Diaphragm curvature modulates the relationship between muscle shortening and volume displacement
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Running head: Determinants of diaphragm volume displacement
ABSTRACT

During physiologic 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 VD is nonlinear and that curvature is a determinant of such relationship. Radiopaque markers were surgically placed on three neighboring muscle fibers in the mid-costal region of the diaphragm in six dogs. The three-dimensional locations were determined using bi-planar 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 to total lung capacity, and during bilateral maximal phrenic nerve stimulation at those same lung volumes. In supine dogs, diaphragm VD was approximately five 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 sub-maximal 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.
INTRODUCTION

During inspiration requiring sub-maximal diaphragm activation, muscle bundles of the diaphragm shorten, the dome descends displacing volume and causing the lungs to inflate. As the lungs inflate and the diaphragm muscle shortens, curvature is constant and transdiaphragmatic pressure (Pdi) decreases proportionately with diaphragm muscle fiber tension (2). It has been generally accepted that during breathing diaphragm volume displacement (VD) is primarily caused by muscle shortening (12, 13, 16, 23). This is evidenced by studies showing a linear relationship between costal shortening and diaphragm VD using geometric diaphragm models incorporating empirical parameters (12, 16). However, these studies only computed VD values during spontaneous breathing (SB) in the supine posture when curvature is constant (4). Dynamic Spatial Reconstruction (DSR) imaging has also been used in the computation of diaphragm VD (13, 23). However, these studies used a small range of muscle shortening and did not determine the relationships between diaphragm VD and muscle shortening. In addition, all the above studies did not include the role of diaphragm curvature or posture as factors in determining the relationship between diaphragm muscle shortening and its VD.

During maximal diaphragm activation caused by maximal phrenic nerve stimulation, Pdi decreases much more rapidly (11) than the diaphragm muscle tension-length curve, presumably because diaphragm curvature decreases (2).
while its line of insertion on the chest wall moves caudally (14). How an alteration in diaphragm curvature and movement of the line of insertion modulate the contribution of diaphragm muscle shortening to its VD is not entirely clear. During maximal activation the diaphragm can no longer be modeled as a piston in a cylinder and the relationship between muscle shortening and diaphragm VD should deviate from a linear relationship. In this study we tested the hypothesis that the relationship between diaphragm muscle shortening and VD is nonlinear and that curvature is a determinant of such relationship. In addition, we hypothesized that this nonlinearity is primarily a consequence of loss of curvature at high levels of muscle shortening.

To test these hypotheses we used a computational modeling approach to fit least squares surfaces to markers sutured to the peritoneal surface along muscle fibers of the mid-costal region of the diaphragm. We quantified diaphragm muscle shortening, VD, and curvature across increasingly active ventilation maneuvers in the prone and supine postures. We determined the relationship of diaphragm muscle shortening to its VD during those breathing modes. The analysis of our data uncovered a novel mechanism by which curvature, in part, modulates the relationship between diaphragm muscle shortening and its VD.

**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 “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 mid-costal 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 three to four weeks.

*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 bi-planar fluoroscopic recording system. Supplemental doses of pentobarbital were titrated to abolish the eyelash reflex and to re-establish 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. Bi-planar 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 (FRC) and at end-inspiration during quiet 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 2-3 seconds at FRC; 8-12 second at FRC+.5 IC, and 15-20 seconds at TLC. Following each occluded effort the airway was then opened and bi-plane 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 Instrument Co., Quincy, MA) to establish supra-maximal stimulation conditions in all dogs. Compound muscle action potential amplitudes reached a maximum at stimulation intensities below 30 V (9). We continuously recorded bi-plane images in the supine posture during bilateral phrenic nerve stimulation at lung volumes spanning the vital capacity: FRC, FRC+.5 IC, and TLC. The phrenic nerve of one dog was not adequately stimulated and the data for this maneuver was not included in the analyses.

**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 by five point grid corresponding to five markers on each of three adjacent fibers of the mid-costal diaphragm muscle. The surface fitting process was repeated for all initial FRC and active final states of the diaphragm muscle. For example, Figure 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 (Figure 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 mid-costal 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 bi-plane 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 in the following form: \( \xi = a\xi^2 + b\eta\xi' + c\zeta + d\xi^2 + e\zeta^2 + F \), was fit to the data by a multiple least squares regression technique. Principal coordinates \( \xi, \eta, \) and \( \zeta \) were determined by removing the cross terms and providing a quadratic in the following form \( \xi = 1/2C_1 \eta + 1/2C_2 \zeta + F \). 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 mid-costal diaphragm along the direction of maximum principal curvature.

Statistical analysis. Statistical analyses were done using the Statistical Package for the Social Sciences (SPSS). Two models were run on the data; a repeated measures two way analysis of variance (ANOVA) design on all active maneuvers excluding supine maximal stimulation data. All sub-maximal and maximal phrenic nerve stimulation maneuvers were then compared within the supine posture using a one way repeated measures ANOVA design. Post-hoc testing was accomplished with Tukey’s Honestly Significant Difference (HSD) or Bonferroni-Dunn corrections, when appropriate.

RESULTS

Diaphragm VD. Data in Figure 3 show the abdominal VD, rib cage VD, and total VD of the mid-costal diaphragm in the prone and supine postures for quiet SB,
large tidal SB during open airway breathing following occluded efforts at FRC, FRC+.5 IC, and TLC. In addition, diaphragm VD values are also shown for maximal bilateral stimulation of the phrenic nerves at three lung volumes (FRC, FRC+.5 IC, and TLC) in the supine posture.

**Abdominal diaphragm VD.** Using a Tukey HSD post-hoc procedure on the abdominal VD values there was a significant difference between open airway breathing after occlusion at FRC + .5 IC and SB, independent of posture (P < 0.05). A one way ANOVA followed by a Bonferroni-Dunn post-hoc analysis within the supine posture showed that all three maximal phrenic nerve stimulation mean values were equal and significantly greater than all other supine active modes.

**Diaphragm rib cage VD.** A one way ANOVA followed by a Bonferroni-Dunn post-hoc analysis on the rib cage VD values showed that the maximal phrenic nerve stimulation at FRC and FRC + .5IC were equal and were significantly smaller than values during open airway breathing after occlusion at FRC (P <0.05).

**Total diaphragm VD.** The total diaphragm VD was the sum of both VD components above. Bonferroni-Dunn post-hoc analysis following an ANOVA analysis showed that the total VD component within the supine posture, open airway breathing after occlusion at FRC + .5IC was greater than both SB and 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 Figure 4 shows the percentage of mid-costal 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 measure 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 Figure 5 show the relationship between mid-costal 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 no 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 sub-maximal 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 was fitted with a quadratic relationship and a strong positive correlation between muscle shortening and VD was found (r² = .78).

Diaphragm curvature. To determine if 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 lab (2). We extended this data to include open airway breathing after occlusion at FRC, FRC + .5IC, and TLC. Figure 6 shows the relationship between mid-costal diaphragm radius of curvature and muscle shortening. A linear exponential equation fit the data well. While the radius of curvature is constant during all sub-maximal 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 sub-maximal and maximal in the supine and prone postures. We showed that during sub-maximal 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 nonlinear increase.

**Computation of diaphragm VD.** Earlier work from our lab has documented significant lateral and caudal displacements of the diaphragm line of insertion on the chest wall 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 sub-maximal activation of the diaphragm (1, 4). We showed that lateral displacement of chest wall 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 sub-maximal activation of the diaphragm and we therefore 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 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 to the supine posture. Consistent with these observations, Chu et al. reported that posture is an important determinant of VD of the rib cage and diaphragm (6). 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 data, Chu et al showed that the lower ribs displaced greater volume in the prone than the supine posture (6). However, this 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 a large 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 sub-maximal 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 five fold greater than those that occurred during sub-maximal diaphragm activation. In addition, we found 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 who also showed equal diaphragm muscle fiber shortening across 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 zone of apposition of the diaphragm decreases markedly so that most of the lower rib cage becomes exposed to the expiratory 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 Krayer et al, who suggested that diaphragm shortening had no inspiratory effect on the rib cage during maximal stimulation (13). The absence of such an inspiratory effect may cause the decreases in curvature observed in our current study as well as 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% ($\Delta L/L_{FRC}$) or more, the total diaphragm VD increases sharply for small increments in muscle shortening. While a polynomial quadratic fit to the data in figure only provided a
modest increase in the adjusted $R^2$ value (linear adjusted $R^2 = .758$ vs Quadratic adjusted $R^2 = .775$), we still obtained a better fit. We believe that the modest increase in $R^2$ is mainly because of the non-linearity 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 2 radiopaque markers sutured along a costal fiber bundle, Petroll et al. and Knight et al. 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 (12,16). It is interesting to note that our results are similar to those reported by both studies even though we only examined the mid-costal region of the diaphragm. However, unlike the above studies we included a wide range of diaphragm muscle shortening that covers both sub-maximal 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 reported that during spontaneous inspiratory efforts against an occluded airway, muscle shortened by 15-40% of length at FRC, but curvature remained nearly unchanged (4). We additionally show that during large tidal breathing following occlusion that curvature still is essentially constant. However, during phrenic nerve stimulation diaphragm muscle shortened by 30 to nearly 50%, and, for shortening approximately 50% or higher, curvature decreased sharply (2). Similarly, our data shows 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. We therefore 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 mid-costal 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 sub-maximal 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, but even more so because of the decrease in curvature.

**Perspectives and Significance** In this study we simultaneously measured mid-costal 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 DeTroyer group has shown a significant caudal displacement during maximal stimulation of the diaphragm (7). The study by DeTroyer et al. predicted that the caudal displacement of the line of insertion contributed 25% of the change in pleural pressure during maximal diaphragm activation (7). 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 in order 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.

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FIGURE CAPTIONS

Figure 1. Two views of the mid-costal diaphragm three-dimensional surfaces for two states of respiration are shown. Figure 1A shows a ventrodorsal/cephalad view and Figure 1B shows a 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 mid-costal region generating a 3 x 5 point grid for each ventilation state. This grid was fitted by a NURBS surface in Rhinoceros. The dark surface represents the initial FRC state for one of the dogs in the prone posture during 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.

Figure 2. Surfaces of two volumetric components representing diaphragm VD divided at the line of intersection. Surfaces to the left of the line of intersection represent the abdominal component of VD as the diaphragm descends caudally. Surfaces to the right of the line of intersection represent rib cage component of VD as the diaphragm is displaced laterally.

Figure 3. Means +/- SEM are shown for large tidal breathing with airway open following occluded efforts in the prone and supine posture at FRC, FRC+ .5 IC, TLC, quiet SB for the two postures, and 50 Hz bilateral stimulation of the phrenic nerves in the supine posture at three different lung volumes. A) The abdominal VD component. $: P < 0.05$: open airway breathing after occlusion at FRC + .5 IC is greater than SB regardless of posture. *: P < 0.05: Comparisons within the supine posture showed that stimulation at all three lung volumes are equal and greater than all other supine breathing modes. B) The rib cage VD component *: P < 0.05: Comparisons within the supine posture showed that stimulation at FRC and FRC + .5 IC were equal and different than open airway breathing after occlusion at FRC. C) The additive total VD component. $: Comparisons within
the supine posture showed that open airway breathing after occlusion at FRC + .5 IC is greater than both open airway breathing after occlusion at FRC (P < 0.05) and SB (P < 0.05). *: P < 0.05: Comparisons made within the supine posture showed that stimulation at all three lung volumes were equal and greater than all other supine breathing modes of ventilation.

Figure 4. A) Mean percentages +/- SEM of mid-costal diaphragm muscle shortening (% ΔL/L_{FRC}) for open airway breathing after occlusion at the three lung volumes (FRC, FRC+ .5 IC, and TLC) and 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 (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). $: (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.

Figure 5. Percentage of mid-costal diaphragm muscle shortening data for each individual dog correlated to diaphragm total VD for all breathing maneuvers across both postures. A quadratic equation fit was constructed and shows a strong non-linear correlation (r^2 = .78) between muscle shortening and total diaphragm VD.

Figure 6. Individual values of radius of curvature and percentage mid-costal muscle shortening plotted against each other. Radius of curvature values were collected without the CW markers so that comparison to previous results from our lab could be made. An equation relating the radius of curvature to percentage of muscle shortening was constructed that took the following form: radius of curvature (cm) = c1 + c2*exp(% ΔL/L_{FRC}) where c1 and c2 were coefficients
determined from a least squares approach. At approximately 40-42% muscle shortening the radius of curvature increases significantly. Coefficients for a linear exponential equation were determined. The resulting equation was fit to the data and is shown as a dashed line.

Figure 7. Effects of muscle shortening (\% ΔL/L_{FRC}) and radius of curvature (cm) on mid-costal diaphragm VD. Using a least squares surface fit routine in MATLAB, the following equation was obtained: $VD = a + b*(\% ΔL/L_{FRC}) + c*(\% ΔL/L_{FRC})^2 + d*\exp(\text{radius of curvature})$, where $a$, $b$, $c$, and $d$ were coefficients determined from the fitting routine. During most sub-maximal 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 was constructed using the raw data from each dog. The solid points represent individual experimental values obtained from dogs.
References


Figure 2
Figure 3A.
Figure 3B.
Figure 3C.
Figure 5.

$R^2 = 0.7837$
Figure 7.