Biaxial constitutive relations for the passive canine diaphragm

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The diaphragm is a curved sheet, and it has been assumed that the sheet carries stress in both directions in the plane of the sheet. Thus the stress-strain relations in two dimensions are pertinent to diaphragm mechanics. Although the biaxial stress-strain relations for membranous connective tissue such as skin and mesentery have been measured (6), the biaxial constitutive relations for diaphragm muscle have not been studied extensively. We know of one study of the mechanical properties of excised diaphragm (9). This study was limited to a single combination of loads in the two directions, and an elastic constitutive relation was obtained by fitting an equation to the data.

We find that the muscle sheet is more compliant and considerably more extensible in the direction of the muscle fibers than in the direction transverse to the fibers. The magnitude of diaphragm compliance in the direction of the muscle bundles is consistent with observations of muscle shortening during passive mechanical ventilation in vivo. However, the compliance that we measured in the transverse direction is contrary to our expectation. Because we have observed that strains in the transverse direction are small during breathing maneuvers in vivo (5), we had assumed that the muscle sheet was inextensible in that direction. The compliance in the transverse direction is larger than we had expected, and it appears that our present model of diaphragm mechanics must be modified in light of these data.

METHODS

Experimental. Left hemidiaphragms were excised from mongrel dogs that had been anesthetized by intravenous injection of pentobarbital sodium and killed by an overdose of pentobarbital sodium. The excised hemidiaphragm was immediately immersed in a muscle bath containing modified Krebs-Ringer solution bubbled with oxygen at room temperature. A 5 × 5-cm-square section was cut from the midcostal region of the left hemidiaphragm with the sides of the square aligned along the direction of the muscle fibers and perpendicular to the fiber direction. The sample included thin sections of connective tissue at the ends of the muscle fibers where the muscle is attached to the central tendon and chest wall; thus the attachment to the connective tissue was left intact. Position markers were attached to the pleural surface of the sample by the following method. A single overhand knot was tied at one end of a piece of size 4–0 black suture thread, the thread was drawn through the diaphragm until the knot was snug against the pleural surface, and the thread was secured to the peritoneal side by a surgical knot. Four markers were placed at the corners of a square with sides of ~1 cm in the center of the sample.

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The biaxial testing apparatus is depicted in Fig. 1. It consists of two motor-transducer pairs aligned at right angles. Each pair contains a stepper motor opposed by a force transducer (model FT10, Grass Instruments). A muscle bath, through which oxygenated Ringer solution was circulated, was situated between the motor-transducer pairs. A video camera was located above the bath, and at the end of each experiment a scale was filmed to calibrate the distance of the camera from the muscle.

The diaphragm sample was transferred to the muscle bath, and 10 small fishhooks were attached at 5-mm intervals along the edges of the sample. Threads from the hooks along each side were led to a carriage, and lines from each of the four carriages were connected to the motor or force transducer that faced the edge. The attachments were adjusted to remove slack from all the lines.

The force in the direction transverse to the fibers was adjusted to zero, and, after one stretch and release along the direction of the muscle, data from both force transducers and the video camera were recorded during stretching along the fiber direction for forces up to 300 g. The transverse stretch was increased, first until the transverse load increased to 200 g, and then to 600 and 1,000 g. At each transverse load, data were obtained for stretch along the fiber direction. Four samples were tested after this protocol. For two additional samples, the force along the fiber direction was fixed at a series of values from 50 to 250 g, and data were obtained during continuous stretch in the transverse direction at each fixed load along the fiber direction.

Marker displacement was monitored by a CCTV video camera (Hitachi HV-720U, Edmund Scientific) and recorded on tape by a video cassette recorder (Sony SLV-620HF). Forces were amplified (Validyne C019A system), collected at 10 Hz with the use of a data-acquisition board (Lab-PC-1200/AI, National Instruments), and recorded with the use of LabVIEW version 5.0 software. The force data were stored in a desktop computer (Dell Pentium 200 MMX). The video of marker displacement was digitally captured with capturing software (Video Work version 1.5 and VidEdit version 1.1) and a video capture card (Captivator PCI version 9.0, Video Logic) with the use of a frame grabber at a sampling rate of 2 Hz. Precise marker coordinates on a Cartesian coordinate plane were obtained by using a unique free-image processing and analysis software (ImageTool version 2.00, http://www.ddsdx.uthscsa.edu). Muscle length was computed from the markers’ coordinates.

Values of membrane stress in the two directions were obtained by dividing the measured force by the unstressed length of the sample, 5 cm. Values of strain were obtained by subtracting the unstressed length from the stressed length and dividing by the unstressed length.

Modeling. The muscle sheet was modeled as a two-dimensional elastic membrane, and the standard scheme (8) for describing the constitutive properties of nonlinear elastic materials was followed. The strain energy function $W(\varepsilon_1, \varepsilon_2)$ is defined as the energy per unit area stored in the sheet due to elastic deformation of the material. This energy density is a function of the principal strains, $\varepsilon_1$ and $\varepsilon_2$. The strains are defined as follows

$$\varepsilon_1 = (L_1 - L_{10})/L_{10}, \quad \varepsilon_2 = (L_2 - L_{20})/L_{20} \quad (1)$$

where $L_1$ and $L_2$ are the distances between the markers along the muscle bundles and transverse to the muscle bun-
strain relations to the incremental change in W yields the following stress- 
eundeformed state. Thus the incremental work done by the stresses 
during the deformation. The energy balance in which the 
changes in the distances between material points in the sheet 
data for dog 4 
transverse stretches: 0, 40, or 100 g/cm. 
data taken while the diaphragm was subjected to 1 of 3 different 
Table 1. 

Fig. 3. Stresses $\sigma_1$ (A) and $\sigma_2$ (B) predicted from Eq. 4 fitted to the data for dog 4 vs. measured stress. Each series of points represents data taken while the diaphragm was subjected to 1 of 3 different transverse stretches: 0, 40, or 100 g/cm.

delles, respectively, and $L_{110}$ and $L_{20}$ are the distances in the undeformed state. Thus $\epsilon_1$ and $\epsilon_2$ are simply the fractional changes in the distances between material points in the sheet during the deformation. The energy balance in which the incremental work done by the stresses $\sigma_1$ and $\sigma_2$ is set equal to the incremental change in W yields the following stress-strain relations

$$\sigma_1 = \partial W/\partial \epsilon_1 \quad \sigma_2 = \partial W/\partial \epsilon_2 \quad (2)$$

where stress $\sigma_1$ is the force in the direction of the muscle bundles per unit length in the transverse direction, and $\sigma_2$ is the same for the transverse direction, mutatis mutandis.

We assumed that the strain energy function was the sum of quadratic and quartic functions of strain

$$W = \frac{1}{2} a_{11} \epsilon_1^2 + a_{12} \epsilon_1 \epsilon_2 + \frac{1}{2} a_{22} \epsilon_2^2$$

$$+ \frac{1}{4} b_{11} \epsilon_1^4 + \frac{1}{2} b_{12} \epsilon_1^2 \epsilon_2^2 + \frac{1}{4} b_{22} \epsilon_2^4 \quad (3)$$

where $a$ and $b$ are coefficients. The corresponding formulas for the stress-strain relations are as follows

$$\sigma_1 = a_{11} \epsilon_1 + a_{12} \epsilon_2 + b_{11} \epsilon_1^3 + b_{12} \epsilon_1 \epsilon_2^2$$

$$\sigma_2 = a_{21} \epsilon_1 + a_{22} \epsilon_2 + b_{12} \epsilon_1^2 \epsilon_2 + b_{22} \epsilon_2^3 \quad (4)$$

We began with a more general strain energy function that contained all quartic terms, but, in fitting the equation to the data, the coefficients of some terms were small and variable among the dogs, and these terms were removed to simplify the equations without serious loss of accuracy.

The coefficients in Eq. 4 were determined by minimizing the sum of the squares of the difference between the measured stress and the stress predicted by Eq. 2 for the full data set for each sample.

RESULTS

A representative set of stress-strain data is shown in Fig. 2. The following features of this example are representative of the data for all dogs. Stress along the muscle fibers increased continuously over the range of strains that were imposed. However, stress transverse to the muscle abruptly increased at a strain of ~0.35 and a stress of ~100 g/cm. That is, for the range of stresses and strains that were tested, no stop was reached in the muscle direction, but a definite stop was reached for extension in the transverse direction.

In fitting Eq. 4 to the data, the data for transverse stress that were beyond the stop in the transverse direction were not included. That is, we did not try to describe the abrupt change of slope in the transverse stress-strain curve at the stop. Thus, for the data shown for dog 4 in Fig. 2, data for three of the four transverse loads were fit. The comparison between the predicted and measured stresses for the three transverse loads for this dog is shown in Fig. 3.

The coefficients that were obtained from the fit of Eq. 4 to the data are listed in Table 1. The data for the first four dogs were obtained by imposing continuous stretch along the muscle and fixed stretches in the transverse direction. For the first three dogs, only two transverse stretches were below the stop, and these cases did not provide enough data to obtain values for $a_{22}$ and $b_{22}$. The data for dog 4 plus the data for dogs 5 and 6, in which continuous stretch was applied in the

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Table 1. Coefficients in Eq. 4 obtained by fitting these equations to the data

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>$a_{11}$</th>
<th>$a_{12}$</th>
<th>$a_{22}$</th>
<th>$b_{11}$</th>
<th>$b_{12}$</th>
<th>$b_{22}$</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>16</td>
<td></td>
<td>120</td>
<td>220</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>32</td>
<td>20</td>
<td></td>
<td>116</td>
<td>220</td>
<td></td>
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<tr>
<td>3</td>
<td>52</td>
<td>18</td>
<td></td>
<td>40</td>
<td>120</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>4</td>
<td>100</td>
<td>76</td>
<td>200</td>
<td>1,600</td>
</tr>
<tr>
<td>5</td>
<td>46</td>
<td>44</td>
<td>64</td>
<td>44</td>
<td>40</td>
<td>1,800</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>32</td>
<td>56</td>
<td>220</td>
<td>180</td>
<td>1,500</td>
</tr>
<tr>
<td>Average ± SD</td>
<td>42 ± 9</td>
<td>22 ± 14</td>
<td>73 ± 23</td>
<td>103 ± 67</td>
<td>163 ± 71</td>
<td>1,633 ± 153</td>
</tr>
</tbody>
</table>

Units of all coefficients ($a_{11}$, $a_{12}$, $a_{22}$, $b_{11}$, $b_{12}$, $b_{22}$) are g/cm.
transverse direction and fixed stretches in the muscle direction, provided sufficient data for determining values for all coefficients.

**DISCUSSION**

From measurements of stress and strain during bi-axial loading of the passive diaphragm, we have obtained a strain energy function and stress-strain laws that describe the mechanical properties of the passive muscle sheet of the midcostal canine diaphragm. The linear behavior of the diaphragm for small strains and stresses is described by the coefficients $a_{11}$, $a_{12}$, and $a_{22}$. Coefficient $a_{11}$ describes the stiffness along the muscle direction for fixed strain in the transverse direction, $a_{22}$ describes the stiffness transverse to the fiber direction, and $a_{12}$ describes the coupling between stress in one direction and strain in the orthogonal direction. The diaphragm is anisotropic. That is, the value of $a_{22}$ is about twice the value of $a_{11}$, and thus, in the linear range, stiffness in the transverse direction is twice that in the muscle direction.

The nonlinear behaviors in the two directions are quite different. The stress-strain relation in the muscle direction is only mildly nonlinear over the sizable range of strains that we have obtained. The nonlinear stiffening in the transverse direction is stronger, and a sharp stop in extensibility occurs at a transverse strain of $\sim 0.35$.

Boriek et al. (5) and Wilson et al. (11) measured muscle shortening in the midcostal region of the diaphragm of dogs during passive inflation from functional residual capacity (FRC) to total lung capacity (TLC), and the stiffness in the muscle direction that we obtained from in vitro testing can be related to these observations of muscle shortening in vivo. In the midcostal region, curvature in the direction perpendicular to the muscle fibers is small, and transdiaphragmatic pressure ($P_{di}$) is related to stress in the muscle, $\sigma_{ir}$, and radius of curvature of the muscle, $r$, by the following equation

$$P_{di} = \sigma_{ir}/r$$

(5)

Assuming that $P_{di} = 5 \text{ cmH}_2\text{O}$ at FRC and using the value $r = 5 \text{ cm}$ (4), we obtain the value $\sigma_{ir} = 25 \text{ g/cm}$ at FRC. Assuming that $e_{2} = 0$ and using the values of $a_{11}$ and $b_{11}$ given in Table 1 yields muscle lengths of 1.42 $L_{10}$ at FRC. If $P_{di} = 0$ and muscle length $= L_{10}$ at TLC, the muscle would shorten by 30%, relative to its length at FRC, during passive inflation from FRC to TLC. Muscle shortening in vivo is $\sim 28 \pm 5 \%$. Thus the in vitro data and the in vivo data on passive muscle shortening agree reasonably well.

The properties of the muscle sheet in the direction transverse to the muscle direction are different from those that we had expected from observations of strains in the diaphragm in vivo. Strains in the direction perpendicular to the muscle are zero during both passive inflation and spontaneous breathing. Our laboratory (1) and others (7, 10) have assumed that the muscle sheet carries stress in both directions, and we thought that the observation of zero strain in the transverse direction implied that the diaphragm is inextensible in that direction. Our in vitro tests show that it is not. If $\sigma_{ir}$ were of the same order as $\sigma_{i}$, we would expect from Eq. 4 that $e_{2} = 0.14$ at FRC and that transverse dimensions would shorten by $\sim 12\%$ during lung inflation. Thus we now conclude that transverse strain in vivo is small because transverse stress is small, not because the diaphragm is inextensible in that direction.

The stress distribution in the diaphragm depends on the shape of the diaphragm. In our modeling of diaphragm shape, we have assumed, for simplicity, that the muscle bundles lay along circular arcs, and, with this assumption, we were able to fit data on the shape reasonably well (2). However, a membrane formed by circular arcs of radius $r$ and loaded by a uniform pressure $P$ carries a transverse stress of $Pr/2$ (3). Thus it appears that the shape of the arcs of the muscle bundles in our model must be modified. The shape of the arcs must be consistent with a diaphragm shape for which transverse stress is negligible. We would like to note that the data reported here describe the properties of the passive diaphragm, and the muscle activation may affect the stress-strain relation in the transverse direction as well as in the direction of the muscle fibers. Therefore, we cannot draw any conclusions about stress in the active diaphragm. However, for the passive state, we conclude that stresses in the transverse direction in vivo are much smaller than stresses along the muscle and that the shape of the diaphragm must be consistent with this particular stress distribution.

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**REFERENCES**


