \) because of the attendant modest fiber hypertrophy \( [S(c,f)] : C = 815 ± 35, E = 987 ± 67 \mu m^2; P < 0.05 \).

Table 1. Morphometric data for diaphragms from emphysematous and control hamsters

<table>
<thead>
<tr>
<th>( l, \mu m )</th>
<th>( Q_s(0), \text{mm}^{-2} )</th>
<th>( Q_s(\pi/2), \text{mm}^{-2} )</th>
<th>K</th>
<th>c(K,0)</th>
<th>j(c,f), mm(^{-2} )</th>
<th>( N_s(c,f) )</th>
<th>( d(c), \mu m )</th>
<th>( S(c,f), \text{cm}^{-1} )</th>
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<tbody>
<tr>
<td><strong>Emphysema group</strong></td>
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<tr>
<td>2.62 ± 0.10</td>
<td>3.253 ± 117</td>
<td>1.319 ± 117</td>
<td>1.83</td>
<td>1.26</td>
<td>4.099</td>
<td>3.1</td>
<td>4.18 ± 0.08</td>
<td>538</td>
</tr>
<tr>
<td>2.74 ± 0.07</td>
<td>3.306 ± 239</td>
<td>1.576 ± 99</td>
<td>1.47</td>
<td>1.34</td>
<td>4.430</td>
<td>2.6</td>
<td>4.45 ± 0.06</td>
<td>619</td>
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<tr>
<td>2.74 ± 0.07</td>
<td>2.706 ± 70</td>
<td>876 ± 64</td>
<td>2.46</td>
<td>1.17</td>
<td>3.166</td>
<td>2.8</td>
<td>4.46 ± 0.08</td>
<td>444</td>
</tr>
<tr>
<td>2.86 ± 0.02</td>
<td>3.004 ± 85</td>
<td>1.155 ± 78</td>
<td>1.97</td>
<td>1.23</td>
<td>3.695</td>
<td>3.3</td>
<td>4.40 ± 0.07</td>
<td>511</td>
</tr>
<tr>
<td>2.52 ± 0.10</td>
<td>2.845 ± 128</td>
<td>1.284 ± 95</td>
<td>1.58</td>
<td>1.31</td>
<td>3.727</td>
<td>2.3</td>
<td>4.37 ± 0.07</td>
<td>512</td>
</tr>
<tr>
<td>2.90 ± 0.02</td>
<td>3.420 ± 67</td>
<td>978 ± 49</td>
<td>2.89</td>
<td>1.13</td>
<td>3.865</td>
<td>2.6</td>
<td>4.02 ± 0.05</td>
<td>488</td>
</tr>
<tr>
<td>2.73 ± 0.06</td>
<td>3.089 ± 115</td>
<td>1.198 ± 103</td>
<td>2.04 ± 0.22</td>
<td>1.24 ± 0.03</td>
<td>3.830 ± 173</td>
<td>2.8 ± 0.1</td>
<td>4.31 ± 0.07</td>
<td>519 ± 24</td>
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<tr>
<td><strong>Control group</strong></td>
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<tr>
<td>2.54 ± 0.01</td>
<td>2.916 ± 95</td>
<td>960 ± 62</td>
<td>2.41</td>
<td>1.17</td>
<td>3.412</td>
<td>2.1</td>
<td>4.61 ± 0.06</td>
<td>494</td>
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<tr>
<td>2.23 ± 0.10</td>
<td>2.226 ± 55</td>
<td>916 ± 70</td>
<td>1.80</td>
<td>1.26</td>
<td>2.805</td>
<td>2.4</td>
<td>4.02 ± 0.09</td>
<td>447</td>
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<tr>
<td>2.35 ± 0.02</td>
<td>3.031 ± 107</td>
<td>724 ± 79</td>
<td>3.69</td>
<td>1.09</td>
<td>3.304</td>
<td>2.6</td>
<td>4.42 ± 0.09</td>
<td>459</td>
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<tr>
<td>3.07 ± 0.01</td>
<td>3.268 ± 294</td>
<td>706 ± 47</td>
<td>4.28</td>
<td>1.07</td>
<td>3.497</td>
<td>2.2</td>
<td>4.22 ± 0.07</td>
<td>464</td>
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<tr>
<td>2.56 ± 0.05</td>
<td>3.195 ± 137</td>
<td>802 ± 51</td>
<td>3.43</td>
<td>1.10</td>
<td>3.515</td>
<td>2.2</td>
<td>4.33 ± 0.07</td>
<td>478</td>
</tr>
<tr>
<td>2.54 ± 0.02</td>
<td>2.539 ± 52</td>
<td>529 ± 26</td>
<td>4.61</td>
<td>1.07</td>
<td>2.717</td>
<td>1.9</td>
<td>4.72 ± 0.11</td>
<td>403</td>
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<tr>
<td>2.61 ± 0.14</td>
<td>2.863 ± 165</td>
<td>772 ± 65</td>
<td>3.37 ± 0.44</td>
<td>1.13 ± 0.03</td>
<td>3.208 ± 145</td>
<td>2.2 ± 0.1</td>
<td>4.56 ± 0.13</td>
<td>457 ± 13</td>
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<tr>
<td><strong>NS</strong></td>
<td><strong>NS</strong></td>
<td>P &lt; 0.01</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.01</td>
<td><strong>NS</strong></td>
<td>P &lt; 0.05</td>
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</table>

Values are means ± SE. \( l \), Sarcomere length; \( Q_s(0) \), number of capillaries per square millimeter transverse sectional area of muscle fibers; \( Q_s(\pi/2) \), number of capillaries per square millimeter longitudinal sectional area of muscle fibers; K, orientation concentration parameter, an index of degree of capillary anisotropy; c(K,0), coefficient relating \( Q_s(0) \) and capillary length per volume of muscle fiber \( j(c,f) \), which permits calculation of additional capillary length derived from capillary tortuosity and branching above that which would obtain if they were straight, unbranched structures oriented parallel to fiber longitudinal axis; \( N_s(c,f) \), capillary-to-fiber ratio; \( d(c) \), mean capillary diameter; \( S(c,f) \), capillary surface area per volume of muscle fiber.
In contrast, capillary counts on longitudinal sections were elevated \( Q_{A}(\pi/2)_{2.5}: C = 775 \pm 54, E = 1,402 \pm 142 \text{ mm}^{-2}; P < 0.01 \), reflecting a change in capillary geometry, i.e., increased tortuosity and branching \( c(K,0)_{2.5}: C = 1.13 \pm 0.02, E = 1.36 \pm 0.05; P < 0.01 \); Figs. 2 and 3. As a result, compared with C values, the contribution of these nonanisotropic components (i.e., tortuosity and branching) to capillary length per fiber volume \( J_{V(c,f)} \) increased by 183%, from 359 \( 6 \) 43 (C) to 1,020 \( 6 \) 158 mm \( ^2 \) (E) (\( P < 0.01 \)) and, consequently, \( J_{V(c,f)}^{2.5} \) increased by 24%, on average (C \( 5 \) 3,115 \( 6 \) 173, E \( 5 \) 3,851 \( 6 \) 219 mm \( ^2 \); \( P < 0.05 \)).

There was no significant difference between C and E hamsters with respect to capillary diameter (C \( 5 \) 4.56 \( 6 \) 0.13, E \( 5 \) 4.31 \( 6 \) 0.07 \( \mu \text{m} \); \( P > 0.05 \)). However, capillary surface area per fiber volume \( S_{V(c,f)} \) increased from 456 \( \pm \) 13 (C) to 519 \( \pm \) 24 (E) \( \text{cm}^{-1} \) (\( P < 0.05 \)).

**DISCUSSION**

The principal original finding of this study is that pulmonary emphysema changes the geometry of the diaphragm capillary bed. Concomitant with the loss of sarcomeres in series (4, 5, 30) and consequent fiber shortening, which occurs in each region of the costal diaphragm, the capillary bed assumes a more tortuous configuration. Thus the contribution of capillary tortuosity and branching to capillary length and surface area per fiber volume increased significantly. Under equivalent conditions of \( Q_{A} \) and \( O_{2} \) delivery, this phenomenon is expected to improve blood-tissue \( O_{2} \) exchange by increasing the capillary surface area available for \( O_{2} \) diffusion.

**Mechanism for Altered Capillary Geometry**

The increased diaphragm capillary-to-fiber ratio indicates that capillary neogenesis occurred and is in agreement with previous reports (12, 31) that support the notion that diaphragm contractile activity is increased in emphysema. This notion is consistent with the elevation of diaphragm oxidative enzymes found in emphysema (12, 30) and the proportional increase in limb muscle capillarity and oxidative enzyme capacity resulting from exercise training (20). It is thought that chronic elevation of \( Q_{A} \) is an important stimulus for capillary proliferation (8). However, it has yet to be demonstrated whether emphysema does actually in-

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**Table 2. Sarcomere number in different regions of costal diaphragm from emphysematous and control animals**

<table>
<thead>
<tr>
<th></th>
<th>Emphysema Group</th>
<th>Control Group</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Ventral</td>
<td>Medial</td>
</tr>
<tr>
<td>3,912</td>
<td>3,340</td>
<td>2,481</td>
</tr>
<tr>
<td>4,106</td>
<td>4,471</td>
<td>3,468</td>
</tr>
<tr>
<td>3,563</td>
<td>3,741</td>
<td>3,287</td>
</tr>
<tr>
<td>4,021</td>
<td>4,633</td>
<td>3,609</td>
</tr>
<tr>
<td>3,671</td>
<td>4,762</td>
<td>4,723</td>
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<tr>
<td>3,811</td>
<td>4,483</td>
<td>3,448</td>
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<tr>
<td>3,847 ( \pm ) 84</td>
<td>4,238 ( \pm ) 230</td>
<td>3,503 ( \pm ) 294</td>
</tr>
</tbody>
</table>

Values are means of left and right hemispheres. Values are means \( \pm \) SE where indicated. There were significantly fewer sarcomeres in each region of diaphragms from emphysematous hamsters compared with control hamsters, \( P < 0.05 \).
crease respiratory muscle $Q\dot{\theta}$ at rest or during exercise above values found in healthy animals.

The elevation of capillary tortuosity at a given sarcomere length is most likely explained on the basis of fiber shortening by means of decreased sarcomere number, with little or no change in the length of individual capillaries. It can be seen in Figs. 2 and 3 that a 16% loss of sarcomeres can account for a substantial increase of $c(K,0)$. Indeed, when we left shifted the sarcomere length values in the $E$ group to account for the effect of the overall muscle shortening, $c(K,0)$ values were close to those from the $C$ data (Fig. 2). This suggests that the length of individual capillaries was largely unchanged with emphysema, i.e., the change in capillary geometry was principally due to the loss of sarcomeres. Besides acute muscle shortening per se (15), we were unaware of any other physiological or pathophysiological conditions that augment capillary tortuosity. It is certainly possible to invoke capillary neogenesis by means of chronic electrical stimulation (16) and exercise training (20) or capillary involution by using streptozotocin-induced diabetes (25) without significantly disrupting the capillary tortuosity-sarcomere length relationship. It remains to be determined whether chronic shortening of limb muscles induces augmented capillary tortuosity at a given sarcomere length.

Relationship to Previous Work

Where there is a precedent, each of the diaphragm gross and microstructural changes observed with emphysema in the present investigation (i.e., fiber length, fiber cross-sectional area, capillary-to-fiber ratio) agrees substantially with previous observations. Specifically, in emphysematous hamster diaphragms, fiber length reportedly decreases by 10–16% (4, 5, 11, 12). This compares with 14–16% in the present study (Table 2) and is consistent with the reduced diaphragm muscle areas found in humans suffering from emphysema (1). In animals and humans, whether emphysema induces fiber hypertrophy leading to increased diaphragm thickness may depend on age at disease onset, state of health, and nutritional intake. Specifically, some animals with elastase-induced emphysema either fail to increase their weight or, toward the latter portion of the 5 mo after elastase instillation, begin to lose weight. One important determining feature of the hypertrophy response appears to be animal age at the induction of the emphysema condition. If emphysema develops in young animals before attainment of mature body weight, the animals usually continue to grow and the diaphragm fibers invariably hypertrophy. However, when emphysema is induced in animals of full adult weight, these animals show evidence of reduced body weight and fiber atrophy in the diaphragm. In most studies (see Ref. 26 for one notable exception) in which emphysematous animals sustain their body weight (11, 12, 31), hypertrophy of either type I or II fibers (11, 31) or just type II fibers (12) is found. Despite overall maintenance of animal body weight in the present study, the 20% increase in fiber cross-sectional area (normalized for sarcomere length) was somewhat less than that reported in these other studies. In contrast, when emphysema reduced body weight, type II fibers atrophied (6). In humans, reports are conflicting. However, two studies did report increased fiber size in the human diaphragm in emphysema (9, 24). In terms of total diaphragm mass, Kelsen et al. (11) found no change in emphysema. Presumably, in this instance the fiber hypertrophy was counterbalanced by reduced fiber length resulting in no net change in mass in these animals in which body weight was preserved.

As pointed out earlier with respect to capillarity, myofibrillar adenosine triphosphatase staining has revealed emphysema-induced capillary neogenesis (increased capillary-to-fiber ratio) in guinea pig (31) and hamster (12) diaphragms. However, as in the present study, this did not lead to elevated capillary densities because of the attendant fiber hypertrophy.

Interpretation of Findings

Quantification of capillary length and surface area per fiber volume gives an appreciation of the maximal capacity for blood-tissue $O_2$ (and substrate) exchange and, as demonstrated herein, these are increased in the diaphragm of hamsters with emphysema. However, whether this capacity can be used depends crucially on factors such as diaphragm bulk $Q\dot{\theta}$ and its regional distribution as well as the matching of red blood cell and thus $O_2$ flux to $O_2$ requirements within individual fibers or motor units. To date, we are unaware of any reports of the effect of elastase-induced emphysema on diaphragm $Q\dot{\theta}$ or microcirculatory dynamics. It is possible that the fiber hypertrophy and greater forces developed in the diaphragm of emphysematous animals alter these variables.

Implications for Diaphragm Function

Capillary length and surface area per fiber volume. As mentioned above, exercise training increases both muscle capillarity and oxidative enzyme capacity in concert (20). Moreover, across training states in rat soleus, capillary length per fiber volume correlates highly with citrate synthase activity. These observations are intuitively reasonable in that the maximal capacities for $O_2$ exchange and utilization appear to be coordinated, at least within a given species, and are elevated in parallel in response to augmented metabolic demands. Previous studies of diaphragm capillarity in emphysema only examined transverse sections (12, 31). Because the increased capillary-to-fiber ratio did not increase capillary density, due to concomitant fiber hypertrophy, it appeared that capillary supply per unit volume of tissue was unchanged. In other words, the capacities for $O_2$ delivery (capillarity) and utilization (oxidative enzymes) appeared to be dissociated in emphysema, suggesting a potential basis for compromised muscle function. However, the present study demonstrates that when the three-dimensional configu-
ration of the capillary bed is accounted for, emphysema induces a substantial increase in capillary length and surface area per fiber volume and thus enhances the potential for O₂ exchange, i.e., diffusing capacity (DO₂). Under equivalent O₂ delivery conditions, we would expect this increased capillary surface area to improve exercising diaphragm O₂ extraction. It is pertinent that O₂ extraction is determined by the proportionality between muscle DO₂ and Q such that

\[ O₂ \text{ extraction} = 1 - e^{-\frac{DO₂}{Q}} \]  

(5)

where \( \beta \) is the slope of the O₂ dissociation curve in the physiological range (22). Exercise training of the limb muscles in humans increases DO₂ to a greater extent than Q. Therefore, O₂ extraction across the leg at maximal exercise is augmented. Whether this occurs in the diaphragm of hamsters with emphysema is not known at present but could potentially be resolved via measurements of microvascular plasma PO₂ (which reflects regional O₂ uptake-DO₂ delivery matching in the region of interest) by techniques such as O₂ phosphorescence quenching.

Capillary diameter. In C rats, capillaries stretch and mean capillary diameter decreases as the diaphragm is passively extended from TLC to FRC or RV (\( r = -0.599, \ P < 0.01, \text{Ref.} \ 18 \)). Despite the substantial variability of capillary diameters, the C hamster diaphragms demonstrated a similar correlation (\( r = -0.512 \)). In contrast, in the diaphragms from the E hamsters, this behavior was not in evidence (\( r = -0.260 \)). These findings appear reasonable in that the shortened fibers and resultant increase in capillary tortuosity will increase capillary “slack,” and therefore the sarcomere length at which capillaries start to stretch will be right shifted. Thus the decrease in capillary diameter with increasing sarcomere length would be reduced or absent over the sarcomere length range considered.

In conclusion, the loss of diaphragm sarcomeres in series found in emphysema increases capillary tortuosity at any given sarcomere length. Coupled with the elevated capillary-to-fiber ratio, this augments capillary length and surface area per fiber volume. At equivalent levels of Q and O₂ delivery, this would be expected to enhance the capacity for O₂ diffusion and improve O₂ extraction. Furthermore, the increase in tortuosity would act to relieve the stretch on the capillary bed at low lung volumes and thereby reduce the tendency for the capillary lumen to narrow at long sarcomere lengths.

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