Mechanism of the lung-deflating action of the canine diaphragm at extreme lung inflation

Dimitri Leduc, Matteo Cappello, Pierre Alain Gevenois, and André De Troyer

Laboratory of Cardiorespiratory Physiology, Brussels School of Medicine; and Chest Service, and Department of Radiology, Erasme University Hospital, Brussels, Belgium

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Leduc D, Cappello M, Gevenois PA, De Troyer A. Mechanism of the lung-deflating action of the canine diaphragm at extreme lung inflation. J Appl Physiol 112: 1311–1316, 2012. First published February 9, 2012; doi:10.1152/japplphysiol.01422.2011.—When lung volume in animals is passively increased beyond total lung capacity (TLC; transrespiratory pressure = +30 cmH₂O), stimulation of the phrenic nerves causes a rise, rather than a fall, in pleural pressure. It has been suggested that this was the result of inward displacement of the lower ribs, but the mechanism is uncertain. In the present study, radiopaque markers were attached to muscle bundles in the midcostal region of the diaphragm and to the tenth rib pair in five dogs, and computed tomography was used to measure the displacement, length, and configuration of the muscle and the displacement of the lower ribs during relaxation at seven different lung volumes up to +60 cmH₂O transrespiratory pressure and during phrenic nerve stimulation at the same lung volumes. The data showed that I) during phrenic nerve stimulation at 60 cmH₂O airway opening pressure increased by 1.5 ± 0.7 cmH₂O; 2) the dome of the diaphragm and the lower ribs were essentially stationary during such stimulation, but the muscle fibers still shortened significantly; 3) with passive inflation beyond TLC, an area with a cranial concavity appeared at the periphery of the costal portion of the diaphragm, forming a groove along the ventral third of the rib cage; and 4) this area decreased markedly in size or disappeared during phrenic stimulation. It is concluded that the lung-deflating action of the isolated diaphragm beyond TLC is primarily related to the invaginations in the muscle caused by the acute margins of the lower lung lobes. These findings also suggest that the inspiratory inward displacement of the lower ribs commonly observed in patients with emphysema (Hoover’s sign) requires not only a marked hyperinflation but also a large fall in pleural pressure.

respiratory muscles; chest wall mechanics; mechanics of breathing; diaphragm configuration; lung-diaphragm interaction

IT IS WELL ESTABLISHED THAT the lung-expanding action of the diaphragm is altered by changes in lung volume. Specifically, when the airways in animals (3, 4, 6, 9, 10, 12, 14–16) and in humans (1, 17) are occluded and the diaphragm is selectively activated by stimulation of the phrenic nerves, the fall in pleural (ΔPpl) or airway opening pressure (ΔPao) that occurs during stimulation decreases progressively as lung volume before stimulation increases from functional residual capacity (FRC) to total lung capacity (TLC; transrespiratory pressure = +30 cmH₂O). Near TLC, the capacity of the diaphragm to generate ΔPpl is almost zero. This decrease is primarily due to the fact that at high lung volumes the muscle during contraction is shorter and generates less force (5).

Earlier studies in rabbits (16) and dogs (14), however, have also shown that, when lung volume is increased above TLC, stimulation of the phrenic nerves causes a rise, rather than a fall, in Pao. Thus the action of the diaphragm at very high lung volumes is expiratory to the lung, and Sant’Ambrogio and Saibene (16) speculated that this action was the result of an inward displacement of the ribs into which the diaphragm inserts. That is, increasing lung volume beyond TLC would produce such a large descent of the dome of the diaphragm that subsequent contraction of the muscle fibers would cause no further descent of the dome; instead, muscle contraction would only pull the lower ribs inward.

This interpretation, however, has not been validated. In addition, measurements of diaphragm length using computed tomography (CT) or sonomicrometry have shown that, when the phrenic nerves in dogs are stimulated at TLC, muscle length is only ~50% of the length during relaxation at FRC (4, 9). It would be expected that increasing lung volume beyond TLC would induce further decrease in diaphragm muscle length and, hence, that the force developed by the muscle would be very small, unable to produce significant displacement of the lower ribs. Based on these grounds, we therefore felt that the rise in Pao was unlikely due to an inward displacement of the lower ribs, and we hypothesized that the primary cause of this pressure rise might lie in a distortion of the diaphragm by the excessively inflated lung and the correction of this distortion by muscle contraction. To test this hypothesis, radiopaque markers were attached both to the tenth rib pair and along muscle bundles in the midcostal region of the diaphragm in dogs. The animals were then placed in a CT scanner, and the phrenic nerves were stimulated while lung volume was increased up to +60 cmH₂O transrespiratory pressure. Therefore, accurate measurements of lower rib position, diaphragm displacement, muscle length, and muscle configuration were obtained, and the pressure changes recorded during stimulation were analyzed as function of these variables.

METHODS

The studies were carried out on seven adult bred-for-research dogs (19–30 kg) anesthetized with pentobarbital sodium (initial dose, 30 mg/kg iv), as approved by the Animal Ethics and Welfare Committee of the Brussels School of Medicine. The animals were placed in the supine position, intubated with a cuffed endotracheal tube, and connected to a mechanical ventilator (Harvard Pump, Chicago, IL). A venous cannula was inserted in the forelimb to give maintenance doses of anesthetic, after which the C5 and C6 phrenic nerve roots were isolated bilaterally in the neck. Two experimental protocols were then followed.

Experiment 1. Five animals were studied first to define the displacement of the diaphragm and its costal insertions, the changes in muscle length, and the pressure-generating capacity of the muscle during phrenic nerve stimulation at very high lung volumes. Thus, in each animal, the abdomen was opened by a midline incision from the
xiphisternum to the umbilicus, and rows of six polyethylene spheres were stitched superficially to a muscle bundle in the midcostal region of both the left and the right hemidiaphragm, using the same method that has been previously described in detail (4, 11). A balloon-catheter system filled with 1.0 ml of air was also placed between the liver and the stomach to measure abdominal pressure (Pab). The abdomen was then closely sutured in two layers, after which a polyethylene sphere identical to those attached to the diaphragm was sutured to the periostium in the lateral aspect of rib 10 on each side. In so doing, we could assess the displacement of material points on the bony ribs into which the diaphragm inserts and, thus, overcome the difficulty in interpreting the displacement of markers attached near the caudal extremity of the diaphragm muscle fibers (4).

The animal was then transferred to a V-shaped board and placed in a four-channel multidetector CT scanner (Somaton Volume Zoom 4; Siemens Medical Solutions, Forchheim, Germany). The C5 and C6 phrenic nerve roots were laid over insulated stainless steel stimulating electrodes, and a differential pressure transducer (Validyne, Northridge, CA) was connected to a side port of the endotracheal tube to measure Pao. The animal was made apneic by mechanical hyperventilation, and a first helical data acquisition starting ~2 cm caudal to the lower rib cage margin and extending ~2 cm cranial to the xiphoid process was performed during relaxation at FRC. The scanning parameters were the same as those used in previous studies (4, 11): 120 kV, 120 effective mA, 0.5 s/revolution scanning time, 1-mm collimation, and 6.9-mm feed/rotation. The animal was reconnected to the ventilator, and CT data acquisitions were obtained after the respiratory system was passively inflated, in random order, to ~10, 20, 30, 40, 50, and 60 cmH2O transrespiratory pressure. After this procedure was completed, the phrenic nerve roots were bilaterally stimulated (0.1-ms duration, 20-Hz frequency, supra-maximal voltage) at the same lung volumes, and a new set of CT data acquisitions was obtained. All stimulations were performed while the animal was apneic and the endotracheal tube was occluded. A final CT acquisition was obtained during relaxation at FRC to check for the presence or absence of pneumothorax; no pneumothorax was seen in any animal.

Experiment 2. Two animals were studied next to assess the potential influence of abdominal surgery on the pressure-generating capacity of the diaphragm during inflation at very high lung volumes. The C5 and C6 phrenic nerve roots in each animal were stimulated at FRC and after passive inflation to 10, 20, 30, 40, 50, and 60 cmH2O transrespiratory pressure, as described in experiment 1. In these animals, however, the abdominal wall was kept intact, and Pab was measured by using a balloon-catheter system positioned in the stomach through the esophagus.

The animals in both experiments were maintained at a constant, rather deep, level of anesthesia throughout the study. Thus at no time in the experiment did they have a corneal reflex or movements of the fore- or hindlimbs. Rectal temperature was maintained constant between 36 and 38°C with infrared lamps. At the conclusion of the experiment, the animal was given an overdose of anesthetic (30–40 mg/kg iv).

Data analysis. Analysis of the CT data was made as previously described (4, 11). Thus, for each lung volume in each animal, 1.25-mm-thick transverse CT sections during relaxation and during phrenic nerve stimulation were reconstructed at 1.0-mm intervals using a 360° linear-interpolation algorithm and a standard kernel (AB 40f; Siemens Medical Solutions). Sagittal and coronal images were also reconstructed, and these multiplanar reconstructions were used in a workstation (Leonardo; Siemens Medical Solutions) to define the three-dimensional coordinates of each diaphragm and rib marker and to measure the length of each hemidiaphragm. By convention, the coordinates of the different diaphragm markers along the craniocaudal axis were expressed in millimeters relative to the relaxed FRC position of the markers situated near the central tendon; a negative sign indicates a caudal displacement of the markers relative to that position. Similarly, the coordinates of the markers attached to the lateral aspect of rib 10 along the craniocaudal and laterolateral axes were expressed in millimeters relative to the markers’ own relaxed FRC position; a positive sign indicates a cranial or outward displacement, respectively. The length of the muscle bundles was also expressed in millimeters. To allow comparison between the different animals, however, muscle lengths during relaxation and during phrenic nerve stimulation at the different lung volumes were then expressed as percentages of muscle length during relaxation at FRC (L-FRC).

Statistical analysis. There were no consistent interhemidiaphragmatic differences in the position of the markers situated near the central tendon or in muscle lengths. Also, there were no consistent differences in the position of the markers attached to the right and left rib 10. For each condition, therefore, the values for the right and left sides were averaged for each individual animal, and they were then averaged across the animal group. The values of ΔPao and ΔPab recorded during phrenic nerve stimulation at the different lung volumes were also averaged across the animal group, and they are presented as means ± SE. Statistical assessments of the effect of increasing lung volume on pressure, marker position, and diaphragm length were made by ANOVA with repeated measures, and multiple comparison testing of the mean values was performed, when appropriate, using Student-Newman-Keuls tests. The criterion for statistical significance was taken as P < 0.05.

RESULTS

Pressure. The pressure changes measured during phrenic nerve stimulation at the different lung volumes in the five animals of experiment 1 are shown in Fig. 1. In agreement with previous studies (3, 4), ΔPao decreased progressively (P < 0.001) from −31.5 ± 2.9 to −3.2 ± 1.0 cmH2O as transrespiratory pressure (Prs) increased from 0 to +29.6 ± 0.5 cmH2O. However, when Prs was set at +39.6 ± 1.0 cmH2O, ΔPao became zero in one animal and slightly positive in two animals, and this trend continued as lung volume increased further. As a result, when Prs was +62.0 ± 2.0 cmH2O, ΔPao was zero in two animals and positive (range, +2.0 to +3.4 cmH2O) in
three animals. For the animal group, therefore, \( \Delta \text{P}_{\text{ao}} \) at this pressure was +1.5 ± 0.7 cmH\(_2\)O.

\( \Delta \text{P}_{\text{ab}} \) during phrenic nerve stimulation also decreased progressively and continuously \((P < 0.001)\) with increasing lung volume. When \( \text{Prs} \) was +62.0 ± 2.0 cmH\(_2\)O, therefore, two animals had a slightly positive \( \Delta \text{P}_{\text{ab}} \) value (+1.0 cmH\(_2\)O), and two had a slightly negative value (−0.5 cmH\(_2\)O). \( \Delta \text{P}_{\text{ab}} \) for the animal group was +0.2 ± 0.4 cmH\(_2\)O.

**Position of diaphragm dome and muscle length.** The axial position of the markers situated near the central tendon during relaxation at the different lung volumes and during phrenic nerve stimulation at the same lung volumes is shown for the five animals in Fig. 2, and the corresponding values of diaphragm muscle length are shown in Fig. 3. With the animal relaxed, the markers situated near the central tendon were gradually displaced in the caudal direction as lung volume increased, and the muscle progressively shortened \((P < 0.001)\) with increasing lung volume. However, muscle shortening was still 14.0\% of LFRC during stimulation at 62.0 cmH\(_2\)O (Fig. 3).

**Shape of diaphragm.** The diaphragm showed a smooth curvature with a caudal concavity in all animals as \( \text{Prs} \) was passively increased from 0 to +30 cmH\(_2\)O, and in one animal, the muscle retained its overall shape as \( \text{Prs} \) was increased to +60 cmH\(_2\)O. This animal was also one of the two in which \( \Delta \text{P}_{\text{ao}} \) was zero during phrenic nerve stimulation at this pressure.

Phrenic nerve stimulation caused additional caudal displacement of the central tendon and additional muscle shortening at all lung volumes. The displacement of the central tendon during stimulation, however, decreased progressively \((P < 0.001)\) as lung volume increased. When \( \text{Prs} \) before stimulation was set at 62.0 cmH\(_2\)O, the central tendon, in fact, was essentially stationary during stimulation (Fig. 2). The amount of muscle shortening during stimulation also decreased \((P < 0.001)\) with increasing lung volume. However, muscle shortening was still 14.0\% of LFRC during stimulation at 62.0 cmH\(_2\)O (Fig. 3).

**Position of the diaphragm dome and muscle length.** The diaphragm showed a smooth curvature with a caudal concavity in all animals as \( \text{Prs} \) was passively increased from 0 to +30 cmH\(_2\)O, and in one animal, the muscle retained its overall shape as \( \text{Prs} \) was increased to +60 cmH\(_2\)O. This animal was also one of the two in which \( \Delta \text{P}_{\text{ao}} \) was zero during phrenic nerve stimulation at this pressure. In the other four animals, however, when \( \text{Prs} \) was passively increased to +40 cmH\(_2\)O and beyond, areas with a cranial concavity appeared in the ventral third of the costal portion of the muscle. As shown by the coronal sections obtained in a representative animal in Fig. 4A, these areas developed bilaterally at the periphery of the muscle, in the immediate vicinity of the rib cage, so as to form a groove along the ventral third of the rib cage. At 60 cmH\(_2\)O \( \text{Prs} \), this groove in the four animals was 1.6 ± 0.2 cm in width and 1.4 ± 0.2 cm in depth. During phrenic nerve stimulation, however, this area decreased markedly in size in two animals and completely disappeared in the other two (Fig. 4B).

**Position of the lower ribs.** As shown in Fig. 5, the markers attached to rib 10 were progressively displaced cranially and outward as the lungs were passively inflated to +60 cmH\(_2\)O \((P < 0.001)\) for both). During phrenic nerve stimulation at FRC, +10 cmH\(_2\)O, and +20 cmH\(_2\)O, the markers moved inward relative to their relaxation position; they also moved caudally during stimulation at FRC and +10 cmH\(_2\)O. However, during phrenic nerve stimulation at all lung volumes between +30 and +60 cmH\(_2\)O, the markers remained stationary.

**Pressure in animals with intact abdomen (experiment 2).** The \( \Delta \text{P}_{\text{ao}} \) and \( \Delta \text{P}_{\text{ab}} \) values recorded during phrenic nerve stimulation in the two animals studied with the abdomen intact were close to those obtained in experiment 1 (shown in Fig. 1). Thus, as \( \text{Prs} \) in the first animal increased from 0 to +60 cmH\(_2\)O, \( \Delta \text{P}_{\text{ao}} \) gradually decreased from −31.5 to +1.5 cmH\(_2\)O and \( \Delta \text{P}_{\text{ab}} \) decreased from +6.8 to +0.3 cmH\(_2\)O. In the second animal, \( \Delta \text{P}_{\text{ao}} \) similarly decreased from −29.0 to +4.4 cmH\(_2\)O and \( \Delta \text{P}_{\text{ab}} \) decreased from +6.0 to −0.1 cmH\(_2\)O.

![Fig. 2. Axial position of the diaphragm markers situated near the central tendon during relaxation (○) and during phrenic nerve stimulation (●) at different lung volumes. Values are means ± SE obtained from 5 animals. Values are expressed relative to the marker position during relaxation at FRC; negative values denote more caudal position.](J Appl Physiol • doi:10.1152/japplphysiol.01422.2011 • www.jappl.org)
DISCUSSION

The results of this study confirmed that in the dog isolated stimulation of the phrenic nerves at lung volumes above TLC commonly causes a rise in Pao and, thus, that the diaphragm at such lung volumes has an expiratory action on the lung. The magnitude of this action in our animals was similar to that previously reported in rabbits by Sant’Ambrogio and Saibene (see Fig. 2 in Ref. 16), but it was much smaller than that observed in dogs by Minh et al. (14). Specifically, these investigators reported that, when lung volume before stimulation was increased by applying a transrespiratory pressure of 42.0 cmH2O, Pao during stimulation was 9.0 cmH2O (see Table 2 in Ref. 14), and when transrespiratory pressure was further increased to 56.0 cmH2O, ΔPao during stimulation was 13.0 cmH2O. Such high ΔPao values were not observed in any animal of the present study, and when transrespiratory pressure was 62.0 cmH2O, ΔPao during stimulation was, on average, 1.5 cmH2O (Fig. 1).

This difference in the magnitude of ΔPao between the study of Minh et al. (14) and the present study cannot be attributed to the mode of activation of the diaphragm, as muscle contraction in both studies was induced by stimulating the C5 and C6 phrenic nerve roots in the neck. On the other hand, the chest wall in the study of Minh et al. (14) was kept intact, whereas in our animals, the abdominal wall was opened in the midline to stitch radiopaque markers to the diaphragm and was subsequently sutured in several layers. The possibility existed that the suture would induce an increase in the elastance of the abdominal compartment and would thereby reduce the lung-deflating action of the diaphragm at elevated lung volumes (see below). In the second experiment, therefore, the phrenic nerve roots were stimulated in two animals with the abdominal wall intact. The rises in Pao recorded in these animals, however, were hardly greater than those obtained in the first experiment, thus indicating that the difference between the study of Minh et al. (14) and the present study is not related to a difference in the animal preparation.

Regardless of this quantitative difference, the primary result of this study is the observation that the lung-deflating action of the diaphragm was smaller in dogs than in rabbits.
the canine diaphragm at high lung volumes is not the result of the action of the muscle on the lower ribs. Thus, in agreement with our previous studies (3, 4), the descent of the dome during phrenic nerve stimulation in our animals decreased gradually as lung volume increased from FRC to TLC, and this trend continued as lung volume increased above TLC. As a result, when stimulation was performed at transrespiratory pressures of +50 and +60 cmH₂O, the dome of the diaphragm was essentially stationary (Fig. 2), in agreement with the hypothesis of Sant’Ambrogio and Saibene (16). ΔPab, as measured by a balloon-catheter system placed in the midline in the upper part of the abdominal cavity, was almost zero as well (Fig. 1). However, contrary to the hypothesis of Sant’Ambrogio and Saibene’s (16), phrenic nerve stimulation at elevated lung volumes continued to produce significant shortening of the muscle fibers (Fig. 3) and did not cause any inward or caudal displacement of the lower ribs (Fig. 5).

On the other hand, with passive inflation above TLC, prominent, reproducible alterations occurred in the configuration of the diaphragm. Indeed, in four of five animals, a curved groove developed in the periphery of the ventral third of the muscle, which then disappeared or decreased in size with phrenic nerve stimulation (Fig. 4). In other words, because the muscle fibers of the relaxed diaphragm are very short at lung volumes above TLC (Fig. 3), they are very compliant and cannot oppose the force exerted on them by the lung. Consequently, the acute margins of the lower lung lobes invaginate into the ventral part of the costal portion of the muscle. However, when the muscle fibers are subsequently activated, they develop tension and shorten further (Fig. 3), so that the invaginated portions of the lung are pushed back cranially. This cranial displacement must result locally in a rise in pleural surface pressure, and it is reasonable to assume that this pressure rise is then transmitted to the entire pleural cavity. The finding that the only animal of the study that did not show any invagination of the diaphragm during passive inflation was also one of the two animals with a ΔPao of zero during nerve stimulation at 60 cmH₂O transrespiratory pressure fully supports this idea.

As a corollary, it would be expected that the rise in Ppl during phrenic nerve stimulation above TLC would be greater as the volume of the lung invaginated during passive inflation is greater. It would also be expected that the volume of the invagination would be greater when the elastance of the diaphragm-abdomen compartment is lower and/or the elastance of the rib cage is greater. Perhaps the greater lung-deflating action of the diaphragm in the study of Minh et al. study (14) is related to such differences in chest wall elastance, but there are no data available to support this hypothesis.

One final issue deserves consideration. Patients with severe chronic obstructive pulmonary disease (COPD) and hyperinflation commonly have an inward displacement of the lateral portions of the lower rib cage during resting inspiration. This paradoxical displacement (Hoover’s sign) has traditionally been attributed to the direct action of the diaphragm on the lower ribs (7). Thus it is considered that because the diaphragm is low in these patients and the zone of apposition of the muscle to the lower rib cage is very small, the muscle fibers of the diaphragm would be radially oriented and would therefore pull the lower ribs inward, rather than cranially during contraction. In our animals, diaphragm contraction at low lung volumes caused an inward displacement of the lower ribs, and a recent study (2) has shown that this displacement is entirely the result of the large fall in Ppl. However, when transrespiratory pressure was set at +40 cmH₂O and beyond, diaphragm contraction caused little or no fall in Ppl and no displacement of the lower ribs (Fig. 5), thus indicating that in the dog a very low diaphragm with a zone of apposition reduced to zero (as shown in Fig. 4) does not generate enough force to pull the lower ribs inward. To be sure, acute lung inflation in dogs does not reproduce all facets of COPD. Also, diaphragm contraction in this study was induced by stimulation of the phrenic nerves, and lower rib displacements during phrenic nerve stimulation and spontaneous diaphragmatic contraction may be different (2). Consequently, the present observations cannot be extended to patients with COPD without caution. Nonetheless, in agreement with the findings in patients reported by Gorman et al. (8) and recently reviewed by McKenzie et al. (13), these observations suggest that Hoover’s sign requires not only a severe hyperinflation with a marked decrease in the zone of apposition but also a high enough neural drive to the inspiratory muscles to cause a large fall in pleural pressure.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: D.L., M.C., P.A.G., and A.D.T. performed experiments; D.L. and A.D.T. analyzed data; D.L., M.C., P.A.G., and A.D.T. approved final version of manuscript; A.D.T. conception and design of research; A.D.T. interpretation of results of experiments; A.D.T. prepared figures; A.D.T. drafted manuscript; A.D.T. edited and revised manuscript.

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