Sarcomeres Are Added in Series to Emphysematous Rat Diaphragm After Lung Volume Reduction Surgery*

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Study objectives: The diaphragm adapts to its shortened state in experimental emphysema primarily by losing sarcomeres in series, thus reducing its optimal operating length. One would expect improved diaphragmatic function after lung volume reduction surgery (LVRS) only if the muscle can readapt to its elevated, lengthened postoperative position by either adding back sarcomeres or lengthening sarcomeres. We used a model of elastase-induced emphysema in rats to test the hypothesis that sarcomere addition occurs following LVRS.

Design: A cohort of emphysematous rats was created by the intratracheal instillation of elastase. Five months after the instillation, one group of rats underwent measurement of in situ costal diaphragm length via laparotomy, the determination of optimal muscle fiber operating length (Lo) on stimulated diaphragm strips in vitro, and the measurement of sarcomere length by electron microscopy on strips fixed at Lo. Another group of rats underwent LVRS or sham sternotomy 5 months after the instillation, and 5 months following the operation these animals underwent the same series of diaphragmatic studies.

Results: Lo was significantly greater in rats that underwent LVRS than those that underwent sternotomy (mean [± SE] Lo after LVRS, 2.50 ± 0.08 cm; mean Lo after sternotomy, 2.27 ± 0.06 cm; p = 0.013). There was no significant difference in sarcomere lengths between the two groups (2.95 ± 0.04 vs 3.04 ± 0.04 μm, respectively; p = 0.10). Using Lo as the length basis, the mean sarcomere number was calculated to be 8,712 ± 192 in animals that had undergone LVRS and 7,144 ± 249 in animals that had undergone sternotomy (p < 0.001).

Conclusion: Sarcomere length is not significantly altered but sarcomeres are added in series following LVRS in this experimental model of emphysema/LVRS. It is likely that this sarcomere addition is a prerequisite to the improvement in inspiratory muscle function that has been observed following LVRS in humans.

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Key words: diaphragm; emphysema; respiratory muscles; sarcomeres

Abbreviations: EM = electron microscopy; Lo = optimal muscle fiber operating length; LVRS = lung volume reduction surgery

The pulmonary hyperinflation of emphysema causes diaphragmatic flattening and shortening. It is widely accepted that that these changes reduce diaphragmatic efficiency and that this plays a major role in the dyspnea experienced by emphysema patients. In elastase-induced emphysema in rodents, it has been demonstrated that at least most of the diaphragmatic adaptation to this shortening, adaptation that allows the muscle to function at close to the optimal point on its length-tension curve, is attributable to the loss of sarcomeres in series. While two of the groups that addressed this issue found that sarcomere length is unchanged, one group found that in emphysema there was a significant decrease in sarcomere length. In humans, it has also been reported that diaphragmatic sarcomeres are significantly shorter in emphysema patients who suffer from a high degree of air trapping.7
Lung volume reduction surgery (LVRS) is an experimental operation that improves expiratory airflows and ameliorates dyspnea in selected patients with emphysema. A major explanation for diminished dyspnea after the operation is improved inspiratory muscle function, and this has been demonstrated to occur in patients. It has also been shown that LVRS lengthens the human diaphragm and that this lengthening correlates with indexes of postoperative physiologic improvement. Diaphragmatic function would be likely to improve following LVRS, however, only if the mechanically advantageous return of the diaphragm to a more elevated position occurs in conjunction with a readaptation of the length-tension relationship of the fibers such that sarcomeres are not simply overstretched.

We have previously reported that LVRS in emphysematous rats alters the diaphragmatic length-tension relationship such that the muscle continues to function close to the optimal muscle fiber operating length (Lo) in its elevated, lengthened, post-LVRS position. If the diaphragm failed to undergo adaptation in sarcomere number or length following LVRS, then diaphragmatic sarcomeres would be overstretched following the procedure, with the likely result of decreased diaphragmatic function. In this study, then, we set out to test the hypotheses that length adaptation in the diaphragm following LVRS results from the addition of sarcomeres in series and that there is not a significant increase in sarcomere length after LVRS.

Materials and Methods

Emphysema Induction

Emphysema was induced in 3-month-old Sprague-Dawley rats by a single intratracheal instillation of porcine pancreatic elastase (ICN Biochemicals; Cleveland, OH), 25 U per 100 g body weight, diluted in 0.60 mL normal saline solution, as described previously. Approximately 20% of the animals died within 1 h of the instillation from pulmonary hemorrhage. The surviving animals were returned to the animal-care facility and were managed routinely until 5 months following induction. Control animals underwent an identical procedure using 0.60 mL normal saline solution without elastase.

In subsequent analyses, the investigator determining the Lo was blinded to the type of animal being studied.

Technique of Lung Volume Reduction

Lung volume reduction was performed on a cohort of emphysema rats 5 months following emphysema induction. The animals were anesthetized and intubated with a 16-gauge, nonocclusive IV catheter. While ventilated with a rodent ventilator (CWE Inc; Ardmore, PA), the rats underwent median sternotomies. The upper lobe on the right and approximately the upper one third of the left lung were resected, and the stumps were ligated with 000 polyglycolic acid ties. A 16-gauge IV catheter was placed into each hemithorax through separate intercostal stab incisions –2 cm H2O suction. The incision was closed in layers with running sutures. The animals were awakened and extubated. There were no air leaks following the cessation of positive-pressure ventilation, and chest tubes were removed when the animals began to ambulate (3 to 5 min after extubation).

Sham sternotomies were performed on another cohort of emphysema animals 5 months following emphysema induction in precisely the same manner as LVRS but without the lung resection.

Measurement of In Situ Diaphragm Fiber Length

Five months following the induction of emphysema (in 8-month-old rats) and 5 months following LVRS or sham sternotomy (in 13-month-old rats), the animals were killed by CO2 inhalation and immediately underwent a laparotomy. The pleural space was not entered in order to maintain as close to the physiologic diaphragmatic geometry as possible. A soft 0.5-mm scale was applied to the abdominal surface of the diaphragm without disturbing the muscle’s position, and the length from the central tendon to the costal insertion was measured on the right hemidiaphragm at a point 0.5 cm anterior to the phrenic nerve insertion.

Determination of Lo

The apparatus for the in vitro study of muscle contractile properties was the same as that described previously. After measurement of the in situ length, the diaphragm was removed en bloc with the rib cage and was placed in oxygenated, buffered Ringer’s solution. One muscle strip that was 7.5 mm wide, and that extended from the central tendon to rib attachments, was dissected under magnification from the same location on the right where the in situ length had been measured. Care was taken to dissect parallel to the fibers. A second strip was taken from the same position in the left hemidiaphragm to be fixed at the Lo for electron microscopy (EM).

Each strip was mounted horizontally in a bath of circulating, oxygenated solution at a temperature of 23 ± 1°C. The costal end of the strip was sutured to a fixed post, while the central tendon was affixed to the arm of a servomotor system (motor model 6450, electronics model 300B; Cambridge Technology; Watertown, MA) on a movable platform. The muscles were stimulated via platinum electrodes (S44 stimulator; Grass Instruments; Quincy, MA) with pulses that were 1.5 times above those needed to achieve maximal twitch force (70 V; 5-ms pulses). A series of twitches generated at incrementally different muscle lengths was used to identify the point of maximal force generation (ie, the Lo). This length then was measured along the middle of the strip, from the point of tendon insertion to the point of costal insertion, using calipers. A length-tension curve then was generated on the right diaphragm strips using five twitches at each muscle length between 70% and 120% of the previously determined Lo. The data presented represent the mean of these five twitches. Muscle length (using the servomotor), stimulator pulse timing, and data collection were under computer control using custom software developed in our laboratory. A computer with a high-speed processor (Pentium; Intel; Santa Clara, CA) with data acquisition board (DT21-EZ; Data Translation; Marlboro, MA) controlled the experiment and recorded all data to a disk for later analysis.

The right hemidiaphragm strips were used for further physiologic studies; the left hemidiaphragm strips were immediately fixed at Lo for EM studies as described below.
Lung Volume Determination

After the measurement of the in situ diaphragm length and the dissection of the muscle strips from which the Lo was determined, the lung was excised with the trachea intact and was inflated to a distending pressure of 25 cm H₂O. This volume (at the total lung capacity) was measured by water displacement.

Determination of Sarcomere Length

Measurements were made by EM in a manner similar to the method of Poole et al. In brief, the left costal diaphragm strips were fixed at the Lo by immersion in a solution of 4% paraformaldehyde/1% glutaraldehyde and were stored at 5°C. A fragment of muscle from the mid-portion of each strip was embedded in medium (Poly/Bed 812; Polysciences, Inc; Warrington, PA). Sections (1 μm thick) were obtained at an angle that was estimated to be parallel to the muscle fibers (0°) and at five, 1°, incremental inflections in each direction from 0°. Each of these sections was stained with 1% toluidine blue and 1% borate solution, and examined under light microscopy (×1,000) against a grid. Five series of 10 consecutive sarcomeres at random locations were measured from the section at each angle. The angle at which the mean sarcomere length was shortest was defined as the angle providing a section longitudinal to the fibers. Thin sections (80 nm) then were obtained at this angle from the original specimen block and were stained with 3% uranyl acetate solution followed by staining with a bismuth subnitrate solution (ie, 2N NaOH, 4% sodium tartrate, and 2.5% bismuth subnitrate). Photomicrographs (final magnification, approximately ×8,000) were made using an electron microscope (model 7000; Hitachi Instruments; Tokyo, Japan) [Fig 1]. Photomicrographs were obtained by random sampling of areas showing Z lines in register. Standard calibration techniques were employed to correct the lengths measured from the photomicrographs to actual size. Eight series of 10 consecutive sarcomeres were measured to obtain the mean sarcomere length on each specimen. The number of sarcomeres was calculated on each diaphragm strip by obtaining the mean sarcomere length on each specimen. The size. Eight series of 10 consecutive sarcomeres were measured to estimate the lengths measured from the photomicrographs to actual lengths. 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Statistical Analysis

A software package (SPSS Base 8.0 for Windows; SPSS Inc; Chicago, IL) was used for all statistical analysis. The independent-samples t test was used to compare the means for each variable from each group. A p value of < 0.05 was considered to be significant.

The use of laboratory animals in this protocol was approved by the animal-care committees of the University of Pennsylvania and the Philadelphia Veterans Affairs Medical Center. All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals that is published by the National Institutes of Health.

Results

As shown in Table 1, the lung volume was greater in 8-month-old rats with emphysema (n = 8) than in controls (n = 8; mean [± SE] lung volume, 29.9 ± 1.2 vs 25.1 ± 0.67 mL; p = 0.003), and it was lower in 13-month-old emphysematous animals that had undergone LVRS (n = 11) than in those that had undergone sham sternotomy (n = 7; 29.0 ± 1.4 vs 34.4 ± 1.4 mL; p = 0.035). Body weights were not significantly different between the 8-month-old animals with emphysema and either the control animals (p = 0.56) or the 13-month-old animals with emphysema that had had undergone LVRS or those that had undergone sternotomy (p = 0.36). Thus, pulmonary hyperinflation was created by elastase instillation, and LVRS successfully reduced this hyperinflation.

We have previously shown that both the in situ diaphragm length and the Lo are reduced in this emphysema model. In situ length was 2.24 ± 0.11 cm in the controls 5 months following emphysema induction (n = 8), and was 1.99 ± 0.04 cm in rats with emphysema (n = 8; p = 0.001). The Lo was 2.48 ± 0.09 cm in the controls and 2.25 ± 0.06 cm in the rats with emphysema (p = 0.038) [Table 2]. Also shown in Table 2, 5 months following surgery (at age 13 months), both the in situ length (2.13 ± 0.06 vs 1.83 ± 0.02 cm, respectively; p < 0.001) and the Lo (2.50 ± 0.08 vs 2.27 ± 0.06 cm, respectively; p = 0.013) were longer after LVRS than after sham sternotomy.

Also seen in Table 2, sarcomere lengths determined by EM were not significantly different between the rats with preoperative emphysema and the control animals or between the animals who had undergone LVRS and sham sternotomy. In the preoperative animals, the mean sarcomere length was 2.87 ± 0.10 μm in controls and 2.98 ± 0.06 μm in the animals with emphysema (p = 0.95). Animals that had undergone surgery had mean sarcomere lengths of 3.04 ± 0.04 μm 5 months following sternotomy and 2.95 ± 0.04 μm 5 months following LVRS (p = 0.10). One can then estimate (using the Lo as the length basis) the mean number of sarcomeres in series in each group (Table 2). Note that the diaphragms of the animals that had undergone

![Figure 1](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21972/ on 06/27/2017)
LVRS added a mean of 1,568 sarcomeres (22%) over the diaphragms of the controls that had undergone sternotomy (p < 0.001).

Figures 2 demonstrates that while the muscle fiber length-tension curve is shifted to the right in animals that have undergone LVRS vs sternotomy, their sarcomere length-tension curves are not similarly shifted. The sarcomere length for this figure was assumed to vary linearly with diaphragm length and was calculated from the EM-determined sarcomere length at the Lo. The difference in the mean peak twitch tension that is shown in these figures between the two groups does not reach statistical significance (p = 0.42).

**DISCUSSION**

We present data herein confirming that sarcomeres are deleted in series from the diaphragm of animals with elastase-induced emphysema and establishing, for the first time, that sarcomeres are added following LVRS. We found no significant difference between the sarcomere lengths in rats with emphysema that underwent LVRS and those with emphysema that underwent sham sternotomy, despite significantly longer muscle lengths in the LVRS group. The calculated total number of sarcomeres in series in the LVRS group was 22% greater than that in the sternotomy group. This addition of sarcomeres as the diaphragm lengthens or after following LVRS maintains the muscle at close to its Lo. It allows the optimal sarcomere length to occur at a longer fiber length. Furthermore, this study shows that adaptation in sarcomere number occurs within 5 months of operation in this model.

Adaptation of the sarcomere number, with the addition of sarcomeres in chronically stretched limb muscles and the loss of sarcomeres in shortened limb muscles, is well-established. It has also been shown that sarcomeres are lost in series in the shortened diaphragm of emphysematous hamsters, but it has been controversial whether there is also a shortening of sarcomere length in this model. Whether the shortened, emphysematous diaphragm that has already adapted by losing sarcomeres can, after LVRS, readapt by adding sarcomeres, has not been studied previously. Furthermore, the possibility that length adaptation following LVRS occurs by an increase in the length of the sarcomeres rather than or in addition to an increase in the number of sarcomeres has been suggested by both the findings of some of the initial studies of sarcomere adaptability in hamsters and by a report that diaphragmatic sarcomeres from emphysema patients with air trapping are, in fact, shorter than those from control subjects.

**Table 2—Diaphragm Lengths, Sarcomere Length, and Sarcomere Number**

<table>
<thead>
<tr>
<th>Variable</th>
<th>8-Mo-Old Rats</th>
<th>13-Mo-Old Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n = 8)</td>
<td>Emphysema (n = 8)</td>
</tr>
<tr>
<td>Length in situ, cm†</td>
<td>2.24 ± 0.11</td>
<td>1.99 ± 0.041</td>
</tr>
<tr>
<td>Lo, cm†</td>
<td>2.48 ± 0.09</td>
<td>2.25 ± 0.061</td>
</tr>
<tr>
<td>Sarcomere length, μm</td>
<td>2.87 ± 0.10</td>
<td>2.88 ± 0.06</td>
</tr>
<tr>
<td>Sarcomere number‡</td>
<td>8,718 ± 331</td>
<td>7,613 ± 3401</td>
</tr>
</tbody>
</table>

*Values given as mean ± SEM.
†Right hemidiaphragm strip lengths shown.
¶p < 0.05 compared with 8-mo-old controls.
§p < 0.05 compared with 13-mo-old rats with emphysema that had undergone sternotomy.
||p < 0.001 compared with 13-mo-old rats with emphysema that had undergone sternotomy.

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These are important questions because following LVRS the diaphragm is returned to a more elevated, mechanically advantaged position. If an increase in diaphragm length after LVRS occurred without a corresponding increase in the Lo, then one would expect the muscle to function less effectively on the descending portion of its length-tension curve. This might result in a diaphragm that is less effective, rather than more effective, at generating inspiratory force following LVRS. In initially addressing this issue, we reported14 that in post-LVRS in emphysematous rats, there is a concurrent increase in both the \textit{in situ} length and the Lo of the costal diaphragm. We thought that the most likely mechanism of this increase in the Lo after LVRS would be the addition of sarcomeres in series, although a change in the myofilament and, thus, the sarcomere length also would be possible. The current data support the former mechanism.

One anomalous finding of this study is that our EM-determined sarcomere lengths at the Lo are slightly greater than those given in most previous reports of sarcomere length in rodent skeletal muscle. This may be explained by the fact that the Lo at which the specimens were fixed for EM was determined by twitch contractions, and twitch contractions have been shown to have longer optimum sarcomere lengths than tetanic contractions.\textsuperscript{17} The finding that both the postoperative sternotomy and LVRS animals had slightly longer, though not significantly longer, sarcomeres than the preoperative animals may be related to some aspect of the postoperative state itself.

One potential criticism of this study is our use of the rat rather than the hamster as the animal model. Although hamsters with elastase-induced emphysema do develop more consistently impressive increases in lung volume, there is an extensive literature establishing that there are also significant and often dramatic increases in lung volumes and in compliance and reductions in expiratory flows in elastase-induced emphysema in rats.\textsuperscript{18–23} We add to this literature with the demonstration of significant increases in lung volume in this cohort. We chose rats because their larger size allows the required complex surgical manipulations to be performed with low mortality rates. Furthermore, given the smaller hamster trachea, even modest airway edema postintubation could reduce the cross-sectional area of the airway significantly, and this might have led not only to high mortality rates following volume reduction but might also have confounded results by placing an inspiratory load on the diaphragm.

Marchand et al\textsuperscript{24} recently reported on physiologic and anatomic adaptation in the diaphragm of emphysematous hamsters following LVRS. Although they found no significant difference in Lo between animals that had undergone LVRS and those that had undergone sham-sternotomy, the greater diaphragm length following LVRS nearly reached significance (p = 0.1) when a single outlier was removed from the statistical analysis. Notably, in the study by Marchand et al\textsuperscript{24} animals were evaluated only 8 weeks following the operation, while in our study the animals were evaluated 20 weeks following the operation. It is possible that 8 weeks was an insufficient time to allow the full diaphragmatic length adaptation to occur. Such an interpretation is supported by the finding of Lahrmann et al\textsuperscript{25} that in humans, increases in diaphragmatic function following LVRS do not reach statistical significance until 6 months following the procedure. Furthermore, all other studies\textsuperscript{3–6} of length adaptation in hamsters with emphysema have allowed 20 weeks at a minimum for adaptation to occur. Finally, in the study by Marchand et al,\textsuperscript{24} thoracostomy tubes were not placed in

![Figure 2. Diaphragm muscle fiber (top) and sarcomere (bottom) active length-tension curves in emphysema animals undergoing sternotomy (squares) and emphysema animals undergoing LVRS (circles). Note that the shift of the tension curve to the right, evident in the LVRS group when plotted as a function of fiber length, is lost when plotted as a function of sarcomere length. Differences in peak twitch tension between the groups do not reach statistical significance (values are means; error bars were omitted for clarity).](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21972/ on 06/27/2017)
the animals following LVRS, and the possibility that these animals developed chronic pneumothoraces, which would tend to reduce diaphragmatic length, was not ruled out.

In summary, we have shown that the basis of increased diaphragmatic Lo following LVRS in emphysematous rats is the addition of sarcomeres in series, while sarcomere length remains unchanged. What precise combination of mechanical and chemical signaling leads to the laying down of sarcomeres in this situation is unknown and merits further study.

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REFERENCES

16 Tabary JC, Tabary C, Tardieu C, et al. Physiological and structural changes in the cat’s soleus muscle due to immobilization at different lengths by plaster casts. J Physiol (Lond) 1976; 224:231–244