Function of the canine inspiratory muscle pump in pleural effusion: influence of body position

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Leduc D, De Troyer A. Function of the canine inspiratory muscle pump in pleural effusion: influence of body position. J Appl Physiol 114: 941–947, 2013. First published February 7, 2013; doi:10.1152/japplphysiol.01392.2012.—Pleural effusion, a complicating feature of many diseases of the lung and pleura, adversely affects the pressure-generating capacity of the diaphragm in supine dogs. The objective of the present study was to assess the impact of body position on this effect and to evaluate the adaptation to effusion of the inspiratory muscle pump during breathing. Two experiments were performed. In the first, progressively increasing effusion was induced in anesthetized animals, and the changes in pleural (ΔPpl) and abdominal (ΔPab) pressure were measured during isolated phrenic nerve stimulation while the animals were placed in both the supine and the 45° head-up posture. In the second experiment, graded pleural effusion was also performed, and ΔPpl, ΔPab, and the electromyogram of the parasternal intercostal muscles were measured while the vagotomized animals were breathing spontaneously in the same two postures. The data showed that with effusion 1) ΔPpl during phrenic nerve stimulation was substantially lower in the animals in the head-up than in the supine posture; 2) this postural effect was primarily the result of the decrease in muscle length in the head-up posture; 3) during spontaneous breathing, however, parasternal intercostal inspiratory activity increased and ΔPpl remained unaltered while ΔPab decreased; and 4) the decrease in ΔPab and in the ΔPab/ΔPpl ratio was much larger in the head-up than in the supine posture. It is concluded that in the presence of pleural effusion, the pressure contribution of the inspiratory intercostals muscles during breathing increases and compensates for the shortening of the diaphragm, particularly in the upright posture.

mechanics of breathing; respiratory muscles; diaphragm; inspiratory intercostal muscles

Pleural effusion accompanies many diseases of the lung and pleura, and its occurrence is frequently associated with dyspnea. Pulmonary function studies have been performed in patients in an attempt to assess the mechanism(s) underlying this symptom, and these have shown that the increase in lung volume after thoracentesis is only one-third of the total volume of liquid removed (3, 12, 23). This implies that pleural effusion causes not only a partial deflation of the lung but also a substantial expansion of the chest wall and, with it, a shortening of the inspiratory muscles.

Using computed tomography, Dechman et al. (5) confirmed that the introduction of saline into the pleural space of supine dogs leads to an expansion of the rib cage. More recently De Troyer et al. (8) showed that pleural effusion in supine dogs produces both a cranial displacement of the ribs and a prominent caudal displacement of the diaphragm. As result, the diaphragmatic muscle fibers are shorter, and the capacity of the muscle to generate pressure during isolated contraction is markedly reduced. Compared with passive inflation, the descent of the relaxed diaphragm during effusion is, in fact, greater than the cranial displacement of the ribs (8). One would expect, therefore, that the adverse effect of effusion on the pressure-generating capacity of the inspiratory intercostal muscles would be less than on the diaphragm and, thus, that with increasing effusion, the contribution of the inspiratory intercostals to the pressure (or lung volume) changes during breathing would gradually increase relative to that of the diaphragm (8).

It is also well established that a change from the supine to the upright posture causes a caudal displacement and shortening of the diaphragm. In pleural effusion, the height of the column of pleural liquid acting on the diaphragm should be greater in the upright posture, causing further muscle shortening. In contrast, the hydrostatic forces applied on the cranial portion of the rib cage should be less in the upright posture, and indeed, Krell and Rodarte (21) detected no change in the orientation of the ribs during graded pleural effusion in head-up dogs. It would therefore be expected that in the presence of a given effusion, the pressure-generating capacity of the diaphragm would be smaller in the upright than in the supine posture, whereas the pressure-generating capacity of the inspiratory intercostals muscles would be relatively unaffected by posture. Consequently, it would also be expected that, as the amount of effusion increases, the pressure contribution of the inspiratory intercostals during breathing would increase more in the upright than in the supine posture.

The objective of the present study was to test the following hypotheses: 1) with pleural effusion, the pressure-generating capacity of the diaphragm is more impaired in the upright than in the supine posture; 2) with increasing effusion, the pressure contribution of the inspiratory intercostals during breathing in the supine posture increases relative to that of the diaphragm; and 3) the increase in the pressure contribution of the inspiratory intercostals during breathing with increasing effusion is greater in the upright than in the supine posture. To test these hypotheses, two sets of experiments were performed. In the first experiment, a graded pleural effusion was induced in anesthetized dogs, and the phrenic nerves were selectively stimulated with the animals in both the supine and the head-up posture. In the second experiment, the animals were breathing spontaneously in the same postures, and the relative contributions of the diaphragm and inspiratory intercostals to breathing were evaluated using the analysis of the pleural and abdominal pressure swings described by Macklem et al. (22).
METHODS

The studies were carried out on 12 adult bred-for-research dogs (20–26 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 supine on a tilt table and intubated with a cuffed endotracheal tube, and a venous cannula was inserted in the forelimb to give maintenance doses of anesthetic (3–5 mg·kg⁻¹·h⁻¹). The abdomen was opened by a 4-cm-long midline incision cranial to the umbilicus, and a balloon-catheter system filled with 1.0 ml of air was placed between the liver and the stomach to measure the changes in abdominal pressure (ΔPab). After the abdomen was closely sutured, the rib cage and intercostal muscles were exposed on both sides of the chest from the second to fifth rib by reflection of the skin and superficial muscle layers, and a pleural cannula, 3 mm outer diameter (Lepine Soc., Lyon, France) was introduced in the pleural space through the third right or left intercostal space near the costochondral junction. The cannula was held in place with a purse-string suture and connected to a catheter and a three-way stopcock so that a graded pleural effusion could be induced later. The animal’s head, shoulders, and pelvis were then firmly tethered to avoid postural changes not intended in the study, after which two experimental protocols were followed.

Experiment 1. Five animals were studied first to assess the influence of body position on the impact of pleural effusion on the pressure-generating capacity of the diaphragm. In each animal, the C5 and C6 phrenic nerve roots were isolated bilaterally in the neck and laid over insulated stainless-steel stimulating electrodes. A differential pressure transducer (Validyne Engineering, Northridge, CA) was then connected to a side port of the endotracheal tube to measure airway opening pressure (Pao), after which the animal was made apneic by mechanical hyperventilation (Harvard Apparatus, Holliston, MA). After the ventilator was stopped, the endotracheal tube was occluded at resting end expiration (functional residual capacity, FRC), and square pulses of 0.1-ms duration and supra-maximal voltage were applied in duplicate at a frequency of 20 impulses/s to the left and right phrenic nerves. The animal was then reconnected to the ventilator and tilted to the 45° head-up posture, the position of the phrenic nerve roots on the stimulating electrodes was adjusted, and stimulation was repeated. After the animal was brought back to the supine posture, a volume of 10 ml isosmotic liquid (Dianeal glucose 1.36%; Baxter, Deerfield, IL) per kilogram of body weight was introduced into the pleural space, and after a 10- to 15-min recovery period, stimulation of the phrenic nerve was repeated, when appropriate, using Tukey’s highly significant difference tests. Statistical assessments of the effects of increasing pleural effusion and posture on ΔPao, ΔPab, and ΔPab/ΔPao during phrenic nerve stimulation were made by analysis of variance with repeated measures, and multiple comparison testing of the mean values was performed, when appropriate, using Tukey’s highly significant difference tests. Statistical assessments of the effects of effusion and posture on ΔPpl, ΔPab, ΔPab/ΔPpl, and parasternal intercostal EMG
activity during breathing were made similarly. The criterion for statistical significance was taken as $P < 0.05$.

RESULTS

Pressure-generating capacity of the diaphragm. The $\Delta P_{\text{ao}}$ values obtained during phrenic nerve stimulation in the presence of increasing pleural effusion in the supine and the 45° head-up posture are shown for the five animals in Fig. 1A. With the animals in the supine posture, $\Delta P_{\text{ao}}$ gradually and continuously decreased ($P < 0.001$) from $46.2 \pm 6.6$ cm H$_2$O during control to $18.8 \pm 3.3$ cm H$_2$O with a 40 ml/kg effusion. A large fall in $\Delta P_{\text{ao}}$ was also seen when the animals were in the head-up posture ($P < 0.001$). In this posture, however, $\Delta P_{\text{ao}}$ in the control condition was only $22.9 \pm 3.8$ cm H$_2$O, i.e., $49.0 \pm 4.2\%$ of the corresponding value in the supine posture ($P < 0.001$), and this difference persisted during effusion so that with a 40 ml/kg effusion, $\Delta P_{\text{ao}}$ was only $-3.7 \pm 1.0$ cm H$_2$O.

When the $\Delta P_{\text{ao}}$ values recorded during effusion were expressed as percentages of the values recorded during control, the fall in pressure with increasing effusion was substantially greater ($P < 0.001$) in the head-up than in the supine posture (Fig. 1B). When effusion was set at 40 ml/kg, for example, $\Delta P_{\text{ao}}$ in the supine posture was $40.8 \pm 5.8\%$ of the control value, whereas in the head-up posture, it was only $16.1 \pm 3.3\%$ ($P < 0.001$).

As shown in Fig. 2, $\Delta P_{\text{ab}}$ also decreased progressively in both postures as effusion increased ($P < 0.001$ for both). The $\Delta P_{\text{ab}}$ value measured during control in the head-up posture was similar to that measured during control in the supine posture, but the values measured with effusion in the head-up posture were smaller than those measured in the supine posture ($P < 0.05$ or less). Consequently, whether the values were expressed as absolute values (Fig. 2A) or as percentages of the control values (Fig. 2B), the fall in $\Delta P_{\text{ab}}$ with increasing effusion was also greater ($P < 0.001$) in the head-up than in the supine posture.

As a result of these changes, the ratio of $\Delta P_{\text{ab}}$ over $\Delta P_{\text{ao}}$ during phrenic nerve stimulation in the supine posture slightly

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Fig. 1. Effects of graded pleural effusion on the changes in airway opening pressure ($\Delta P_{\text{ao}}$) during isolated stimulation of the C5 and C6 phrenic nerve roots in the supine (○) and the 45° head-up (●) posture. Means ± SE values obtained from 5 animals. $\Delta P_{\text{ao}}$ values are expressed as absolute values in A and as percentages of the values obtained in the control condition in B. $\Delta P_{\text{ao}}$ decreased gradually and continuously as the volume of effusion increased, in particular in the head-up posture.

Fig. 2. Effects of graded pleural effusion on the changes in abdominal pressure ($\Delta P_{\text{ab}}$) during isolated stimulation of the C5 and C6 phrenic nerve roots in the supine (○) and the 45° head-up (●) posture. Means ± SE values obtained from 5 animals. $\Delta P_{\text{ab}}$ decreased progressively with increasing effusion, more markedly so in the head-up than in the supine posture.
increasing \( (P < 0.05) \) from \(-0.18 \pm 0.04\) during control to \(-0.29 \pm 0.08\) with a 40 ml/kg effusion (Fig. 3). Also, the ratio in the control condition was greater \( (P < 0.001) \) in the head-up than in the supine posture, and the increase in the ratio with increasing effusion was also greater in the head-up posture. With a 40 ml/kg effusion in this posture, \( \Delta P_{ab}/\Delta P_{ao} \) amounted to \(-0.70 \pm 0.13\).

**Pressure development during breathing.** The effects of pleural effusion on \( \Delta P_{pl} \) and \( \Delta P_{ab} \) during inspiration in the supine and the 45° head-up posture are illustrated by the records from a representative animal in Fig. 4, and the pressure changes obtained with the different amounts of effusion are shown for the seven animals in Fig. 5. No alteration in \( \Delta P_{pl} \) occurred with effusion in either the supine or the head-up posture (Fig. 5A). As effusion increased, however, \( \Delta P_{ab} \) in the supine posture progressively decreased \( (P < 0.001) \) from \(+2.3 \pm 0.3\) cmH\(_2\)O during control to \(+1.6 \pm 0.3\) cmH\(_2\)O with 40 ml/kg effusion, and \( \Delta P_{ab} \) in the head-up posture decreased even more \( (P < 0.001) \), from \(+2.5 \pm 0.6\) cmH\(_2\)O during control to \(0.0 \pm 0.2\) cmH\(_2\)O with 40 ml/kg effusion \( (P < 0.001) \) (Fig. 5B). With a 40 ml/kg effusion, in fact, \( \Delta P_{ab} \) in the head-up posture was zero in one animal and had a slightly negative value in three animals.

As is shown in Fig. 6, therefore, the \( \Delta P_{ab}/\Delta P_{pl} \) ratio during breathing gradually decreased \( (P < 0.001) \) with increasing effusion in both postures, but the decrease was much larger in the head-up than in the supine posture. Thus, when effusion in the animal group was set at 40 ml/kg, \( \Delta P_{ab}/\Delta P_{pl} \) in the supine
posture was still negative at $-0.27 \pm 0.06$, whereas in the head-up posture, it was $+0.01 \pm 0.04$ ($P < 0.001$).

Figure 4 also shows the effects of pleural effusion on the EMG activity of the parasternal intercostal muscles. As effusion increased, parasternal intercostal inspiratory activity gradually increased, in particular in the head-up posture. At 40 ml/kg, the peak height of the integrated EMG signal in the supine and the head-up posture was, respectively, 122 ± 12 and 140 ± 11% of the control value ($P < 0.001$).

DISCUSSION

The measurements obtained in the first experiment of the present study confirmed our previous observation (8) that in supine dogs, pleural effusion induces a marked decrease in the capacity of the diaphragm to generate pressure, in particular pleural pressure. In addition, this experiment showed that in the presence of a given effusion, the lung-expanding capacity of the diaphragm is further reduced when the animals are tilted from the supine to the head-up posture. This postural effect was such that, with a large effusion (40 ml/kg), $\Delta P_{\text{ao}}$ (or $\Delta P_{\text{ppl}}$) during phrenic nerve stimulation in the supine posture was $-19$ cmH$_2$O or 40% of the control value, whereas in the head-up posture, it was only $-3.7$ cmH$_2$O or 16% of the control value. In the following sections, we will first analyze the mechanism of the influence of posture on isolated diaphragm function in pleural effusion, and we will then discuss how the inspiratory muscle pump adapts to this setting and performs the act of breathing.

Mechanism of the postural influence on diaphragm function in pleural effusion. It is well established that muscle length is a primary determinant of the pressure-generating capacity of the diaphragm and that the relationship between pressure and muscle length is particularly steep when isolated, tetanic stimulation of the phrenic nerves causes muscle length to decrease to $\sim 60\%$ of the optimal length and below (9, 11, 19). At such short muscle lengths, small changes in length lead to large changes in pressure, and we have recently shown that the detrimental effect of pleural effusion on the diaphragm pressure-generating capacity in the supine posture is well accounted for on the basis of the decrease in muscle length (8). Because of the effects of gravity on the abdominal contents and the pleural liquid, it would be expected that for a given effusion, the diaphragm would be more caudal and shorter when the animals are in the head-up than in the supine posture. Therefore, even if the adoption of the head-up posture caused only a small decrease in diaphragm length, it should produce a substantial decrease in pressure during phrenic nerve stimulation.

In addition, with a change from the supine to the head-up posture, the caudal displacement of the abdominal contents induces an outward displacement of the ventral wall of the abdomen and, with it, a lengthening of the abdominal muscles. In the head-up posture, therefore, these muscles should develop passive tension, and this tension should increase as muscle length is further increased by pleural effusion and phrenic nerve stimulation. In agreement with this view, $\Delta P_{\text{ab}}/\Delta P_{\text{ao}}$ during phrenic nerve stimulation in our control animals was greater in the head-up than in the supine posture (Fig. 3). This ratio also increased progressively with increasing effusion in both postures but increased more in the head-up posture, thus confirming that abdominal compliance in this posture was lower. It would be expected that in this condition, the descent of the diaphragm during contraction would be reduced and, thus, that the capacity of the muscle to generate $\Delta P_{\text{pl}}$ would be lower than anticipated on the basis of the postural decrease in muscle length alone.

The relative contributions of these two mechanisms to the marked decrease in the diaphragm capacity to generate $\Delta P_{\text{ao}}$...
during pleural effusion can be estimated as follows. As effusion increased to 40 ml/kg, \(\Delta P_{\text{Pao}}\) in the supine posture decreased by 59% from -46.2 to -18.8 cmH\(_2\)O. If abdominal compliance were unaltered, \(\Delta P_{\text{Pab}}\) would also decrease by 59%, i.e., from +7.3 to +3.0 cmH\(_2\)O. Instead, \(\Delta P_{\text{Pab}}\) decreased from +7.3 to +4.7 cmH\(_2\)O. In other words, the decrease in abdominal compliance induced by pleural effusion in the supine posture would account for a 1.7 cmH\(_2\)O fall in \(\Delta P_{\text{Pao}}\), which corresponds to 6% of the total pressure loss. Similarly, as effusion in the head-up posture increased to 40 ml/kg, the decrease in abdominal compliance was responsible for a 1.2 cmH\(_2\)O fall in \(\Delta P_{\text{Pao}}\), whereas the total loss in \(\Delta P_{\text{Pao}}\) was 19.2 cmH\(_2\)O. The conclusion can be drawn, therefore, that the prominent decrease in the lung-expanding capacity of the diaphragm that occurs with pleural effusion in both the head-up and the supine posture is primarily the result of the decrease in active muscle length.

Adaptation of the inspiratory muscle pump to pleural effusion. Because the diaphragm is the main inspiratory muscle, it would be expected that a marked decrease in its lung-expanding action, as observed in pleural effusion, would have a detrimental effect on lung expansion during breathing and, thus, would cause a fall in \(\Delta P_{\text{Ppl}}\). Alternatively, it would elicit a compensatory increase in inspiratory neural drive so as to maintain \(\Delta P_{\text{Ppl}}\) constant. Furthermore, because the pressure-generating capacity of the inspiratory intercostals would be relatively preserved during effusion, it would also be expected that the pressure contribution of these muscles during breathing would increase relative to that of the diaphragm. These alterations should occur in both the supine and the head-up posture but the magnitude of the changes should be larger in the head-up posture.

The recordings obtained in the second experiment of the study confirmed these predictions in all respects. Thus, in these vagotomized animals, no decrease in \(\Delta P_{\text{Ppl}}\) during breathing was observed with effusion (Fig. 5), but a gradual increase in parasternal intercostal activity occurred as effusion increased, thus indicating that the inspiratory neural drive was increased (6, 7). At the same time, a progressive decrease in \(\Delta P_{\text{Ppl}}\) occurred, so that the \(\Delta P_{\text{Pab}}/\Delta P_{\text{Ppl}}\) ratio decreased. Finally, the increase in parasternal intercostal inspiratory activity during effusion was larger in the head-up than in the supine posture (Fig. 4), and the decrease in \(\Delta P_{\text{Pab}}\) in the head-up posture was so prominent that with a 40 ml/kg effusion, the \(\Delta P_{\text{Pab}}/\Delta P_{\text{Ppl}}\) ratio was approximately zero (Fig. 6). Yet, during isolated stimulation of the phrenic nerves (i.e., when the contribution of the diaphragm to \(\Delta P_{\text{Ppl}}\) was 100%), the \(\Delta P_{\text{Pab}}/\Delta P_{\text{Ppl}}\) ratio progressively increased, rather than decreased, with increasing effusion, and for any volume of liquid, the ratio in the head-up posture was greater, rather than smaller, than in the supine posture (Fig. 3). Collectively, these findings indicate that the contribution of the inspiratory intercostals to \(\Delta P_{\text{Ppl}}\) during breathing progressively increases with increasing effusion, whereas that of the diaphragm decreases; they also indicate that the decrease in the diaphragm contribution to \(\Delta P_{\text{Ppl}}\) is larger in the head-up than in the supine posture.

No attempt was made to record diaphragmatic EMG activity in our animals because moving viscera in the upper abdominal cavity and implanting electrodes in the diaphragm may induce inhibition of the diaphragm (14, 15, 24). Also, when recorded with intramuscular electrodes, diaphragmatic EMG activity shows artifactual changes with alterations in the conductivity of the environment surrounding the electrodes (2) and with changes in length of the muscle fibers (16, 20). Therefore, the possibility that diaphragm activity decreased with pleural effusion cannot be formally excluded. However, similar to neural drive to the canine parasternal intercostals (6, 7), neural drive to the diaphragm is primarily governed by supraspinal control mechanisms, and measurements of motoneuron synchronization in cats by Vaughan and Kirkwood (27) showed that phrenic motoneurons and parasternal intercostal motoneurons receive common monosynaptic inputs. On this basis, it would therefore be reasonable to assume that effusion induced a compensatory increase in neural drive to both the parasternal intercostals and the diaphragm.

The analysis of the changes in transdiaphragmatic pressure (\(\Delta P_{\text{Pdi}} = \Delta P_{\text{Pab}} - \Delta P_{\text{Ppl}}\)) recorded in our animals also supports the idea that activation of the diaphragm during breathing increased with increasing pleural effusion. That is, the data shown in Figs. 1 and 2 indicate that, with the animals in the supine posture, \(\Delta P_{\text{Pdi}}\) during phrenic nerve stimulation decreased by 56% of its control value as effusion increased to 40 ml/kg. In contrast, \(\Delta P_{\text{Pdi}}\) during inspiration decreased by only 5% (Fig. 5). Similarly, with a large effusion in the head-up posture, \(\Delta P_{\text{Pdi}}\) during phrenic nerve stimulation and during spontaneous breathing decreased, respectively, by 80% and 14% of the control value. To be sure, the diaphragm shortens more during isolated phrenic nerve stimulation than during spontaneous breathing, and the relationship between \(P_{\text{di}}\) and muscle length is steeper when length decreases to 60% of the optimal muscle length and below (9, 11, 19). This slope difference in the relationship could partly account for the relative maintenance of \(\Delta P_{\text{Pdi}}\) during spontaneous breathing, so no quantitative estimate can be made of the increase in diaphragm activation during effusion.

In summary, the present studies showed that 1) pleural effusion impairs the lung-expanding capacity of the diaphragm; 2) this effect is larger in the head-up posture because the muscle is shorter in this posture; 3) this impairment is compensated for by an increase in neural drive to the inspiratory intercostals and the diaphragm, so that the pleural pressure swings during breathing remain unaltered; and 4) because the action of the diaphragm during effusion is impared more than that of the inspiratory intercostals, the relative pressure contribution of these muscles is increased, particularly in the head-up posture.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: D.L. and A.D.T. performed experiments; D.L. and A.D.T. analyzed data; D.L. and A.D.T. interpreted results of experiments; D.L. and A.D.T. drafted manuscript; D.L. and A.D.T. approved final version of manuscript.

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