Diaphragm muscle shortening modulates kinematics of lower rib cage in dogs

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Running head: Effects of diaphragm shortening on rib cage kinematics
ABSTRACT
We tested the hypothesis that diaphragm muscle shortening modulates volume displacement and kinematics of the lower rib cage in dogs, and that posture and mode of ventilation affect such modulation. Radiopaque markers were surgically attached to the lower three ribs of the ribcage and to the mid-costal region of the diaphragm in six dogs of ~8 kg, and the locations of these markers were determined by biplane fluoroscopy system. Three-dimensional software modeling techniques were used to compute volume displacement and surface area of the mid-costal diaphragm and the lower three ribs during quiet spontaneous breathing, mechanical ventilation, and bilateral phrenic nerve stimulation at different lung volumes spanning the vital capacity. Volume displaced by the diaphragm relative to that displaced by the lower ribs is disproportionately greater under mechanical ventilation than during spontaneous breathing in the supine position (P<0.05). At maximal stimulation, diaphragm volume displacement grows disproportionately larger than rib volume displacement as lung volume increases (P<0.05). Surface area of both the diaphragm and the lower ribs during maximal stimulation of the diaphragm is reduced compared to that at spontaneous breathing (P<0.05). In the prone posture, mechanical ventilation results in a smaller change in diaphragm surface area than spontaneous breathing (P<0.05). Our data demonstrate that during inspiration the lower rib cage move not only through the pump- and bucket-handle motion, but also rotate around the spine. Taken together, these data support the observation that the kinematics of the lower rib cage and its mechanical interaction with the diaphragm are more complex than previously known.
INTRODUCTION

Over the past three decades, other investigators have extensively analyzed the mechanical relationship and interaction of the diaphragm and rib cage (2, 7, 8, 9). However, the precise relationship between the volume displacement and surface area of the diaphragm and how they modulate the kinematics of the lower ribs are unknown. In particular, the specific effect of the magnitude of diaphragm muscle shortening on volume displacement of the lower rib cage is not well understood. Thus, to demonstrate the coupling between the lower rib cage and the diaphragm, we measured volume displacement and quantified muscle shortening of the diaphragm. In addition, we measured surface area and volume displacement of the lower rib cage. We experimented with six anesthetized beagle dogs, each with radiopaque markers attached to the midcostal diaphragm and the lower three ribs. Biplane images were taken at end-expiration and end-inspiration during quiet spontaneous breathing (SB), mechanical ventilation (MV), bilateral stimulation of the diaphragm at the following lung volumes: functional residual capacity (FRC), FRC + ½ inspiratory capacity (IC), and total lung capacity (TLC). Volume displacement and surface area of both the ribs and the diaphragm were calculated using Rhinoceros 3-D modeling software. Although an observed inward deflection of the lower ribcage during inspiration in COPD patients with severe hyperinflation is contrary to diaphragm behavior in dogs, in both human patients with COPD and dogs at high lung volumes and high muscle activations, diaphragm flattening is augmented by larger rings of insertion (1). In this study we tested the hypothesis that diaphragm muscle shortening modulates kinematics and volume displacement of lower rib cage in dogs, and that posture and mode of ventilation affect such modulation.

METHODS

Imaging: Six bred-for-research beagle dogs with body masses of ~ 8 kg were studied using the same methods that we have used previously (1). 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 Committee of Baylor College of Medicine and Mayo Clinic. Briefly, radiopaque markers were attached to the diaphragm and lower three ribs by the following procedure. The abdomen was opened by midline laparotomy, and 2-mm beads were stitched to the peritoneal surface of muscle bundles in the mid-costal 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, one at its insertion on the chest wall, and three at equal intervals along the muscle bundle. Radiopaque markers in the
form of 4 mm titanium screws were also attached to the left, lower three ribs of the same six dogs. The dogs were allowed 3-4 weeks for recovery from surgery. The dogs were then anesthetized with pentobarbital sodium (30 mg/kg), incubated with a cuffed endotracheal tube, and placed in the test field of a biplane fluoroscopy system. This high spatial (±0.5 mm) and temporal (30 Hz) resolution system was used to record displacement of the radiopaque metallic markers.

**Inducing SB and MV:** 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 frequency pattern of quiet SB was obtained, 5 breaths were recorded, and the average end-expiration (FRC) and end-inspiration coordinates were obtained. To induce MV, a volume mechanical ventilator at the same tidal volume and slightly higher frequency than that during quiet SB was utilized. We inspected airway opening pressure, reduced the pressure tracings to match quiet SB and concluded that there were no spontaneous respiratory efforts in any of the dogs during MV.

**Bilateral Stimulation:** The spinal roots of the phrenic nerves (C5 and C6) were identified and isolated on both sides of the neck. Insulated hook electrodes were then placed under the nerve roots and the phrenic nerves were stimulated at supramaximal synchronous tetanic stimulations at frequencies between 1 and 50 Hz using a Grass S 88 nerve stimulator. The tetanic stimulation parameters used were 6V amplitude, 1 ms pulse duration, and 500 ms pulse train duration. We recorded biplane images in the supine position before and after bilateral phrenic nerve stimulation and after unilateral stimulation of the left and right phrenic nerves at three lung volumes spanning the vital capacity: FRC, FRC + ½ IC, and TLC.

**Fitting Diaphragm Surface:** Coordinates in the form of a 3 by 5 point grid corresponding to five markers on each of three diaphragm muscle fibers were imported into the 3D modeling software (Rhinoceros). A reference surface of the diaphragm at the passive state was fitted through the point grid at FRC (Figure 1A). We fitted the surface at the active state of the diaphragm at end of inspiration during either SB or MV, and at maximal stimulation. The surfaces of the diaphragm at the passive and active states were superimposed on each other (Figure 1B).

**Computation of the Volume Displacements:** We found that the midcostal diaphragm VD has two primary components. One component of the VD, the abdominal component, is in the caudal direction and consequently displaces the abdominal wall outward. The other component of the VD, the rib cage component, is a lateral displacement of the diaphragm insertion on the chest wall causing an inspiratory action on the rib cage. The total volume displacement was computed as the summation of these two components.
Statistical analysis: Statistical analysis was done with one- and two-factor ANOVA tests for differences between ventilation states, lung volumes, posture, and mode of ventilation were examined by Tukey’s multiple comparison tests. One- and two-factor tests were used to determine overall significance of posture and mode of ventilation on volume displacement (VD) and surface area (SA). Multiple comparison Tukey tests were performed to determine significant differences between individual groups.

RESULTS

Rib Cage Motion: As shown in Figure 2A and Figure 2B, bucket-handle and pump-handle motions were observed from FRC to TLC. The bucket angle, $\beta$, was $69.02\pm2.1^\circ$ and the pump angle, $\alpha$, was $-13.07\pm5.68^\circ$. Bucket-handle and pump-handle motions are observed for rotation of the ribs in caudal and cephalad directions, respectively. From FRC to TLC, we detected an additional motion of the ribs around the spine in supine dogs. As shown in Figure 2C, the observed rotation around the spine was $-2.36\pm9.29^\circ$.

Volume Displacement of Lower Ribs: The volume displacement of the lower three ribs and mid-costal region of the diaphragm under maximal bilateral stimulation of the diaphragm (50 Hz) and during SB were computed. Volume displacement of the lower ribs and diaphragm with increasing lung volume from FRC to TLC is shown in Figure 3A. Volume displacement during maximal stimulation of the diaphragm at lung volumes varying from FRC to TLC was 4-5 times fold of that displaced during SB (P<0.05).

The data in Figure 3B show the ratio of diaphragm VD to lower rib cage VD at SB and at maximal stimulation. The data demonstrate a disproportionately greater volume displacement of the diaphragm relative to the volume displacement of the lower ribs with increasing lung volumes (P<0.05).

Effect of Posture on Volume Displacement: Total volume displaced by the diaphragm and lower rib cage in the supine and prone postures during mechanical ventilation and during spontaneous breathing are shown in Figure 4A. Posture and mode of ventilation significantly affect volume displaced by the diaphragm and by the lower ribs. Volume displacement of the ribs during mechanical ventilation is decreased in the supine position relative to the prone position (P<0.05).

In Figure 4B, total volume displacement of the diaphragm as a fraction of the volume displaced by the lower ribs under two ventilation states, mechanical ventilation and spontaneous breathing, is shown. Volume displaced by the diaphragm relative to that of the lower ribs was disproportionately greater during MV than during SB in the supine but not in the prone position (P<0.05).

Surface Area, Posture, and Mode of Ventilation: In Figure 5A, surface area of the mid-costal diaphragm and lower rib cage during bilateral maximal stimulation of the diaphragm is compared to quiet spontaneous breathing in the supine dog. During spontaneous breathing, the surface areas of the diaphragm and the lower ribs follow an inverse relationship. Diaphragm muscle contraction causes a decrease in the surface
area of the diaphragm whereas expansion of the lower three ribs increases the surface area of the lower ribs. When maximally stimulated, the surface areas of both the diaphragm and the lower rib cage tend to decrease. The decrease in surface area at maximal stimulation at FRC is greater compared to that at SB (P<0.05). In Figure 5B, the surface area of the mid-costal diaphragm and lower rib cage during MV is compared to quiet SB in both supine and prone positions. Changes in diaphragm surface area are smaller during MV than during SB in the prone posture (P<0.05), whereas in the supine position, the change in diaphragm surface area during mechanical ventilation is nearly the same as during spontaneous breathing. The surface area encompassed by the lower three ribs appears to be unaffected by mode of ventilation in either posture.

DISCUSSION

The purpose of our study is to quantitatively assess the contribution of diaphragm muscle shortening to volume displacement, surface area, and overall kinematics of the lower ribcage. More precisely we determined the effect of different levels of diaphragm muscle activation on volume displacement and surface area of the diaphragm and lower rib cage. Other studies have shown the mechanical coupling of the rib cage and the diaphragm by measuring the inspiratory effect of the diaphragm on the rib cage. In one such study, Boynton et al used a linear translational transformer model to support the mechanical coupling of the diaphragm, rib cage and abdominal wall (2). Many investigators have also studied rib cage kinematics and have quantified the displacement of the ribs through assessment of the bucket-handle and pump-handle motions. Using a CT scan, Margulies et al examined the orientation of the ribs at total lung capacity (TLC) and functional residual capacity (FRC) in beagle dogs (8). They showed that the ribs did not lie in the planes fitted to each rib with the z-axis oriented cephalocaudally and the sagittal midplane corresponding to the x-z plane. Their data demonstrated that rib displacement from TLC to FRC varied between individual ribs, as measured by changes in the pump (Δα) and bucket (Δβ) handle angles. Although there was a steady decrease in pump handle angle with increasing rib number, the change in bucket handle angle decreased across the same ribs. Wilson et al observed a similar relationship in human ribs, but reported a more gradual bucket-handle angle change (10).

Though data in their study were obtained only for mechanically ventilated dogs, Margulies et al acknowledged the possible effect of mode of ventilation on rib motion. De Troyer and Kelly discovered that the sternum moves caudally during inspiration in spontaneously breathing dogs but cephalad in mechanically ventilated dogs (4). Da Silva et al attributed these contrasting movements of the sternum to the straightening of the costal cartilage and an increase in the caudal angle between the sternum and costal cartilage in reaction to the caudal shift of the sternum during spontaneous breathing (3). Margulies et al. also speculated that posture
could have an effect on orientation and kinematics of the ribs. In our study, we quantified the effects of posture and mode of ventilation on the lower three ribs and on the midcostal region of the diaphragm.

**Effect of Posture and Mode of Ventilation on VD of Ribs and Diaphragm:** Our data revealed that, depending on posture, mode of ventilation may or may not have a significant effect on volume displacement. That is posture affects the way mode of ventilation modulates volume displacement. Data shown in Fig. 4A clearly demonstrate that posture is an important determinant of volume displacement of the diaphragm and lower rib cage. The data suggest that the prone posture is more advantageous in that diaphragm volume displacement is greater than in the supine posture. Mode of ventilation in the supine position affected only the volume displaced by the lower ribs and not that displaced by the diaphragm, whereas, in the prone position, only the volume displacement of the diaphragm was affected, not that of the rib cage. Under spontaneous breathing, volume displaced by the diaphragm and the ribs was greater in the prone posture relative to the supine posture. Our data in Figure 4B showed that mechanically ventilated dogs in the supine position showed about a two and half fold increase in the ratio of diaphragm to lower rib cage volume displacement compared to SB. This disproportionate displacement of the diaphragm and ribs in the supine posture shows that work done by the diaphragm does not entirely translate to an increase in inspiratory rib motion leading to altered volume displacement of the lower ribcage.

**Effect of Bilateral Maximal Stimulation on VD of Ribs and Diaphragm:** Krayer et al used a dynamic spatial reconstructor (DSR) to quantify the effect of ribcage and diaphragm motion on tidal volume (6). Their results showed that the diaphragm did not have a significant inspiratory effect on ribcage motion to force inflation of the lungs. In particular, they found no significant correlation of ribcage motion to diaphragm position during spontaneous breathing in anesthetized supine dogs. Though they did examine the relationship between the diaphragm and ribcage to a small degree, their conclusion was based only on spontaneously breathing dogs. We, however, examined the effect of maximal phrenic nerve stimulation at varying lung capacities on the volume displacement of the diaphragm and the lower ribs. In Figure 3A, starting at maximal stimulation of the diaphragm at FRC, there is a constant decrease in volume displacement of the ribs in the direction of increasing lung volume. This is consistent with Mead’s conclusion that the zone of apposition of the diaphragm to the rib cage decreased as lung volume increased (9). From a force balance point of view, Loring and Mead also reached the same conclusion, showing that the diaphragm’s mechanical effect on the ribcage is greatest at low lung volumes (7). Our results for maximal stimulation of the diaphragm showed that diaphragm volume displacement was independent of lung volume at which phrenic stimulation occurred. Furthermore, maximal stimulation of the diaphragm muscle at all three lung volumes displaced the diaphragm 4-5 times more than that during spontaneous quiet breathing.
We computed a ratio of volume displaced by the diaphragm to that by the ribs under both maximal stimulation and spontaneous breathing. With increasing lung volume, diaphragm volume displacement became disproportionately larger than rib volume displacement. Interestingly, differences in these ratios were only significant between maximal stimulation at TLC and spontaneous breathing.

**Effects of Posture and Mode of Ventilation on SA of Diaphragm and Ribcage:** We measured the change in surface area of the diaphragm and the ribs in supine and prone postures under spontaneous breathing and mechanical ventilation. The change in surface area of both the diaphragm and the ribs was computed relative to the relaxed state at FRC. In the prone posture, the decrease in diaphragm surface area from FRC during mechanical ventilation was significantly smaller than during spontaneous breathing (P<0.05). Posture and mode of ventilation appeared to have little effect on the surface area of the rib cage. The effect of maximal stimulation on the surface areas of the diaphragm and the lower rib cage is shown in Fig. 5A. There appears to be a correlation between lung volume and diaphragm surface area, marked by a constant decrease in surface area with increasing lung volume. Maximal stimulation of the diaphragm induced a smaller decrease in rib surface area under TLC than under either FRC or FRC + ½ IC.

**Kinematics of the Lower Rib Cage:** Previous investigators, such as Margulies and Wilson have described the geometry and movements of the rib cage through pump and bucket handle motions (8,10). However, the precise kinematics and trajectory of the individual ribs and their relationship to the muscle fibers of the diaphragm are still unknown. Our data showed that, during inspiration, the lower ribs move not only through the pump- and bucket-handle angles, but also rotate around the spine. This rotation provides an additional degree of freedom in the kinematics of the rib cage that was previously ignored. Additionally, caudal and cephalic rotations of the lower rib cage appeared to be coupled with a rotation toward the sagittal midplane.

**Perspectives and Significance:** Our study provides a detailed quantitative assessment of the volume displacement of the diaphragm and the lower rib cage at varying levels of diaphragm activation in the supine and prone postures. Dogs exhibited a greater decrease in surface area of the diaphragm breathing spontaneously in the prone position than during mechanical ventilation. The mechanical effect of the diaphragm on the lower rib cage was a function of the level of stimulation of the diaphragm muscle. Volume displacement of the ribs was dependent on posture, although surface area appeared to have no such dependency in either mode of ventilation. When mechanically ventilated, volume displaced by the ribs was smaller in the supine position than in the prone position. Our data showed volume displaced by the diaphragm is greater during spontaneous breathing than during mechanical ventilation irrespective of posture. Finally, posture and mode of ventilation are important determinants in the interaction between the diaphragm and the lower rib cage. The observed rotation of the rib cage around the spine, in addition to pump and bucket handle motions, highlights an
additional degree of freedom to the kinematics of the lower rib cage. Our data support the observation that the
kinematics and mechanical interaction of the diaphragm and the lower rib cage is more complex than previously
known.

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FIGURE CAPTIONS

Figure 1A. This figure creates a reference for the following two figures by showing the deformed surfaces of the lower 3 ribs and the mid-costal diaphragm under end-inspiration at FRC. The surface markers represented 5 markers on 3 mid-costal diaphragm muscle fibers and were represented as a 3 x 5 point grid for each state. This grid was created using the software Rhinoceros.

Figure 1B. This figure shows the reference surface at the end of expiration at FRC and the deformed surface after inspiration at FRC of the same dog shown in Figure 1. We see an apparent decrease in surface area of the diaphragm as it contracts and an increase in surface area of the lower ribs as it expands. This decrease in surface area shows the precise kinematics of the ribs relative to the diaphragm at this particular stimulation. This figure is shown to obtain a better grasp of the Rhino software and how we are able to model diaphragm and rib interaction.

Figure 1C. This figure represents the volume displacement of the lower 3 ribs and the mid-costal diaphragm of a dog in the supine position at FRC, at end-inspiration and end-expiration. Volume displacement from the reference surface to the deformed surface is shown clearly by the semi-translucent solid generated between the two surfaces.

Figure 2A. The bucket movement of the lower three ribs. Rib location is shown in relation to a dog lying in a supine position on the lateral-cephalad plane. Two configurations of the same ribs are displayed. One is magnified to enhance detail. The bucket angle was 69.02±2.1° at FRC. The blue colored ribs show the bucket orientation of the ribs at FRC. The reddish-brown colored ribs show the bucket orientation at bilateral maximal stimulation of the diaphragm at TLC. The ribs are rotated caudally.
**Figure 2B.** This figure shows the pump movement of a supine dog. The ribs are fitted inside a dog in the supine position on the dorsoventral-cephalad plane. Two configurations of the same ribs are displayed. One is magnified to enhance detail. The pump angle was -13.07±5.68° at FRC. The blue colored ribs show the pump orientation of the ribs at FRC. The reddish-brown colored ribs show the orientation of the ribs at bilateral maximal stimulation of the diaphragm at TLC. The ribs are rotated cephalad. From this view it becomes apparent that the ribs expand as lung volume and diaphragm stimulation increase.

**Figure 2C.** The orientation of the ribs around the spine in a supine dog is shown. Rib orientation is modeled by outlining a figure of a dog lying in the supine position rotated into the dorsoventral-lateral planar field. Two configurations of the same ribs are displayed. One is magnified to enhance detail. The rotation around the spine was -2.36±9.29° at FRC. The blue colored ribs show the orientation of the ribs around the spine at FRC. The brown colored ribs show the orientation of the ribs at bilateral maximal stimulation of the diaphragm at TLC. The ribs are rotated toward the sagittal midplane.

**Figure 3A.** Volume displacement (VD) at 50 Hz bilateral maximal stimulation compared to quiet spontaneous breathing (SB) in the mid-costal diaphragm and the lower three ribs in the supine dog. Volume displacement is significantly greater during maximal stimulation of the diaphragm than during SB (P<0.05). There is very little increase in percent VD of the lower ribs during maximal stimulation at the lung volume of FRC+1/2 inspiratory capacity (IC) and at total lung capacity (TLC) when compared to SB. Although not statistically significant VD of the lower ribs appears to be greater than SB.

**Figure 3B.** Ratio of volume displaced by the diaphragm to volume displaced by the ribs due to 50 Hz bilateral stimulation compared to SB is shown. Volume displacement by the diaphragm at increasing lung volumes is larger than the corresponding volume displacement by the lower rib cage. The volume displacement of the diaphragm is disproportionately greater relative to the volume displacement of lower rib with increasing lung volumes (P <0.05).

**Figure 4A.** Total volume displacement (VD) of the diaphragm muscle and lower rib cage is shown in the supine and prone postures during mechanical ventilation (MV) and spontaneous breathing (SB). An asterisk denoted significant differences in values relative to posture within the same mode of ventilation. In relation to SB, VD by the ribs during MV is increased from the supine to prone position (*, P<0.05). The symbol $ denotes significant changes in VD due to mode of ventilation. In the supine posture, VD by the lower ribs during SB is significantly greater than volume displaced by the ribs under MV ($, P<0.05). Posture appears to be an important determinant of volume displacement of the diaphragm irrespective of mode of ventilation, though this
is not statistically supported. Posture appears to be an important determinant of volume displacement of the diaphragm irrespective of mode of ventilation, though this is not statistically supported.

**Figure 4B.** Ratio of total volume displaced by the diaphragm to the volume displaced by the ribs during mechanical ventilation (MV) compared to spontaneous breathing (SB). The volume displacement of the diaphragm is disproportionately greater relative to the volume displacement of lower rib during MV than during SB in the supine posture (P<0.05).

**Figure 5A.** Decrease in surface area of the mid-costal diaphragm and lower rib cage during bilateral maximal stimulation of the diaphragm compared to quiet spontaneous breathing of the supine dog. During spontaneous breathing the surface area of the diaphragm decreases and the surface area of the ribs increases. During maximal stimulation of diaphragm, surface area in both diaphragm and lower rib cage decrease. The decrease in SA during maximal stimulation at FRC is significantly greater than alteration in SA during SB for the diaphragm and the rib cage (*, P<0.05). Maximal stimulation at TLC shows a decrease in SA of the lower ribs much less than the decrease in SA of the diaphragm.

**Figure 5B.** Decrease in surface area of the mid-costal diaphragm and lower rib cage during mechanical ventilation of the diaphragm compared to quiet spontaneous breathing of the supine and prone dog positions. In the prone position the diaphragm surface area is decreased less during MV in comparison to SB. However, in the supine position the diaphragm surface area is essentially the same in both modes of ventilation. The surface area of the ribs appears to be unaffected by mode of ventilation in either the prone or supine postures. In the prone position, the increase in lower rib surface area is significantly more for SB than for MV ($, P<0.05).
REFERENCES:


Fig. #1A

- Lower 3 Ribs at the End of Expiration
- Origin of the Central Tendon
- Midcostal Diaphragm at the End of Expiration
- Chest Wall Insertion
Fig. #1C
Fig. #2B
Rotation Around the Spine, \( \delta \)

- Y
  - Left, Lateral
  - X
    - Dorsal

FRC - Max Stim.
Max Stim. at TLC

(Ventral)
+X
(Right, Lateral)
+Y
(X)
(Dorsal)

Fig. #2C
Figure 3A

Total Volume Displacement (cm³)

- **SB**
- **50 Hz FRC**
- **50 Hz FRC + 1/2 IC**
- **50 Hz TLC**

**Supine, Diaphragm**

**Supine, Ribs**

* indicates statistical significance.
Figure 3B

Bar graph showing diaphragm to rib volume displacement in the supine position.

- **SB**
- **50 Hz FRC**
- **50 Hz FRC + 1/2 IC**
- **50 Hz TLC**

* indicates a significant difference.
Figure 4A
Figure 4B

Diaphragm to Rib Volume Displacement

- Supine
- Prone

SB
MV

$ *
Figure 5A
Figure 5B

Decrease in Surface Area (cm²)

- Supine, Diaphragm
- Prone, Rib
- Supine, Rib
- Prone, Diaphragm

Legend:
- SB
- MV

Figure 5B