Diaphragm muscle shortening modulates kinematics of lower rib cage in dogs

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Chu I, Fernandez C, Allen Rodowicz K, Lopez MA, Lu R, Hubmayr RD, Boriek AM. Diaphragm muscle shortening modulates kinematics of lower rib cage in dogs. Am J Physiol Regul Integr Comp Physiol 299: R1456–R1462, 2010. First published August 25, 2010; doi:10.1152/ajpregu.00016.2010.—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 rib cage and to the midcostal region of the diaphragm in six dogs of ~8 kg body masses, and the locations of these markers were determined by a biplane fluoroscopy system. Three-dimensional software modeling techniques were used to compute volume displacement and surface area of the midcostal 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 with 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 moves not only through the pump- and bucket-handle motion, but also rotates 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.

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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 midcostal region of the left diaphragm. Five markers were placed along each of three nearby muscle bundles: one at the origin of each muscle bundle on the central tendon, 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 wk 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 SB, and biplane images were obtained at end expiration. The frequency pattern of quiet SB was obtained, five 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 6 V 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 + one-half IC, and TLC.

Fitting diaphragm surface. Coordinates in the form of a $3 \times 5$ 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 (Fig. 1A). We fitted the surface at the active state of the diaphragm at the 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 (Fig. 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 (VD) 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 VD and surface area. Multiple-comparison Tukey tests were performed to determine significant differences between individual groups.

RESULTS

Rib cage motion. As shown in Fig. 2, A and B, bucket-handle and pump-handle motions were observed from FRC to TLC. The bucket angle, $\beta$, was $69.02 \pm 2.1$ degrees, and the pump angle, $\alpha$, was $13.07 \pm 5.68$ degrees. 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 Fig. 2C, the observed rotation around the spine was $-2.36 \pm 9.29$ degrees.

VD of lower ribs. The VD of the lower three ribs and midcostal region of the diaphragm under maximal bilateral stimulation of the diaphragm (50 Hz) and during SB were computed. VD of the lower ribs and diaphragm with increasing lung volume from FRC to TLC is shown in Fig. 3A. VD during maximal stimulation of the diaphragm at lung volumes varying from FRC to TLC was four to five-fold of that displaced during SB ($P < 0.05$).

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

Effect of posture on VD. Total volume displaced by the diaphragm and lower rib cage in the supine and prone postures during MV and during SB are shown in Fig. 4A. Posture and mode of ventilation significantly affect volume displaced by the diaphragm and by the lower ribs. VD of the ribs during MV is decreased in the supine position relative to the prone position ($P < 0.05$).

In Fig. 4B, total VD of the diaphragm as a fraction of the volume displaced by the lower ribs under two ventilation states, MV and SB, 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 Fig. 5A, surface area of the midcostal diaphragm and lower rib cage during bilateral maximal stimulation of the diaphragm is compared with quiet SB in the supine dog. During SB, the surface areas of the diaphragm and the lower ribs tend to decrease. The decrease in surface area at maximal stimulation at FRC is greater compared with that at SB ($P < 0.05$). In Fig. 5B, the surface area of the midcostal diaphragm and lower rib cage during MV is compared with 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 MV is nearly the same as during SB. The surface 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 VD, surface area, and overall kinematics of the lower rib cage. More precisely, we determined the effect of different levels of diaphragm muscle activation on VD and surface area of the diaphragm and lower rib cage. Our 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. (2) used a linear translational transformer model to support the mechanical coupling of the diaphragm, rib cage, and abdominal wall. 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. (8) examined the orientation of the ribs at TLC and FRC in beagle dogs. 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
Their data demonstrated that rib displacement from TLC to FRC varied between individual ribs, as measured by changes in the pump and bucket 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. (10) observed a similar relationship in human ribs but reported a more gradual bucket-handle angle change.

Although data in their study were obtained only for mechanically ventilated dogs, Margulies et al. (8) acknowledged the possible effect of mode of ventilation on rib motion. De Troyer and Kelly (4) discovered that the sternum moves caudally during inspiration in spontaneously breathing dogs but cephalad in mechanically ventilated dogs. Da Silva et al. (3) attributed these contrasting movements of the sternum to the straightening of the costal cartilage and an increase in 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 and bucket 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. (10) observed a similar relationship in human ribs but reported a more gradual bucket-handle angle change.
caudad angle between the sternum and costal cartilage in reaction to the caudad shift of the sternum during SB. Marguilies et al. (8) 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 VD. That is, posture affects the way mode of ventilation modulates VD. Data shown in Fig. 4A clearly demonstrate that posture is an important determinant of VD of the diaphragm and lower rib cage. The data suggest that the prone posture is more advantageous in that diaphragm VD 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 VD of the diaphragm was affected, not that of the rib cage. Under SB, volume displaced by the diaphragm and the ribs was greater in the prone posture relative to the supine posture. Our data in Fig. 4B showed that mechanically ventilated dogs in the supine position showed about a 2.5-fold increase in the ratio of diaphragm to lower rib cage VD compared with 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 VD of the lower rib cage.

**Effect of bilateral maximal stimulation on VD of ribs and diaphragm.**

Krayer et al. (6) used a dynamic spatial reconstructor to quantify the effect of rib cage and diaphragm motion on tidal volume. Their results showed that the diaphragm did not

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Fig. 3. A: VD at 50 Hz bilateral maximal stimulation compared with quiet spontaneous breathing (SB) in the midcostal diaphragm and the lower three ribs in the supine dog. VD is significantly greater during maximal stimulation of the diaphragm than during SB ($P < 0.05$). There is very little increase in %VD of the lower ribs during maximal stimulation at the lung volume of FRC + one-half IC and at TLC compared with SB. Although not statistically significant, VD of the lower ribs appears to be greater than SB. B: ratio of volume displaced by the diaphragm to volume displaced by the ribs due to 50 Hz bilateral stimulation compared with SB is shown. VD by the diaphragm at increasing lung volumes is larger than the corresponding VD by the lower rib cage. The VD of the diaphragm is disproportionately greater relative to the VD of lower rib with increasing lung volumes ($P < 0.05$).

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Fig. 4. A: total VD of the diaphragm muscle and lower rib cage is shown in the supine and prone postures during mechanical ventilation (MV) and SB. *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 ($P < 0.05$); $significant changes in VD due to mode of ventilation. In the supine posture, VD by the lower ribs during SB is significantly greater than volume displaced by the ribs under MV ($P < 0.05$). Posture appears to be an important determinant of VD of the diaphragm regardless of mode of ventilation, although this is not statistically supported. B: ratio of total volume displaced by the diaphragm to the volume displaced by the ribs during MV compared with SB. The VD of the diaphragm is disproportionately greater relative to the VD of lower rib during MV than during SB in the supine posture ($P < 0.05$).
Perspectives and Significance

Our study provides a detailed quantitative assessment of the effect of VD of the diaphragm on the kinetics of 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 MV. The mechanical effect of the diaphragm on the lower rib cage was a function of the level of stimulation of the diaphragm muscle. VD 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 SB than during MV, regardless of posture. Finally, the observed rotation of the rib cage around the spine, in addition to pump- and bucket-handle motions, highlights an additional degree of freedom of 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.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES