Length and curvature of the dog diaphragm

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Boriek, Aladin M., Ben Black, Rolf Hubmayr, and Theodore A. Wilson. Length and curvature of the dog diaphragm. J Appl Physiol 101: 794–798, 2006; doi:10.1152/japplphysiol.00865.2004.—Transdiaphragmatic pressure is a result of both tension in the muscles of the diaphragm and curvature of the muscles. As lung volume increases, the pressure-generating capability of the diaphragm decreases. Whether decrease in curvature contributes to the loss in transdiaphragmatic pressure and, if so, under what conditions it contributes are unknown. Here we report data on muscle length and curvature in the supine dog. Radiopaque markers were attached along muscle bundles in the midcostal region of the diaphragm in six beagle dogs of ~8 kg, and marker locations were obtained from biplanar images at functional residual capacity (FRC), during spontaneous inspiratory efforts against a closed airway at lung volumes from FRC to total lung capacity, and during bilateral maximal phrenic nerve stimulation at the same lung volumes. Muscle length and curvature were obtained from these data. During spontaneous inspiratory efforts, muscle shortened by 15–40% of length at FRC, but curvature remained unchanged. During phrenic nerve stimulation, muscle shortened by 30 to nearly 50%, and, for shortening exceeding 52%, curvature appeared to decrease sharply. We conclude that diaphragm curvature is nearly constant during spontaneous breathing maneuvers in normal animals. However, we speculate that it is possible, if lung compliance were increased and the chest wall and the diameter of the diaphragm ring of insertion were enlarged, as in the case of chronic obstructive pulmonary disease, that decrease in diaphragm curvature could contribute to loss of diaphragm function.


Here we report the results of studies that were undertaken to test this hypothesis. In these studies, L and curvature were measured using the same techniques that were used in the previous work (1, 2). Radiopaque markers were attached along three muscle bundles in the midcostal region of the diaphragms of six dogs. The three-dimensional positions of the markers were calculated from the positions of the markers in biplanar video fluoroscopic images, and L and curvature were obtained from these data. Images were obtained at resting end expiration [functional residual capacity (FRC)]; during inspiratory efforts (IE) against a closed airway at FRC, FRC + 1/2 inspiratory capacity (IC), and total lung capacity (TLC); and during maximum phrenic nerve stimulation at the same three lung volumes. For the spontaneous IE at different volumes, muscle shortening, as a fraction of length at FRC, ranged from 15 to 40%. For phrenic nerve stimulation, shortening ranged from 30 to nearly 50%. We found that, for muscle shortening up to 52%, curvature is nearly constant, but for shortening greater than 52%, curvature decreased abruptly.

METHODS

Six bred-for-research beagle dogs with body masses of ~8 kg were studied, using the same methods that we have used previously (1, 2).
Dogs were maintained according to the National Institutes of Health “Guide for the Care and Use of Laboratory Animals,” and all procedures were approved in advance by the Institutional Review Board of Baylor College of Medicine and Mayo Clinic. Radiopaque markers were attached to the diaphragm by the following procedure. The abdomen was opened by midline laparotomy, and 2-mm silicon-coated beads were stitched to the peritoneal surface of muscle bundles in the midcostal region of the left diaphragm. Five markers were placed along each of three nearby muscle bundles: one at the origin of each muscle bundle on the central tendon (CT), one at its insertion on the CW, and three at equal intervals along the muscle bundle. The animals were allowed to recover for at least 3 wk.

The animals were anesthetized with pentobarbital sodium (30 ml/kg), intubated with a cuffed endotracheal tube, and placed in the test field of a biplanar fluoroscopic system. This high spatial (≥0.5 mm) and temporal (30 Hz) resolution system was used to record displacement of the radiopaque metallic markers. The animal was mechanically ventilated to apnea, and IC was measured by inflating the animal to TLC, defined as the volume at an airway pressure of 30 cmH2O. The animal was allowed to resume spontaneous breathing, and biplane images were obtained at end expiration. The airway was then occluded at FRC, or the lungs were inflated and the airway was occluded at either FRC + 1/2 IC or TLC. The change in airway opening pressure (ΔPao) was measured during IE against the occluded airway, and, when ΔPao reached a plateau, usually during the fifth or sixth IE, biplane images were recorded at the peak of the IE.

**In vivo phrenic nerve stimulation.** The roots of the phrenic nerve were stimulated using the same technique as that used by a number of other investigators (3, 6, 7, 8). Briefly, the spinal roots of the phrenic nerves (C5, C6) were identified and isolated on both sides of the neck. Insulated hook electrodes were placed under the nerve roots, and the preparation was covered with mineral oil. Supramaximal synchronous tetanic stimulations at frequencies between 1 and 50 Hz were applied to the nerve roots using a Grass S 88 nerve stimulator. The tetanic stimulation parameters used are the following: amplitude (6 V), pulse duration (1 ms), delay between pulses (0.01 ms), pulse frequency (1–50 Hz), and pulse train duration (500 ms). We recorded biplane images in the supine position before and after bilateral stimulation at lung volumes spanning the vital capacity: FRC, FRC + 1/2 IC, and TLC.

**Data analysis.** The coordinates of the markers in the two biplane images for each maneuver were determined, and the three-dimensional coordinates of the markers were computed. Examples of anterior-posterior views of the marker locations are shown in Fig. 1, *left*.

It was apparent from plots like that shown in Fig. 1, *bottom left*, that the markers on the CW did not lie on the curve drawn through the other four markers on each muscle bundle. In addition, the distance between the markers on the CW and the adjacent markers along a muscle bundle decreased less than the distances between other markers. These discrepancies suggest that the markers on the CW lay caudal to the effective line of insertion of the diaphragm. Defining the line of insertion is not straightforward. The diaphragm is anchored to the rib cage along a line that lies cranial from the line of attachment to the abdominal muscles. We placed the markers along the line connecting the diaphragm to the abdominal muscles on the abdominal side of the diaphragm, and it appeared that this line lay caudal to the

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**Fig. 1.** Examples of the data on marker locations for one dog at functional residual capacity (FRC) (*top*) and during bilateral phrenic nerve stimulation with the airway occluded at FRC (*bottom*). In each panel, the anterior-posterior view of the data in laboratory coordinates ($x$, $y$, $z$) is shown on the left. The $z$-axis lies in the cranial direction, and the $y$-axis in the lateral direction. The muscle bundles extend from the insertion on the central tendon (CT) to the insertion on the chest wall (CW). The view in the coordinate system ($\xi$, $\eta$, $\zeta$) of the plane fit to the data is shown on the right. $\eta$ is perpendicular to the $\xi$, $\zeta$ plane. The $\zeta$-axis lies in the direction perpendicular to the plane fit to the markers, and the $\xi$-axis lies in the direction of maximum curvature. The scale of the $\zeta$-axis is expanded relative to that of the abscissa. Circular arcs fit to the data are shown by dashed lines.
effective line of insertion on the CW. We, therefore, ignored the data for the markers on the CW in the subsequent analysis. The lengths of each muscle bundle were determined by adding the three distances between the four markers on each muscle bundle. Lengths during IE and phrenic nerve stimulation were expressed as fractions of the length at FRC, and the values for the three bundles were averaged.

A plane was fit to the coordinates of the 12 markers, and the marker coordinates were transformed to a coordinate system based on that plane with the $\xi'$ and $\eta'$ axes of the coordinate system in the plane and the $\zeta$ direction normal to the plane. The plane was fit to all markers, including those on the CW, by minimizing the sums of the squares of the distances between the marker locations and the plane. The marker coordinates in a new coordinate system with the $\eta$ and $\zeta$ axes in the plane and the $\xi$-axis normal to the plane were determined. A quadratic, $\xi = a\zeta^2 + b\zeta^2 + c\zeta^2 + d\zeta^2 + e\zeta^2 + f$, was fit to the data by a multiple least squares regression technique. Principal coordinates $\xi$, $\eta$, and $\zeta$ were determined, for which the quadratic has the form, $\xi = 1/2C1\eta + 1/2C2\zeta + F$. The coefficients $C1$ and $C2$ were the principal curvatures of the quadratic fit to the data. The $\xi$ was chosen as the coordinate with the largest curvature. The data were then transformed to a third coordinate system ($\xi, \eta, \zeta$), in which the $\xi$-axis lay along the direction of maximum curvature. Examples of the marker locations in this coordinate system are shown in Fig. 1, right.

For most conditions, the line of the muscle bundles lay nearly parallel to the direction of maximum curvature, the $\xi$ direction, and the curvature in the transverse direction, the $\eta$ direction, was small. In those cases, the $r$ is the inverse of the curvature in the $\xi$ direction. In a few cases, the line of the muscle bundle lay at an angle to the $\xi$-axis. In those cases, the curvature along the muscle bundle and the corresponding $r$ were used.

Modeling. A simplified version of the model for diaphragm kinematics that our laboratory presented in an earlier paper (2) is shown in Fig. 2. In this model, the shape of the midcostal region of the diaphragm is pictured as being an arc of a circular cylinder that extends between two fixed points on the CW that lie at distances $a$ from the midplane of the cylinder. The center of the circular arc lies at point $O$ on the midplane, and the arc of the diaphragm between the CW and the midplane subtends an angle $\phi$. This arc is composed of muscle with variable length that lies between CW and CT, and CT with fixed length $c$ that lies between CT and the midplane. The $L$ and the $r$ are related to $a$, $c$, and $\phi$ by the following equations.

$$L = a\phi/\sin \phi - c$$  \hspace{1cm} (1)

$$r = a/\sin \phi$$  \hspace{1cm} (2)

For given values of $a$ and $c$, these equations describe $r$ as an implicit function of $L$.

Statistical analysis. Based on the mathematical model described in Eqs. 1 and 2, we simulated a data set and generated two statistical models. Figure 3 shows lines plotted for the mathematical model as well as the two statistical models. The mathematical model was fit to the data by defining the average values for the two ends of the range of $L$ at FRC ($L_{FRC}$) and solving for the constants $a$ and $c$ in Eqs. 1 and 2. The first statistical model was found by generating a complex function that best fit the relationship between $L/L_{FRC}$ and $r$ as defined by Eqs. 1 and 2, with an adjusted error value of $<1\%$. The second statistical model showed the same form of an equation fit to the raw data of $L/L_{FRC}$ and $r$. Figure 3 shows the three models plotted as lines over the raw data.

RESULTS

Individual values of $L_{FRC}$, length as a fraction of $L_{FRC}$, and $r$ are listed in Table 1. The $r$ of the muscle bundles vs. $L$ as a fraction of $L_{FRC}$ for $a = 4.4$ cm, $e = 1.6$ cm, and $L_{FRC} = 5.3$ cm is plotted in Figure 3. In the mathematical model and in the statistical models, muscle shortening $(1 - L/L_{FRC})$ is always above 48%. More specifically, $r$ is approximately constant for $L/L_{0} > 0.52$ and increases sharply for $L/L_{0} < 0.52$. On the other hand, the data-based model takes into account the inherent variability in the data and has a more conservative estimate of the location of the break point, the point at which the radius rises sharply. It predicts that $r$ is approximately constant for $L/L_{0} > 0.517$ and increases sharply for $L/L_{0} < 0.517$. The measurements came from six different dogs, and the fit of the curves representing the individual dogs are not shown; however, none of the data points fell to the left of the minimum value of $L/L_{FRC}$, and the general shape of the relationship did not contradict the fit shapes for individual dogs. We conclude that the data-based model is conservative enough for the purpose of estimating a statistically significant rise in $r$ for $L/L_{0} < 0.52$.

DISCUSSION

Our objective in this study was to test the hypothesis that diaphragm curvature would decrease at muscle shortening greater than the shortening that occurred during the spontaneous breathing maneuvers that we studied in our previous work. We obtained data on diaphragm curvature as a function of muscle length for more extreme muscle shortening, and we
modified our model of diaphragm kinematics to explain the relation between \( L \) and curvature for the range of muscle shortening that was tested.

\( L \) and curvature were measured by the radiopaque marker technique, and this technique is well suited to the objectives of measuring \( L \) and curvature. The location of the markers is measured to within an accuracy of \( \pm 0.5 \) mm, and this precision is needed to obtain accurate values of the \( r \). As can be seen in Fig. 1, at FRC, the positions of the markers deviate from the best fit plane by distances of the order of 2–3 mm. The distances of the markers from the plane constitute the signal from which curvature is obtained. Thus, at FRC, the signal-to-noise ratio is small. The uncertainty in the curvature of the quadratic fit to the data at FRC is \( \pm 10\% \).

Given the relationship of curvature and its radius: \( \kappa = 1/r \), if the range of \( \kappa \) is \((0.9 \, [\kappa]_0, 1.11 \, [\kappa]_0)\), the uncertainty of \( r \) ranges between \( 0.91 \, r_0 \) and \( 1.11 \, r_0 \), where \( r_0 = 1/[\kappa]_0 \). During phrenic nerve stimulation, the absolute magnitude of the uncertainty in the quadratic fit is about the same, but, because the curvature is smaller, the normalized relative uncertainty of \( r \) (divided by \( r_0 \)) is the same, although the magnitude of the uncertainty is bigger.

The primary results of this study, as shown in Fig. 3, are the following. For muscle shortening of up to \( \sim 52\% \), the \( r \) of the muscle bundles appears to remain nearly constant, and for muscle shortening \( >52\% \), \( r \) appears to increase slightly. We explained the relation between \( r \) and \( L/L_{FRC} \) in a simple model for diaphragm kinematics. In such a model, the diaphragm is pictured as lying on the arc of a circular cylinder that extends between fixed end points. As the center of the arc descends, the muscle shortens, curvature remains nearly constant until \( L/L_{FRC} \) reaches \( \sim 0.52 \), and then curvature decreases sharply for greater shortening. The model can be used to predict the dependence of curvature on other parameters. In particular, curvature is sensitive to the diameter of the ring of insertion that is described by the parameter \( a \). If \( a \) were to increase by 10\% and the other parameters, \( c \) and \( L_{FRC} \), were unchanged, \( r \) at FRC would increase by 10\%, but, more importantly, the point at which \( r \) increases sharply would shift from \( L/L_{FRC} \sim 0.52 \) to \( L/L_{FRC} \sim 0.62 \).

\( P_{DI} \) is proportional to both muscle tension and curvature. Because curvature is nearly constant for \( L/L_{FRC} > 0.52 \), the curve of \( P_{DI} \) vs. \( L/L_{FRC} \) would have the same shape as the curve of muscle force vs. \( L/L_{FRC} \) for \( L/L_{FRC} > 0.52 \). Because curvature decreases sharply for \( L/L_{FRC} < 0.52 \), the curve of \( P_{DI} \) vs. \( L/L_{FRC} \) would be steeper than the force-length curve for \( L/L_{FRC} < 0.52 \). This behavior is consistent with the data for \( P_{DI} \) vs. \( L/L_{FRC} \) reported by Hubmayr et al. (6).

Diaphragm muscle shortening of \( >40\% \) would not occur in normal dogs during spontaneous breathing. In particular, our data demonstrated that muscle shortening was less during spontaneous breathing than that during phrenic nerve stimulation \( (P < 0.05) \). The high degree of muscle shortening that occurred during maximal phrenic nerve stimulation is non-physiological for two reasons. First, the diaphragm is never maximally activated during spontaneous respiratory efforts (5). Second, during phrenic nerve stimulation in our preparation, the muscles of the rib cage were silent. If these muscles were active, as they are during spontaneous breathing, the load on the diaphragm would be greater and diaphragm descent and muscle shortening would be smaller than they were in our experiments. However, in chronic obstructive respiratory disease, lung compliance is increased so that the load on the diaphragm is decreased (9, 11), and the rib cage is enlarged so that the diameter of the ring of insertion is increased (4, 10, 12). In that situation, a decrease in diaphragm curvature could contribute to a loss in diaphragm function.

![Graph](image_url)

**Table 1. Muscle lengths and radii of curvature**

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>( L ) cm</th>
<th>( L/L_{FRC} )</th>
<th>IE</th>
<th>MPNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.7</td>
<td>0.87</td>
<td>0.65</td>
<td>0.63</td>
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<td>2</td>
<td>4.5</td>
<td>0.89</td>
<td>0.72</td>
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<td>3</td>
<td>4.9</td>
<td>0.84</td>
<td>0.68</td>
<td>0.59</td>
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<td>4</td>
<td>4.0</td>
<td>0.86</td>
<td>0.70</td>
<td>0.53</td>
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<td>4.2</td>
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<td>0.53</td>
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<tr>
<td>6</td>
<td>4.1</td>
<td>0.82</td>
<td>0.61</td>
<td>0.54</td>
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<tr>
<td>Average</td>
<td>4.4</td>
<td>0.86</td>
<td>0.67</td>
<td>0.56</td>
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</table>

<table>
<thead>
<tr>
<th>( r ) cm</th>
<th>( L/L_{FRC} )</th>
<th>( L/L_{FRC} )</th>
<th>( L/L_{FRC} )</th>
<th>( L/L_{FRC} )</th>
</tr>
</thead>
<tbody>
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<td>6.3</td>
<td>3.5</td>
<td>3.5</td>
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</tr>
<tr>
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<td>2.6</td>
<td>2.6</td>
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<td>2.8</td>
<td>2.8</td>
<td>8.4</td>
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<tr>
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<td>4.2</td>
<td>4.8</td>
<td>7.0</td>
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<tr>
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<td>3.8</td>
<td>4.6</td>
<td>4.0</td>
</tr>
<tr>
<td>6</td>
<td>3.1</td>
<td>3.7</td>
<td>4.5</td>
<td>4.2</td>
</tr>
<tr>
<td>Average</td>
<td>4.3</td>
<td>4.0</td>
<td>4.1</td>
<td>4.7</td>
</tr>
<tr>
<td>SD</td>
<td>( \pm 0.58 )</td>
<td>( \pm 0.02 )</td>
<td>( \pm 0.06 )</td>
<td>( \pm 0.08 )</td>
</tr>
</tbody>
</table>

\( L_{FRC} \), \( l / L_{FRC} \); IE, end of inspiratory effort; MPNS, maximal phrenic nerve stimulation; TLC, total lung capacity; IC, inspiratory capacity; \( r \), radius of diaphragm curvature; SD, standard deviation.
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