Diaphragm curvature modulates the relationship between muscle shortening and volume displacement

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Greybeck BJ, Wettergreen M, Hubmayr RD, Boriek AM. Diaphragm curvature modulates the relationship between muscle shortening and volume displacement. Am J Physiol Regul Integr Comp Physiol 301: R76–R82, 2011. First published March 23, 2011; doi:10.1152/ajpregu.00673.2010.—During physiological spontaneous breathing maneuvers, the diaphragm displaces volume while maintaining curvature. However, with maximal diaphragm activation, curvature decreases sharply. We tested the hypotheses that the relationship between diaphragm muscle shortening and volume displacement (VD) is nonlinear and that curvature is a determinant of such a relationship. Radiopaque markers were surgically placed on three neighboring muscle fibers in the midcostal region of the diaphragm in six dogs. The three-dimensional locations were determined using biplanar fluoroscopy and diaphragm VD, curvature, and muscle shortening were computed in the prone and supine postures during spontaneous breathing (SB), spontaneous inspiration efforts after airway occlusion at lung volumes ranging from functional residual capacity (FRC) to total lung capacity, and during bilateral maximal phrenic nerve stimulation at those same lung volumes. In supine dogs, diaphragm VD was approximately two- to three-fold greater during maximal phrenic nerve stimulation than during SB. The contribution of muscle shortening to VD nonlinearly increases with level of diaphragm activation independent of posture. During submaximal diaphragm activation, the contribution is essentially linear due to constancy of diaphragm curvature in both the prone and supine posture. However, the sudden loss of curvature during maximal bilateral phrenic nerve stimulation at muscle shortening values greater than 40% (ΔL/LFRC) causes a nonlinear increase in the contribution of muscle shortening to diaphragm VD, which is concomitant with a nonlinear change in diaphragm curvature. We conclude that the nonlinear relationship between diaphragm muscle shortening and its VD is, in part, due to a loss of its curvature at extreme muscle shortening.

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Various physiological maneuvers (e.g., airway occlusion and maximal phrenic nerve stimulation) were used to elicit maximal contractions of the diaphragm in vivo. The three-dimensional locations of the markers were determined by biplanar fluoroscopy and a computational modeling approach (1, 12, 13, 16, 23). A total of 18 dogs were used for this study, and the results were analyzed using a series of statistical tests. The results indicate that the relationship between diaphragm muscle shortening and volume displacement is nonlinear and that curvature is a determinant of such a relationship. In addition, we hypothesized that this nonlinearity is primarily a consequence of loss of curvature at high levels of muscle shortening.

Methods

Imaging. Six bred-for-research beagle dogs with body masses ranging from 7.0 to 8.6 kg were studied using the same methods that were used in earlier studies (2–4). Dogs were maintained according to the National Institutes of Health’s “Guide for the Care and Use of Laboratory Animals,” and all procedures were approved in advance by the Institutional Animal Care and Use Committees at Baylor College of Medicine and at Mayo Clinic. The dogs were anesthetized with pentobarbital sodium (60–80 mg/kg), and 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 hemidiaphragm. Five markers were placed along each of three nearby muscle bundles: one at the origin of each muscle bundle on the central tendon, one at its insertion on the chest wall, and three markers were placed at equal intervals along each muscle bundle. The animals were then allowed to recover for 3 to 4 wk.
Inducing ventilation maneuvers. The animals were anesthetized with pentobarbital sodium (30 mg/kg), intubated with a cuffed endotracheal tube, and placed in the supine or prone posture in the test field of a biplanar fluoroscopic recording system. Supplemental doses of pentobarbital were injected to abolish the eyelash reflex and to reestablish regular breathing. Given that adequate ventilation, measured by chest wall movement, and a normal appearance of the mucous membranes, we did not independently monitor arterial oxygenation. Biplanar images were recorded continuously using high spatial (± 0.5 mm) and temporal (30 Hz) resolution of the radiopaque metallic markers. Images during five spontaneous breaths were recorded and average marker locations were collected at end-expiration (functional residual capacity, or FRC) and at end-inspiration during quiet spontaneous breathing (SB). Mechanical ventilation was then initiated with a volume mechanical ventilator that was adjusted to provide the same tidal volume and essentially the same frequency as that during quiet SB. The lungs were then inflated, and the airway was occluded successively at three different lung volumes [FRC, FRC+ .5 inspiratory capacity (IC), and total lung capacity (TLC)]. The airway was held occluded until the change of airway pressure reached a plateau, usually during the fifth or sixth inspiratory effort at each lung volume. The occlusion time varied between 2 and 3 s at FRC, 8 and 12 s at FRC+ .5 IC, and 15 and 20 s at TLC. Following each occluded effort, the airway was then opened, biplane images were recorded, and the average marker positions obtained during five spontaneous efforts were computed. The animal was then rotated to the opposite posture, and the procedure was repeated.

Bilateral phrenic nerve stimulation. The spinal roots of the phrenic nerves (C5, C6) were identified and isolated on both sides of the neck in the supine posture. Insulated hook electrodes were placed under the nerve roots, and the preparation was covered with mineral oil. Synchronous twitch stimulations (pulse duration of 0.2 ms) were applied to the nerve roots using a dual nerve stimulator (S 88; Grass Instruments, Quincy, MA) to establish supramaximal stimulation conditions in all dogs. Compound muscle action potential amplitudes reached a maximum and the procedure was repeated.

Computation of diaphragm VD. Diaphragm VD was computed by importing the three-dimensional positions of each marker into Rhinoceros, a modeling software package. The diaphragm surface was generated at FRC by fitting a surface through the three-dimensional coordinates of the markers. The coordinates were in the form of a three × five point grid, corresponding to five markers on each of three adjacent fibers of the midcostal diaphragm muscle. The surface fitting process was repeated for all initial FRC and active final states of the diaphragm muscle. For example, Fig. 1 shows the corresponding surfaces at initial FRC and at end of inspiration (i.e., active final state for SB) in a representative dog. As shown, the two surfaces intersect due to lateral and caudal displacements of the line of insertion on the chest wall. We define the volume enclosed by the surfaces at the initial FRC state and final active state as the total volume displaced by the diaphragm. This volume has two components; the first, we termed rib cage VD and is due to the action of the diaphragm on the rib cage. The second component, termed the abdominal VD, is due to the action of the diaphragm on the abdominal contents. To calculate these two components of diaphragm VD, we generated two different volumetric solids (Fig. 2) by using line segments to connect the markers between the initial FRC state and the final active state. One solid represents the rib cage VD component, and the other represents the abdominal VD component.

Computation of diaphragm muscle shortening. The lengths of the three adjacent muscle fibers in the midcostal region of the diaphragm were computed at the initial FRC and final active states of each ventilation maneuver using the three-dimensional coordinates obtained from the biplane images. Distances between adjacent markers were calculated and then summed to represent the muscle fiber length. The fractional muscle shortening was computed as the ratio of the change in muscle fiber length between the initial FRC and final active states to the muscle fiber length at FRC.

Computation of diaphragm radius of curvature. Muscle curvature was computed based on previous methods (2, 3). Briefly, a quadratic equation in the following form: \( \zeta = a_0 x^2 + b_0 y^1 + c_0 x^0 + d_0 y^0 + e_0 y^0 + F \) was fit to the data by a multiple least-squares regression technique. Principal coordinates \( \xi^0, \eta^0, \zeta^0 \) were determined by removing the cross terms and providing a quadratic in the following form \( \zeta^0 = 1/2C_1\xi^2 + 1/2C_2\eta^2 + F^0 \). The coefficients \( C_1 \) and \( C_2 \) were the principal curvatures of the quadratic fit to the data, and the maximum curvature was inverted to provide the radius of curvature of the midcostal diaphragm along the direction of maximum principal curvature.

Statistical analysis. Statistical analyses were done using the Statistical Package for the Social Sciences. Two models were run on the data; a repeated-measures two-way ANOVA design on all active maneuvers, excluding supine maximal stimulation data. All submaximal and maximal phrenic nerve stimulation maneuvers were then

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**Fig. 1.** Two views of the midcostal diaphragm three-dimensional surfaces for two states of respiration are shown. **A**: ventrodorsal/cephalad view. **B**: lateral/cephalad view. The configuration of the diaphragm was obtained by fitting a surface to 15 markers attached to three adjacent muscle fibers of the midcostal region generating a 3 × 5 point grid for each ventilation state. This grid was fitted by a nonuniform rational b-spline, or NURBS, surface in Rhinoceros. The dark surface represents the initial functional residual capacity (FRC) state for one of the dogs in the prone posture during spontaneous breathing (SB). The light surface represents the final end of inspiration state for the same dog in the prone posture. The two surfaces intersected in most of the ventilation maneuvers.
open airway breathing after occlusion at FRC (P < 0.05). Furthermore, a one-way ANOVA analysis within the supine posture showed that each of the maximum phrenic nerve stimulation mean total diaphragm VD values were significantly greater than each of the other physiological active modes of ventilation (P < 0.05).

Diaphragm muscle shortening. Data in Fig. 4 show the percentage of midcostal diaphragm muscle shortening relative to muscle fiber lengths at FRC during quiet SB and during open airway breathing after occlusion at FRC, FRC + .5 IC, and TLC in the prone and supine posture. Using a two-way repeated-measures ANOVA, there was a main effect of mode of ventilation. Tukey HSD post hoc testing showed that SB was significantly different than open airway breathing after occlusion at TLC independent of posture (P < 0.05). Using a one-way ANOVA analysis within each posture showed that open airway breathing after occlusion at FRC + .5 IC was significantly different than SB for both postures (P < 0.05). However, in the supine posture, there was a significant difference between open airway breathing after occlusion at FRC + .5 IC and open airway breathing after occlusion at FRC (P < 0.05).

Relationship between diaphragm muscle shortening and total VD. Data in Fig. 5 show the relationship between midcostal diaphragm total VD and its muscle shortening. Our data show that total VD and muscle shortening were independent of posture. Furthermore, mode of ventilation had an effect on both VD and muscle shortening. Therefore, we had a sufficient data range to determine a relationship between diaphragm VD and its muscle shortening. The VD continuously and nonlinearly increased over the range of examined muscle shortening. While a linear line provided a good fit to the submaximal stimulation modes (data not shown), it did not capture the nonlinear increase of VD with muscle shortening values obtained from bilateral maximal phrenic nerve stimulation. The raw data were fitted with a quadratic relationship and a strong positive correlation between muscle shortening and VD was found (r² = 0.78).

Diaphragm curvature. To determine whether the significant increase in diaphragm VD with its muscle shortening during bilateral maximal phrenic nerve stimulation might be affected by diaphragm shape, we quantified the diaphragm radius of curvature according to procedures developed previously in our laboratory (2). We extended these data to include open airway breathing after occlusion at FRC, FRC + .5 IC, and TLC. Figure 6 shows the relationship between midcostal diaphragm radius of curvature and muscle shortening. A linear exponential equation fit the data well. While the radius of curvature is constant during all submaximal active breathing maneuvers, there is a nonlinear increase in the radius of curvature with the onset of maximal bilateral phrenic nerve stimulation.

DISCUSSION

The purpose of this study was to identify novel determinants of the relationship between diaphragm muscle shortening and its VD. Previous studies have used geometric models and various imaging methods to investigate diaphragm VD and its relationship to muscle shortening (12, 13, 16, 23). However, these studies did not include a broad range of muscle shortening values and were limited to a single posture. Furthermore, diaphragm curvature was not measured. Here, we measured diaphragm curvature, muscle shortening, and VD of the mid-
costal diaphragm during both submaximal and maximal activation in the supine and prone postures. We showed that during submaximal activation diaphragm VD is essentially linearly dependent on muscle shortening, independent of posture. However, during maximal activation, there is a change in the contribution of muscle shortening to VD, resulting in a non-linear increase.

Computation of diaphragm VD. Earlier work from our laboratory has documented significant lateral and caudal displacements of the diaphragm line of insertion on the CW during passive inflation to TLC in supine dogs (5). In addition, we previously assessed the effect of muscle shortening on the lateral and caudal displacements of the diaphragm’s line of insertion in a kinematic model during submaximal activation of the diaphragm (1, 4). We showed that lateral displacement of the CW insertion is a mechanism by which diaphragm curvature is maintained (1). Consistent with these studies, our data demonstrated similar lateral and caudal movements of the diaphragm line of insertion during submaximal activation of the diaphragm, and therefore, we were able to compute two components of diaphragm VD. The more lateral diaphragm VD component, termed rib cage VD, is consistent with observations of lateral and cephalic movement of the diaphragm’s

Fig. 3. Means ± SE are shown for large tidal breathing with airway open following occluded efforts in the prone and supine posture at FRC, FRC + 5 IC, total lung capacity (TLC), quiet SB for the two postures, and 50-Hz bilateral phrenic nerve stimulation at the occluded lung volumes. Open airway breathing after occlusion at FRC + 5 IC is greater than SB regardless of posture and were greater than supine and prone SB (&P < 0.05), and supine open airway breathing after occlusion at FRC (&P < 0.05). Comparisons made within the supine posture showed that stimulation at FRC + 5 IC and TLC were equal and greater than all other supine breathing modes (*P < 0.05). Comparisons made within the supine posture showed that stimulation at FRC is equal to the other two lung volumes at which stimulation occurs and is greater than all other supine breathing modes except open airway breathing after occlusion at FRC + 5 IC (SP < 0.05).

Fig. 4. Means ± SE of midcostal diaphragm muscle shortening (% ΔL/LFRC) for open airway breathing after occlusion at the three lung volumes (FRC, FRC + 5 IC, and TLC) in SB in the prone and supine postures and for 50-Hz bilateral phrenic nerve stimulation at the occluded lung volumes. Open airway breathing after occlusion at FRC + 5 IC and at TLC are equal regardless of posture and were greater than supine and prone SB (&P < 0.05), and supine open airway breathing after occlusion at FRC (SP < 0.05). Comparisons made within the supine posture showed that stimulation at FRC + 5 IC and TLC were equal and greater than all other supine breathing modes (*P < 0.05). Comparisons made within the supine posture showed that stimulation at FRC is equal to the other two lung volumes at which stimulation occurs and is greater than all other supine breathing modes except open airway breathing after occlusion at FRC + 5 IC (SP < 0.05).

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line of insertion on the chest wall during physiological SB (4). During maximal stimulation of the phrenic nerves, caudal displacement of the diaphragm line of insertion appears to become significantly greater than any lateral movement (14). This is accompanied by significant decreases in diaphragm curvature (2) and is consistent with our results that show a large abdominal VD component with essentially no rib cage VD during maximal phrenic nerve stimulation.

Effect of posture and mode of ventilation on diaphragm VD and muscle shortening. Our results show that posture did not significantly affect the abdominal VD, rib cage VD, or total VD components. However, at low lung volume ventilation maneuvers (i.e., SB and open FRC), there was a pattern of greater diaphragm abdominal VD, total VD, and less rib cage VD in the prone posture compared with the supine posture. Consistent with these observations, Chu et al. (6) reported that posture is an important determinant of VB of the rib cage and diaphragm. However, only SB and maximal stimulation were analyzed in that study. Our data suggest that at higher lung volumes and greater levels of muscle activation, posture continues to be an important determinant of lower rib cage kinematics. In contrast to our diaphragm rib cage VD data, Chu et al. (6) showed that the lower ribs displaced greater volume in the prone than the supine posture. However, their VD component is the combination of three different rotations around the spinal axis, and there may be greater cephalic displacement of the lower ribs in the prone posture causing the large diaphragm rib cage VD component shown in our study. The pattern of decreased diaphragm rib cage VD component found in our study in the prone posture could be due to posture differences in chest wall compliance causing rib and diaphragm line of insertion movement during submaximal ventilation modes to be different between postures (1).

In addition, our results show that diaphragm muscle shortening was independent of posture consistent with results from Sprung et al. (20), who found similar diaphragm shortening between the prone and supine postures during quiet SB. Our results additionally show similar diaphragm muscle shortening between supine and prone postures at ventilation maneuvers with higher levels of diaphragm muscle activation. Effect of maximal phrenic nerve stimulation on diaphragm VD. Maximal phrenic nerve stimulation maximally activates the diaphragm, allowing for increased muscle shortening and dome descent with a reduced diaphragm load given that the active muscles of the rib cage are silent. This condition is similar to those during chronic obstructive pulmonary disease (COPD) where diaphragm load is decreased and the rib cage is expanded (8, 18). In this study, we demonstrated that during bilateral phrenic nerve stimulation, the percentage of diaphragm muscle shortening, abdominal VD, and total VD values were two- to three-fold greater than those that occurred during submaximal diaphragm activation. In addition, we found nearly equal diaphragm muscle shortening values and equal VD across the three lung volumes at which stimulation occurred. These results are consistent with a study by Hubmayr et al. (10), who also showed equal diaphragm muscle fiber shortening occurring during maximal bilateral stimulation of the diaphragm with the airway occluded at lung volumes spanning the IC in the supine dog (10).

We also found that the diaphragm rib cage VD component during bilateral phrenic nerve stimulation was virtually absent at the three different lung volumes. During higher levels of diaphragm activation, the height of the zone of apposition of the diaphragm decreases markedly so that most of the lower rib cage becomes exposed to the inspiratory effect of pleural pressure, rather than the inspiratory effect of abdominal pressure. However, during bilateral maximal phrenic nerve stimulation, this inspiratory effect appears to be absent, and the rib cage moves inward and caudally, consistent with the absent rib cage VD component shown in this study and decreased rib VD found previously (6). This is also consistent with a study by Krayser et al. (13), who suggested that diaphragm shortening had no inspiratory effect on the rib cage during maximal stimulation. The absence of such an inspiratory effect may
cause the decreases in curvature observed in our current study, as well as in our previous study (2).

**Relationship between total VD and muscle shortening.** We show a continuous, linear increase in total VD of the diaphragm as a function of muscle shortening up to 40% ($\Delta L/L_{FRC}$). With shortening of nearly 40% or more, the total diaphragm VD increases sharply for small increments in muscle shortening. While a polynomial quadratic fit to the data in the figure only provided a modest increase in the adjusted $R^2$ value (linear adjusted $R^2 = 0.758$ vs. quadratic adjusted $R^2 = 0.775$), we still obtained a better fit. We believe that the modest increase in $R^2$ is mainly because of the nonlinearity present at muscle shortening near 40% ($\Delta L/L_{FRC}$). At levels of diaphragm muscle activation that are less than maximal, curvature is essentially constant (Fig. 6), and therefore, diaphragm shape is independent of either its VD or muscle shortening, which is consistent with findings by Road et al. (17). The linear portion of the relationship between diaphragm VD and its muscle shortening is also consistent with previous studies investigating the relationship between diaphragm VD and muscle shortening (12, 16). Using geometric estimates of diaphragm-swept volume, empirically determined parameters from anteroposterior fluoroscopic images, and muscle lengths from two radiopaque markers sutured along a costal fiber bundle, Petroll et al. (16) and Knight et al. (12) reported moderately strong coefficient of correlations ($r^2 = 0.74$, $r^2 = 0.82$, respectively) between diaphragm VD and costal muscle shortening during quiet SB in supine dogs. It is interesting to note that our results are similar to those reported by both studies, even though we only examined the midcostal region of the diaphragm. However, unlike the above studies, we included a wide range of diaphragm muscle shortening that covers both submaximal and maximal stimulation. While both studies varied the degree of muscle shortening from 0 to 18.4% $L_{FRC}$ for the costal diaphragm, our study provided muscle shortening values up to nearly 50%.

**Diaphragm radius of curvature and total VD.** Boriek et al. (4) reported that during spontaneous inspiratory efforts against an occluded airway, muscle shortened by 15–40% of length at FRC, but curvature remained nearly unchanged. We additionally show that during large tidal breathing following occlusion that curvature still is essentially constant. However, Boriek et al. showed that during phrenic nerve stimulation, diaphragm muscle shortened by 30 to nearly 50%, and for shortening, ~40% or higher curvature decreased sharply (2). Similarly, our data show a sharp decrease in curvature at nearly the same level of muscle shortening. Interestingly, this loss of curvature at high muscle shortening corresponds to a sharp nonlinear change in the contribution of diaphragm muscle shortening to its total VD. Therefore, we speculated that loss of curvature during maximal phrenic nerve stimulation is a modulator of the relationship between diaphragm muscle shortening and its VD, at least in the midcostal region. However, there is also significant caudal displacement of the line of insertion, and this movement may also allow diaphragm VD values to become larger than during any other submaximal mode of ventilation. It appears that the substantial increase in VD with high muscle shortening during phrenic nerve stimulation is not entirely due to muscle shortening, but rather to both the caudal displacement of the line of insertion and because of the decrease in curvature.

**Perspectives and Significance**

In this study, we simultaneously measured midcostal diaphragm muscle shortening, its VD, and its radius of curvature across multiple active breathing maneuvers in the supine and prone postures. Our data support the hypothesis that the relationship between muscle shortening and VD is nonlinear and, at maximal stimulation, curvature is a modulator of the contribution of muscle shortening to VD. We showed that muscle shortening has a greater contribution to total VD at maximum levels of muscle activation where the curvature of the diaphragm decreases sharply, which is accompanied by the nonlinear relationship between muscle shortening and diaphragm curvature. Our previous work and data from the current study showed that during passive and active breathing maneuvers, there was a caudal displacement of the diaphragm line of insertion (5). In addition, the De Troyer group has shown a significant caudal displacement during maximal stimulation of the diaphragm (7). The study by De Troyer et al. (7) predicted that the caudal displacement of the line of insertion contributed 25% of the change in pleural pressure during maximal diaphragm activation. We speculate that diaphragm shape change may facilitate and perhaps contribute to this percentage change. During COPD conditions in humans, there is an enlargement of the ring of insertion. This enlargement may be compensated by the diaphragm becoming flatter with a premature loss of curvature to maintain sufficient VD during breathing maneuvers. This speculation is supported by our data shown in Fig. 7. These data show that for any particular level of muscle shortening there is no increase in diaphragm VD unless the radius of curvature increases beyond 15 cm. The loss of diaphragm curvature could potentially be an additional mechanism that could contribute to the fall in pleural pressure during maximal stimulation of its muscles.

![Fig. 7. Effects of muscle shortening (% $\Delta L/L_{FRC}$) and radius of curvature (cm) on midcostal diaphragm VD. Using a least-squares surface fit routine in MATLAB, the following equation was obtained: VD = $a + b \cdot (\% \Delta L/L_{FRC}) + c \cdot (\% \Delta L/L_{FRC})^2 + d \cdot \exp(\text{radius of curvature})$, where a, b, c, and d were coefficients determined from the fitting routine. During most submaximal ventilation maneuvers the radius of curvature is constant and only muscle shortening has an effect on diaphragm VD. At high radius of curvature values VD increased significantly, independent of the percentage of muscle shortening. The resulting best-fitted surface shown represents diaphragm VD as a function of muscle shortening and the radius of curvature, and it was constructed using the raw data from each dog. The solid points represent individual experimental values obtained from dogs.](http://ajpregu.physiology.org/10.1152/ajpregu.00034.2011)
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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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