Myosin and actin filament lengths in diaphragms from emphysematous hamsters

DAVID C. POOLE, RICHARD L. LIEBER, AND ODILE MATHIEU-COSTELLO
Departments of Medicine and Orthopaedics, University of California, San Diego,
La Jolla, California 92033-0623

Poole, David C., Richard L. Lieber, and Odile Mathieu-Costello. Myosin and actin filament lengths in diaphragms from emphysematous hamsters. J. Appl. Physiol. 76(3): 1220-1225, 1994.-In vitro studies of the diaphragm from emphysematous animals have, in some instances, shown an alteration in its sarcomere length-tension relationship and a decreased maximal specific tension. To our knowledge, it has never been determined whether such functional changes may be indicative of ultrastructural adaptations, e.g., changes in filament lengths and thus cross-bridge number. To address this, we compared filament lengths in diaphragms from hamsters in which emphysema was induced by endotracheal instillation of elastase (E) 5 mo before the hamsters were killed with those from control hamsters (C; saline instillation). Diaphragms were then fixed by vascular perfusion with buffered glutaraldehyde in situ at airway pressures set to approximate the physiological range of lung volumes from residual volume (RV) to total lung capacity (TLC). Ultrathin sections (50-70 nm) were taken parallel to the muscle fiber axis and examined by electron microscopy (~33,000). Sarcomere and filament length measurements were calibrated using an actin periodicity of 39 nm and an M-band width of 86 nm to correct for dimensional changes during preparation. Emphysema increased the change in lung volume from -20 to +25 cmH₂O airway pressure (from RV to TLC) by ~88%, and the displacement volume of excised lung at 0 cmH₂O airway pressure was increased by ~138% on average. Neither myosin (C = 1.592 ± 0.027; E = 1.572 ± 0.036 μm; P = 0.72) nor actin (C = 1.210 ± 0.035; E = 1.221 ± 0.014 μm; P = 0.76) filament lengths were affected by emphysema. Thus, filament length changes do not underlie the diaphragm functional adaptations observed previously in emphysema.

sarcomere; length-tension relationship; specific tension; muscle ultrastructure; chronic obstructive pulmonary disease

PULMONARY EMPHYSEMA results in chronic lung hyperinflation, which flattens the diaphragm, thereby reducing its mechanical efficiency and augmenting diaphragmatic work. A number of the morphological and functional adaptations of the diaphragm to emphysema have been documented. Muscle fiber cross-sectional area increases, which enhances total force production (13, 27, 29; for review see Ref. 16). Sarcomeres are lost in series, yielding a decreased fiber length and restoring a more favorable operating length-tension relationship (3, 27). Oxidative enzyme activities increase in types I and II muscle fibers, and diaphragm muscle bundles become more resistant to fatigue in vitro (2, 4, 16). The possibility of another adaptation, namely, change in the length of the contractile filament(s), is intriguing considering the following conflicting data in the literature. Parkas and Roussos (3) reported that the loss of sarcomeres in series from the emphysematous diaphragm could not account fully for the observed change in the fiber length-tension relationship. Rather, in emphysema there was a significant decrease in the sarcomere length at which optimum force was achieved (L₀) from 2.64 to 2.47 μm. However, Supinski and Kelsen (27) were unable to confirm this finding. Lewis et al. (16) documented a 25% reduction of in vitro maximal specific tension (P₀) generated by fiber bundles from diaphragms of emphysematous hamsters (for review see Ref. 25). Although other available studies do indicate a tendency for P₀ to fall in diaphragm of emphysematous animals, the magnitude of this change was more modest (5-10%) and was not statistically significant (2, 3, 27).

The overlapping arrangement of actin and myosin filaments in vertebrate striated muscles and their interactions with ATP to convert chemical energy into mechanical work or tension are well established. Although not universally accepted, the popular sliding filament-crossbridge model (6) stipulates that a change in muscle length occurs by means of altered filament overlap rather than by any change in filament length (for review see Refs. 10, 22). The relative lengths of actin and myosin filaments define the sarcomere length-tension relationship, and potential P₀ or force developed at a given sarcomere length will thus depend, in part, on the degree of filament overlap and the number of cross-bridge attachments this permits.

Actin filament length varies widely between vertebrate species, and it may also vary between muscles or muscle fiber types within a single animal (7, 20). Because this variation occurs in multiples of ~39 nm, it is possibly the regulation of tropomyosin polymerization that controls filament length (19, 31). It has also been proposed that the giant protein nebulin (600-900 kDa) plays a central role in determining thin filament length (15). In contrast to actin, the myosin filament length appears to be strictly regulated at 1.5-1.6 μm within and between vertebrate species (1, 10, 22, 30, 31), and Huxley (10) defined the maximal isometric tension capability of muscle as the number of myosin filaments per unit area. Both the filament axial repeat (3 myosin molecules for each 14.3-nm repeat) and the lateral spacing (40 nm) appear invariant.

Given that neither the myosin filament packing density nor the myosin molecule axial repeat distance changes, the only mechanism by which P₀ generated per unit area of muscle (i.e., per myofibril) at a given sarcomere length could in theory be increased (or decreased) would be via a change in the filament length.

Chronic length changes in vertebrate muscle due to growth or to limb immobilization in extended or shortened positions are generally thought to occur via addition or removal of sarcomeres in series rather than by altered
length of preexisting filaments within sarcomeres (28, 33; for review see Ref. 5). However, we are unaware of any measurements of filament lengths in the diaphragm of emphysematous animals. This is of particular interest because this muscle shortens while remaining active, which is a very different model than that of limb immobilization.

The purpose of this investigation was to determine whether emphysema induces alterations in costal diaphragm actin and myosin filament lengths and thereby provides an ultrastructural mechanism by which the sarcocere length-tension relationship (3) and/or specific tension (16, 25) might be altered.

METHODS

Emphysema model. Male Syrian Golden hamsters of 125-130 g body wt (7–9 wk old) were divided randomly into control (C) and emphysema (E) groups. All procedures were conducted under deep ketamine (150 mg/kg)-xylazine (7.5 mg/kg) anesthesia administrated intramuscularly as approved by the University of California, San Diego Animal Subjects Committee. Emphysema was induced by means of a one-time instillation of pancreatic porcine elastase (25 IU/100 g body wt; Sigma Chemical, St. Louis, MO) in 0.3 ml of normal saline (2, 16). During this injection, the animal was supported in a head-up position and was rotated from side to side to facilitate a more uniform instillation of the elastic. These doses and procedure have been demonstrated to be effective in producing panacinar emphysema with increased lung compliance, elevated lung volumes, reduced internal surface area, and augmented diaphragm fiber cross-sectional area (16). C hamsters were administered 0.3 ml/100 g body wt of normal saline with use of the same method. Animals were studied 23–24 wk after clastase or saline administration.

The efficacy of the protocol in inducing emphysema was assessed by in vivo measurements of vital capacity, defined as the lung volume change from −20 to +25 cmH₂O airway pressure. In rodents, the start and end of this range define residual volume (RV) and total lung capacity (TLC), respectively (2, 14). Hamsters were tracheostomized under deep anesthesia, and airway pressure was controlled by means of a 60-ml syringe connected in parallel with a Validyne MP 45-26, ±35 cmH₂O pressure transducer (Validyne, Northridge, CA). After a brief period of mechanical hyperventilation, at least two excursions were made from RV to TLC, each taking 4–6 s. In addition, excised lung volume was measured at 0 cmH₂O airway pressure with use of a liquid-displacement technique, the lung being immersed in saline.

Perfusion and fixation procedure. After measurement of RV-to-TLC volume, vascular perfusion of the diaphragm was conducted as follows. A laparatomy was performed, and the liver and gut were reflected to expose the aorta. A PE-50 or PE-90 catheter was placed in an upstream direction into the abdominal aorta immediately rostral to the renal arteries and was secured by nylon ties. The inferior vena cava was tied at this level and gut were reflected to expose the aorta. A PE-50 or PE-90 catheter was placed in an upstream direction into the abdominal aorta immediately rostral to the renal arteries and was secured by nylon ties. The inferior vena cava was tied at this level and was rotated from side to side to facilitate a more uniform instillation of the elastic. These doses and procedure have been demonstrated to be effective in producing panacinar emphysema with increased lung compliance, elevated lung volumes, reduced internal surface area, and augmented diaphragm fiber cross-sectional area (16). C hamsters were administered 0.3 ml/100 g body wt of normal saline with use of the same method. Animals were studied 23–24 wk after clastase or saline administration.

The efficacy of the protocol in inducing emphysema was assessed by in vivo measurements of vital capacity, defined as the lung volume change from −20 to +25 cmH₂O airway pressure. In rodents, the start and end of this range define residual volume (RV) and total lung capacity (TLC), respectively (2, 14). Hamsters were tracheostomized under deep anesthesia, and airway pressure was controlled by means of a 60-ml syringe connected in parallel with a Validyne MP 45-26, ±35 cmH₂O pressure transducer (Validyne, Northridge, CA). After a brief period of mechanical hyperventilation, at least two excursions were made from RV to TLC, each taking 4–6 s. In addition, excised lung volume was measured at 0 cmH₂O airway pressure with use of a liquid-displacement technique, the lung being immersed in saline.

After measurement of RV-to-TLC volume, vascular perfusion of the diaphragm was conducted as follows. A laparatomy was performed, and the liver and gut were reflected to expose the aorta. A PE-50 or PE-90 catheter was placed in an upstream direction into the abdominal aorta immediately rostral to the renal arteries and was secured by nylon ties. The inferior vena cava was tied at this level and was rotated from side to side to facilitate a more uniform instillation of the elastic. These doses and procedure have been demonstrated to be effective in producing panacinar emphysema with increased lung compliance, elevated lung volumes, reduced internal surface area, and augmented diaphragm fiber cross-sectional area (16). C hamsters were administered 0.3 ml/100 g body wt of normal saline with use of the same method. Animals were studied 23–24 wk after clastase or saline administration.
were sectioned and analyzed by light microscopy. Thus, the mean sarcomere lengths given for each animal represent the mean sarcomere length of these blocks. For electron microscopy measurements, we chose the block in which sarcomere length was closest to the mean for all four blocks from that animal. We corrected filament measurements for tissue dimensional changes during preparation by using a standard actin periodicity of 39 nm (30) and an M-band width of 86 nm (21) when appropriate. Actual actin periodicity was measured from consecutive series of 5–15 periodicities at three to five sites per picture. M-band width was measured at four random sites per picture. That portion of the actin filament in the zone of actin-myosin overlap was assumed to shorten to the same extent as the myosin filament (7) and was corrected accordingly.

Statistical analysis. Actin and myosin filament lengths and other dimensional measurements between E and C animals were compared by unpaired t test. A significance level (α) of <0.05 was accepted. All values are presented as means ± SE. Statistical power (1 - β) for all negative conclusions exceeded 50%.

RESULTS

The final group sizes (6 C and 13 E hamsters) reflect an ~14% mortality rate in E hamsters. Autopsy revealed that two E animals died of massive pulmonary hemorrhage without recovering from the anesthesia (within 2–4 h from the instillation of elastase). Also, those diaphragms that did not perfuse adequately (indicated by blood remaining within major vessels) were considered to be inadequately perfused by fixative and were not analyzed (3 diaphragms in all).

Both C and E hamsters gained weight in a similar fashion, with final weights at death being 151.5 ± 12.3 and 152.9 ± 13.6 g, respectively (P = 0.942).

In the E hamsters, vital capacity (the change in lung volume from RV to TLC) was increased by ~88% (C, 5.7 ± 0.1 ml; E, 10.7 ± 0.5 ml; P < 0.001) and excised lung volume (measured by liquid displacement at 0 cm H2O airway pressure) was increased by ~138% (C, 2.4 ± 0.1 ml; E, 5.7 ± 0.5 ml; P < 0.001). In addition, there was abundant gross evidence of emphysema (enlarged and/or coalesced alveoli) that was fairly well distributed between left and right lungs and from apex to base.

Both myosin (C, 1.592 ± 0.027 μm; E, 1.572 ± 0.035 μm; P = 0.672) and actin (C, 1.210 ± 0.035 μm; E, 1.221 ± 0.014 μm; P = 0.762) filament lengths were remarkably similar in diaphragms from C and E animals. The same was also true for Z-line (C, 0.086 ± 0.008 μm; E, 0.090 ± 0.005 μm; P = 0.670) and bare-zone (C, 0.144 ± 0.006 μm; E, 0.134 ± 0.004 μm; P = 0.148) widths (Table 1).

The range of diaphragm sarcomere lengths from RV to TLC in E hamsters was 3.09–2.47 μm compared with 3.07–2.22 μm in C hamsters (Table 2). Although the small total sample size at each lung volume (n = 5, 6, and 6 for RV, FRC, and TLC, respectively) precluded formal statistical analysis at all airway pressures, it is notable that sarcomere lengths at TLC were greater in the E than the C hamster diaphragms (E, 2.54 ± 0.03 μm; C, 2.29 ± 0.07 μm; P < 0.05). At FRC in E hamsters, sarcomere length averaged 2.82 ± 0.03 μm compared with 2.95 ± 0.02 μm in the C hamster (n = 1; Table 2). Sarcomere length measured previously in the healthy rat diaphragm at FRC was 2.79 ± 0.05 μm (23).

Tissue dimensional changes in the longitudinal axis (i.e., parallel to the fiber longitudinal axis) as estimated from measurements of actin periodicity ranged from −5.4 to 3.4%, and those for M band width ranged from −12.8 to 2.9%. The correction for those changes did not affect the final mean values substantially or change the conclusions.

Neither the slope nor the intercept of the relationship between filament overlap and sarcomere length was significantly different between diaphragms from C and E animals (P = 0.832 and 0.945, respectively; Fig. 1). For all diaphragms, there was a negative correlation (r = −0.891, P < 0.001) between filament overlap and sarcomere length. The slope and intercept of that relationship were similar to those predicted assuming constant myosin and actin filament lengths at each sarcomere length.

DISCUSSION

This investigation demonstrated that pulmonary emphysema does not induce filament length changes in the hamster diaphragm. To account for the reported reduction in Lm (2.64–2.47 μm; Ref. 3), the actin filament would have to shorten by 0.085 μm per half sarcomere (2 helical repeats or periodicities). This clearly did not occur. Similarly, to account for a reduction in Pm of 25% (16), the myosin filament would be expected to shorten by ~0.35 μm (8 helical repeats). Again, no evidence for this was found.

Adequacy of emphysema model. The hamster model of elastase-induced pulmonary emphysema has been established as macroscopically and histologically resembling the human condition of panacinar emphysema (12). The elastase dose and instillation procedure used here followed closely those of Lewis et al. (16) and others (2–4, 16, 27). After elastase treatment, lung compliance increases rapidly and achieves near-maximal levels 3–12 wk postinjection (12, 26). Within 4–6 mo, lung volumes at RV, FRC, TLC, and passive vital capacity (RV to TLC, −20 to +25 cm H2O) all increase substantially (2, 3, 16, 18). Also within this time course maximal transdiaphragmatic pressure at a given lung volume increases (18) and diaphragm fibers hypertrophy (16, 29), shorten (3), increase in oxidative capacity, and are less fatigable (2, 4, 16). We documented the presence and severity of emphysema in each animal by measuring passive vital capacity in situ and fluid displacement volume of the excised lung. Both variables increased substantially, although the mean increase in passive vital capacity of ~88% we observed was somewhat lower than the ~120% reported by Farkas and Roussos (2). On the other hand, we found a 138% increase in the volume of the excised lung (at 0 cm H2O airway pressure), which was higher than the 93% reported by Lewis et al. (16) at 5 cm H2O airway pressure.

It is possible that additional diaphragm adaptations could occur after a more prolonged period of emphysema such as the 18 mo used by Supinski and Kelsen (27). However, the main functional changes of interest (i.e., shift in Lm and reduced Pm) reportedly occurred within 4–6 mo of elastase instillation (8, 16), and it was the focus of this study to investigate whether ultrastructural adaptations might explain these specific changes.


### TABLE 1. Dimensions of filament elements

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Myosin Filament Length, µm</th>
<th>Actin Filament Length, µm</th>
<th>Z-Band Width, µm</th>
<th>Actin Periodicity, nm</th>
<th>Bare-Zone Width, µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.461±0.007</td>
<td>1.107±0.015</td>
<td>0.087±0.003</td>
<td>38.4±1.1</td>
<td>0.136±0.004</td>
</tr>
<tr>
<td>2</td>
<td>1.471</td>
<td>1.121</td>
<td>0.088</td>
<td>39</td>
<td>0.137</td>
</tr>
<tr>
<td>3</td>
<td>1.498±0.011</td>
<td>1.226±0.017</td>
<td>0.088±0.004</td>
<td>38.4±0.5</td>
<td>0.137±0.005</td>
</tr>
<tr>
<td>4</td>
<td>1.583</td>
<td>1.264</td>
<td>0.100</td>
<td>39</td>
<td>0.147</td>
</tr>
<tr>
<td>5</td>
<td>1.404±0.008</td>
<td>1.121±0.013</td>
<td>0.086±0.002</td>
<td>37.7±1.1</td>
<td>0.124±0.002</td>
</tr>
<tr>
<td>6</td>
<td>1.006</td>
<td>1.175</td>
<td>0.089</td>
<td>39</td>
<td>0.138</td>
</tr>
<tr>
<td>7</td>
<td>1.536±0.003</td>
<td>1.150±0.014</td>
<td>0.087±0.003</td>
<td>38.6±0.7</td>
<td>0.127±0.004</td>
</tr>
<tr>
<td>8</td>
<td>1.655</td>
<td>1.187</td>
<td>0.088</td>
<td>39</td>
<td>0.138</td>
</tr>
<tr>
<td>9</td>
<td>1.442±0.012</td>
<td>1.188±0.011</td>
<td>0.072±0.002</td>
<td>39.1±1.3</td>
<td>0.130±0.003</td>
</tr>
<tr>
<td>10</td>
<td>1.853</td>
<td>1.244</td>
<td>0.072</td>
<td>39</td>
<td>0.118</td>
</tr>
<tr>
<td>11</td>
<td>1.507±0.009</td>
<td>1.217±0.008</td>
<td>0.103±0.003</td>
<td>38.3±0.6</td>
<td>0.125±0.002</td>
</tr>
</tbody>
</table>

**Group mean ± SE**  
Emphysema  
1.572±0.035  
1.221±0.014  
0.090±0.005  
0.134±0.004

Control  
1.592±0.027  
1.210±0.035  
0.086±0.008  
0.144±0.006

Values are means ± SE (measured values). Bold nos., values corrected for actin periodicity of 39 nm (30) or M-band width of 86 nm (21) as appropriate. There were no significant differences (P > 0.05) between mean values from emphysematous and control conditions.

### TABLE 2. Sarcomere lengths in individual costal diaphragms measured by light microscopy

<table>
<thead>
<tr>
<th>Airway Pressure, cmH₂O</th>
<th>Emphysema</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>−25 to −20 (RV)</td>
<td>2.86±0.02 (4)</td>
<td>3.07±0.01 (10)</td>
</tr>
<tr>
<td></td>
<td>2.90±0.02 (6)</td>
<td>3.09±0.04</td>
</tr>
<tr>
<td></td>
<td>2.90±0.06</td>
<td>2.94±0.05</td>
</tr>
<tr>
<td>Group mean ± SE</td>
<td>2.94±0.05</td>
<td></td>
</tr>
<tr>
<td>0 (FRC)</td>
<td>2.74±0.07 (3)</td>
<td>2.95±0.02 (9)</td>
</tr>
<tr>
<td></td>
<td>2.74±0.07 (9)</td>
<td>2.85±0.07</td>
</tr>
<tr>
<td></td>
<td>2.88±0.09</td>
<td>2.91±0.02</td>
</tr>
<tr>
<td>Group mean ± SE</td>
<td>2.82±0.03</td>
<td></td>
</tr>
<tr>
<td>+12.5 (Intermediate)</td>
<td>2.54±0.01</td>
<td>2.56±0.05 (11)</td>
</tr>
<tr>
<td>+20 to +26 (TLC)</td>
<td>2.62±0.10 (1)</td>
<td>2.22±0.10 (8)</td>
</tr>
<tr>
<td></td>
<td>2.52±0.10 (5)</td>
<td>2.35±0.02 (7)</td>
</tr>
<tr>
<td></td>
<td>2.65±0.04</td>
<td>2.67±0.05</td>
</tr>
<tr>
<td>Group mean ± SE</td>
<td>2.54±0.03</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE of 4 blocks from each hamster in µm. No. in parentheses corresponds to animal no. in Table 1; in all other hamsters only light microscopy analysis of sarcomere length was performed. RV, residual volume; FRC, functional residual capacity; TLC, total lung capacity.

Although not the focus of this study and not testable statistically because of small group sizes, it was interesting that the E hamster diaphragm sarcomere lengths at RV and FRC were close to those of C hamsters and healthy rats (23). If they are a true representation of the emphysematous condition, then these results suggest that muscle length adaptation may be approaching completion after 5 mo of emphysema. If this were not the case, then markedly shorter sarcomere lengths than control would be expected. At TLC, however, there was the...
indication that sarcomere length was not as short in E as in C diaphragms. Thus, at high lung volumes it is possible that the altered geometry in emphysema facilitates achievement of greater lung volumes for a reduced change in sarcomere length from FRC. As described in METHODS, the in situ anatomic positions of the liver and gut were preserved to permit fixation in a condition as close to physiological as possible. However, the 25-cm\(H_2O\) positive pressure needed to achieve TLC could have induced diaphragm inversion. This, in turn, would have resulted in a reduced shortening or an artificial lengthening of the diaphragm muscle fibers and thus sarcomeres. Although macroscopic examination revealed no evidence of inversion, some artificial lengthening of the diaphragm at TLC cannot be discounted. Indeed, in the dog with unilateral emphysema, diaphragm length ceases to decrease beyond the TLC of the control lung, which may reflect some positive pressure-induced diaphragm buckling (8).

For the filament lengths and bare-zone width reported in the present investigation, the classic relationship described by Gordon et al. (6) predicts that the sarcomere length-tension plateau will extend from 2.44 to 2.58 \(\mu m\). Thus, for sarcomere lengths above 2.58 \(\mu m\), i.e., on the descending limb of the length-tension curve, tension is expected to fall linearly with decreasing filament overlap, reaching zero at \(\sim 4.0 \mu m\). For sarcomere lengths from 2.44 to 1.58 \(\mu m\) on the upper portion of the ascending length-tension curve, tension potential falls more gradually to \(\sim 80\%\) of maximum. The reduced range of sarcomere lengths found from RV to TLC in the F hamster diaphragms appears advantageous in that it will reduce potential tension losses at the lower extreme of the achieved sarcomere length range, i.e., at high lung volumes.

**Effect of tissue dimensional changes during preparation.** The potential for tissue fixation and preparation for electron microscopy to cause dimensional changes (7, 19, 20, 30) and to affect our conclusions was clearly ruled out. As already mentioned, all tissues were processed in the same bath, all blocks were sectioned with the microtome knife edge parallel to the fiber longitudinal axis to prevent filament compression artifact, and the measurements were corrected for standard actin periodicity. We followed the technique described by ter Keurs et al. (30), using an actin periodicity of 39 \(nm\) as an internal calibration to correct for actin filament dimensional changes in the I band. This method is based on the X-ray diffraction experiments of Huxley and colleague (9, 11), which show that the meridional reflection arising from the actin filament troponin complexes occurs at \(\sim 38.5\) nm in living frog muscle. As discussed by ter Keurs et al., it is possible that this value is not appropriate for mammalian muscle because of interspecies variation in troponin spacing. Because myosin filaments can shrink to a greater extent than actin filaments in the I band (7), it is important to correct not only myosin filament lengths but also that portion of the actin filament in the overlap zone, which likely shrinks to the same extent as the myosin filament. Using an M-band width of 86 nm (21), we found a modest (5.2 \(\pm\) 1.3\%) degree of myosin filament shrinkage (Table 1). On the basis of the previous study (7), we assumed that that portion of the actin filament in the zone of overlap was affected to the same extent as the myosin filament. Both actin and myosin filament lengths obtained from C and E diaphragms in the present study are in good agreement with published data for rodent skeletal muscle from different laboratories (cf. Refs. 22, 30-32). Evidence of internal consistency in our measurements of actin and myosin filament length is illustrated in Fig. 1, which shows that the actin-myosin overlap decreased in the systematic and linear fashion expected with increased sarcomere length.

In conclusion, we showed that diaphragm functional changes in experimental emphysema do not arise from changes in either actin or myosin filament length. This finding supports the notion that alterations in the fiber length-tension relationship arise solely from reduced sarcomere number.

The authors thank Dr. Michael I Lewis for advice regarding the elastase-induced emphysema model and Dr. Jennifer Fujimoto (University of California, San Diego Office of Animal Resources) for assistance and advice regarding animal treatment. Also, we are grateful to Dr. Gaspar A. Farkas for pertinent insights and helpful discussion. This work was supported in part by Cigarette and Tobacco Surtax Fund of the State of California through the Tobacco Related Disease Research Program of the University of California Grant 2KT-0066 and National Heart, Lung, and Blood Institute Grant HL-17731.

Address for reprint requests: D. C. Poole, Dept. of Medicine, UCSD, La Jolla, CA 92039-0623.

Received 3 May 1993; accepted in final form 30 September 1993.

**REFERENCES**