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Alteration of the Piglet Diaphragm Contractility In Vivo *and Its Recovery after Acute Hypercapnia*

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Background: **The effects of hypercapnic acidosis on the diaphragm and its recovery to normocapnia have been poorly evaluated. The authors studied diaphragmatic contractility facing acute variations of arterial carbon dioxide tension ($Paco_2$) and evaluated the contractile function at 60 min after normocapnia recovery.**

Methods: **Thirteen piglets weighing 15–20 kg were anesthetized, ventilated, and separated into two groups: a control group (n = 5) evaluated in normocapnia (time-control experiments) and a hypercapnia group (n = 8) in which animals were acutely and shortly exposed to five consecutive ranges of $Paco_2$ (40, 50, 70, 90, and 110 mmHg). Then carbon dioxide insufflation was stopped. Diaphragmatic contractility was assessed by measuring transdiaphragmatic pressure variations obtained after bilateral transjugular phrenic nerve pacing at increased frequencies (20–120 Hz). For each level of arterial pressure of carbon dioxide, pressure–frequency curves were obtained *in vivo* by phrenic nerve pacing.**

Results: **In the hypercapnia group, mean ± SD transdiaphragmatic pressure significantly decreased from 41 ± 3 to 29 ± 3 cm H_2O ($P < 0.05$) between the first (40 mmHg) and fifth (116 mmHg) stages of capnia at the frequency of 100 Hz stimulation. The observed alteration of the contractile force was proportional to the level of $Paco_2$ ($r^2 = 0.61$, $P < 0.01$). Normocapnia recuperation allowed a partial recovery of the diaphragmatic contractile force (80% of the baseline value) at 60 min after carbon dioxide insufflation interruption.**

Conclusion: **A short exposure to respiratory acidosis decreased diaphragmatic contractility proportionally to the degree of hypercapnia, and this alteration was only partially reversed at 60 min after exposure.**

ACUTE respiratory acidosis occurs with acute respiratory failure, which can result from any sudden respiratory parenchymal (*e.g.*, pulmonary edema and massive pulmonary embolism),[1–3] airway,[4–10] pleural, chest wall, neuromuscular (*e.g.*, spinal cord injury), or central nervous system (*e.g.*, drug overdose) event.[11–13] Two main situations of hypercapnic acidosis are encountered in patients with acute respiratory failure in the intensive care unit. First, it is observed in weak or comatose patients with a "short and acute" exposure to hypercapnia.[11–14] Second, it is observed in mechanically ventilated patients with a limited tidal volume and plateau pressure ("protective settings").[4,15,16] Although the effects of hypercapnia have been well evaluated on hemodynamic[17–19] and pulmonary functions,[4,20–22] few studies have evaluated *in vivo* effects of both "short" and "prolonged" hypercapnia exposure on diaphragmatic function. To our knowledge, only two studies have evaluated its effect on the diaphragm in animals at rest and without a fatigue protocol preceding hypercapnia.[23,24] These studies[23,24] reported a decrease in diaphragmatic force related to respiratory acidosis, but neither of them evaluated the recovery of contractile diaphragm properties after acute hypercapnia. Nevertheless, approaching the functional recovery of diaphragmatic weakness secondary to acute hypercapnia is important in terms of clinical applications, such as the weaning process.

Therefore, our model, using piglets, was designed to evaluate, first, the effect of short hypercapnia on transdiaphragmatic pressure (Pdi) and, second, the immediate (1-h) recovery of Pdi after the return to normocapnia. Our hypothesis was that a short and acute exposure to hypercapnia would decrease the Pdi, which would not return to its baseline value after 1-h recovery from hypercapnia.

Materials and Methods

This study, including care of the animals involved, was conducted according to the official edict presented by the French Ministry of Agriculture (Paris, France) and the recommendations of the Declaration of Helsinki. Therefore, these experiments were conducted in an authorized laboratory and under the supervision of authorized researchers (J.M., S.J., X.C., and S.M.).

Animal Preparation and Experimental Procedures

Thirteen piglets (15–20 kg) were anesthetized with intravenous pentobarbital sodium (5–6 mg/kg), intubated with a cuffed endotracheal tube, and mechanically ventilated (Onyx Plus®; Tyco, St. Louis, MO), with an inspired fraction of oxygen of 0.35, a tidal volume be-

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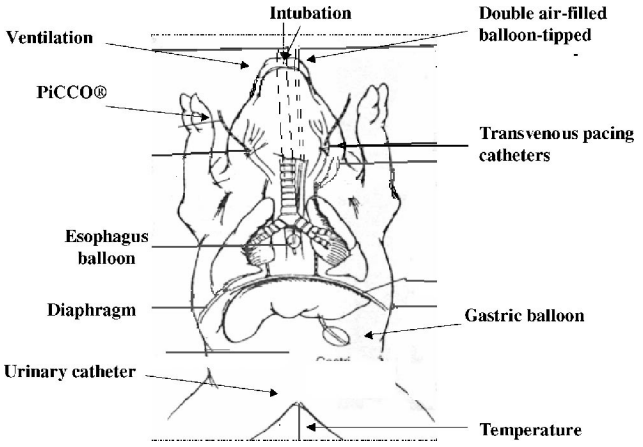

Fig. 1. Diagram of preparation showing jugular and carotid catheters and double air-filled balloon with transvenous jugular phrenic pacing. PiCCO (Pulsion, Munich, Germany) = pulse induced contour cardiac output.

tween 8 and 10 ml/kg, 20 cycles/min to obtain normocapnia, and 4 cm H_2O end-expiratory pressure. In this study, we used the same experimental design described in our previous study[25] (fig. 1). Briefly, the piglets were maintained under anesthesia with continuous intravenous propofol (15–20 mg · kg^{-1} · h^{-1}) and ketamine (3–4 mg · kg^{-1} · h^{-1}). The piglets did not have spontaneous ventilatory activity, as assessed by the esophageal pressure curves. Although, hypercapnia is a strong ventilatory stimulus, the anesthesia could be adapted to inhibit this stimulus. Therefore, we could affirm that the piglets were not in asynchrony with the ventilator. Neuromuscular agents were not used. An oral gastric tube was placed. A vesicostomy was performed and a urine catheter were placed for urine collection. A carotid arterial line (PiCCO; Pulsion, Munich, Germany) was inserted for the monitoring of heart rate, arterial blood pressure, and cardiac output.[26] At the end of the procedure, animals were killed by intravenous injection of potassium chloride.

Design of the Study

Animals were separated into two groups (fig. 2): (1) a control group (n = 5) mechanically ventilated in normocapnia without any intervention in which studied variables were measured each hour over the same time period (6-h) as in the hypercapnic group (time-control experiments) and (2) a hypercapnic group (n = 8) acutely and shortly exposed to five consecutive ranges of arterial carbon dioxide tension ($Paco_2$) in the first part of the study and evaluated at 60 min after normocapnia recovery in the second part of the study. The two groups received the same care except for the capnia levels.

First Part of the Study: Induction of Hypercapnia

Five consecutives ranges of capnia were studied: 35–45, 50–60, 60–70, 90–100, and 110–120 mmHg. Each level of arterial pressure of carbon dioxide was obtained by insufflating carbon dioxide (Aga Medical, Bassens, France) into the inspiratory line of the ventilator. We did not change the respiratory rate or the tidal volume. Arterial pressure of carbon dioxide levels were checked by a capnograph (Deltatrac; Datex-Ohmeda, Helsinki, Finland) and then verified by arterial blood gases (iSTAT®; Abbott, Abbott Park, IL). Steady state at all arterial pressure of carbon dioxide levels was verified by the constancy of end-tidal carbon dioxide for at least 5 min. After obtaining end-tidal carbon dioxide steady state, we performed a hemodynamic evaluation. Arterial blood gases and Pdi measurements were performed after 15 min of additional exposure at this level of capnia. After measurements, the inspiratory flow of carbon dioxide was increased to the next level. We also calculated lung compliance as follows: tidal volume/(plateau pressure − esophageal pressure).

Second Part of the Study: Recovery

After the end of the last studied period, the carbon dioxide insufflation was discontinued. We then mea-

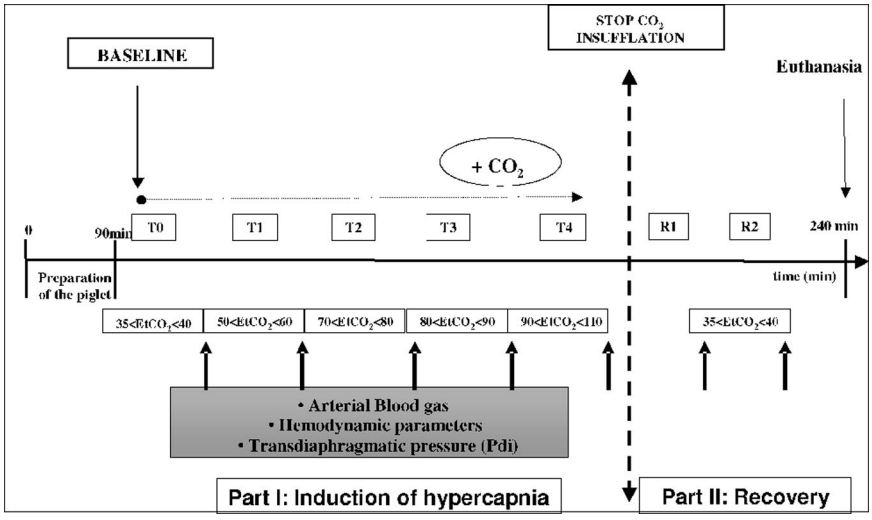

Fig. 2. Design of the study. The hypercapnic group (n = 8) was acutely and shortly exposed to five consecutive ranges of arterial carbon dioxide tension (in the first part of the study and evaluated at 60 min after normocapnia recovery in the second part of the study). CO_2 = carbon dioxide; $ETco_2$ = end-tidal carbon dioxide; Pdi = transdiaphragmatic pressure.

sured the Pdi at 30 and 60 min after the return to normocapnia, which was attained 5–10 min after stopping the insufflation.

In Vivo *Measurement of Transdiaphragmatic Pressure*

Bipolar transvenous pacing catheters were introduced *via* each internal jugular vein and adjusted to achieve stimulation of the phrenic nerve and subsequent contraction of the diaphragm. Double air-filled balloon-tipped catheters were placed transorally in the distal third of the esophagus and in the stomach for measurement of Pdi. Pdi was produced by supramaximal stimulation at frequencies of 20, 40, 60, 80, 100, and 120 Hz in a serial manner and recorded for a given arterial pressure of carbon dioxide. Each train of impulses had a duration of 2,000 ms. Each impulse in the train had duration of 150 ms. A pressure–frequency curve was obtained for each range of arterial pressure of carbon dioxide for each piglet at functional residual capacity, as described in our previous study.[25]

Statistical Analysis

After performing a Kolmogorov-Smirnov test to assure the normality of the variable distribution, we expressed data as mean ± SD. We performed separate analyses of hypercapnia and recovery phases of our study. Comparisons of several means were performed using repeated-measures analysis of variance and the Newman-Keuls test. The difference between the two groups was mainly analyzed by using the interaction between time and group. Comparison of two means was performed using an unpaired Student *t* test. Correlation coefficients (r^2) between $Paco_2$ and Pdi and carbon dioxide were calculated. All *P* values were two-tailed, and a *P* value of less than 0.05 was required to rule out the null hypothesis. Statistical analysis was performed using SAS/STAT software version 8.1 (SAS Institute, Cary, NC).

Results

No significant difference was observed between the control group and the hypercapnia group for all the studied baseline variables. In the hypercapnia group, each range of capnia was attained in the eight piglets. In the first three piglets, the carbon dioxide insufflation was progressively stopped to avoid adverse cardiovascular effects contrary to the five other piglets in which carbon dioxide insufflation was stopped without steps. To avoid confusion, we have not reported the recovery phase in the first three piglets.

The mean tidal volume was 9.2 ± 1.1 ml/kg, and the respiratory rate was 20 ± 2 breaths/min. We did not change the respiratory settings throughout the study. The mean weight of the piglets was 17 ± 0.8 kg. The duration of the study was 240 ± 36 min.

Hemodynamic Variables and Gas Exchange

The hemodynamic and gas exchange variables are reported in table 1. The baseline hemodynamic and gas exchange data of the two groups were not significantly different and did not change significantly in the control group during the 4 h of the time-control experiment in normocapnia ventilation without any intervention. In the hypercapnia group, we observed a progressive increase in the heart rate and arterial blood pressure. There was a significant correlation between arterial pressure of carbon dioxide increase and cardiac output increase ($r^2 = 0.54$, $P < 0.01$). In the second part of the study (recovery), the hemodynamic variables returned to baseline values. The variables of oxygenation were maintained normal during the entire procedure.

In Vivo *Measurement of Transdiaphragmatic Pressure*

The baseline pressure–frequency curves of the two groups were not significantly different (Pdi at 20 Hz: 25.5 ± 1.9 *vs.* 26.9 ± 2.1 cm H_2O; Pdi at 100 Hz: 40.5 ± 2.7 *vs.* 42.8 ± 3.4 cm H_2O) and remained unchanged in the control group after 4 h in normocapnia ventilation without any intervention (Pdi at 20 Hz: 26.9 ± 2.1 *vs.* 25.2 ± 2.3 cm H_2O; Pdi at 100 Hz: 42.8 ± 3.4 *vs.* 38.9 ± 4.3), indicating that anesthetic agents used did not significantly modify the diaphragmatic function.

The pressure–frequency curves in the hypercapnia group, for the five ranges of capnia, are represented in figure 3. Pdi was significantly lower ($P < 0.05$) at all stimulation frequencies between the third, fourth, and fifth levels of capnia and the initial value of Pdi (normocapnia). We found a significant correlation between Pdi decrease and capnia increase ($r^2 = 0.61$, $P < 0.01$). However, we obtained several pair measurements for each piglet, and the correlation coefficient should be interpreted with caution because the experimental design resulted in data that potentially violate the assumptions of correlation analysis. Moreover, for the hypercapnia group, an interaction effect between time and capnia was observed for T2, T3, and T4 (table 1 and figs. 3 and 4).

In the hypercapnia group, 30 and 60 min after the return to normocapnia, Pdi increased but did not return to its baseline value. Pdi reached only 80–85% of initial values and was significantly different from baseline values ($P < 0.05$; fig. 4).

The lung compliance values throughout the study varied from 72 ± 5 to 83 ± 5 ml/cm H_2O with no significant changes between steps, demonstrating that mechanical properties of the respiratory system remained stable throughout the procedure (table 1).

Table 1. Hemodynamic and Respiratory Variables for the Five Ranges of Capnia and the Two Ranges of Recovery Obtained in the Hypercapnia Group and in the Control Group over the Same Period of Time

	Hypercapnia Induction Phase					Between-group Comparisons	Recovery Phase			Between-group Comparisons
	T0	T1	T2	T3	T4		T0	R1	R2	
$Paco_2$, mmHg						—				—
Control	39 ± 6	39 ± 6	41 ± 5	40 ± 6	39 ± 6		38 ± 6	39 ± 6	41 ± 5	
Hypercapnia	40 ± 7	54 ± 6	68 ± 9	95 ± 18	116 ± 16		41 ± 6	43 ± 7	43 ± 5	
HR, beats/min						$P < 0.05$				NS
Control	113 ± 8	107 ± 6	110 ± 9	101 ± 11	107 ± 12		112 ± 8	101 ± 9	99 ± 12	
Hypercapnia	120 ± 4	123 ± 11	137 ± 9	171 ± 13*†	191 ± 11*†		121 ± 3	128 ± 14	125 ± 14	
SBP, mmHg						$P < 0.05$				NS
Control	90 ± 9	88 ± 7	92 ± 7	90 ± 8	93 ± 12		91 ± 8	88 ± 9	91 ± 7	
Hypercapnia	95 ± 7	122 ± 3†	126 ± 4†	144 ± 15*†	132 ± 15*†		94 ± 7	99 ± 4	101 ± 5	
MBP, mmHg						$P < 0.05$				NS
Control	72 ± 4	70 ± 6	78 ± 6	68 ± 5	72 ± 7		73 ± 5	71 ± 5	73 ± 5	
Hypercapnia	83 ± 7	105 ± 2†	108 ± 3†	123 ± 13*†	112 ± 15*†		83 ± 7	87 ± 7	89 ± 2	
CO, l/min						$P < 0.05$				NS
Control	2.8 ± 0.4	3.0 ± 0.2	2.7 ± 0.4	2.8 ± 0.4	3.1 ± 0.3		2.7 ± 0.5	3.0 ± 0.2	2.9 ± 0.5	
Hypercapnia	3.0 ± 0.2	3.5 ± 0.3	3.7 ± 0.5	4.3 ± 0.4†	5.1 ± 0.6*†		3.1 ± 0.1	3.5 ± 0.7	3.6 ± 0.7	
pH						$P < 0.05$				NS
Control	7.45 ± 0.02	7.48 ± 0.03	7.44 ± 0.02	7.46 ± 0.03	7.47 ± 0.03		7.44 ± 0.01	7.46 ± 0.03	7.44 ± 0.03	
Hypercapnia	7.46 ± 0.03	7.34 ± 0.03*	7.27 ± 0.05*†	7.03 ± 0.08*†	6.98 ± 0.01*†		7.45 ± 0.03	7.41 ± 0.06	7.42 ± 0.07	
Pao_2, mmHg						NS				NS
Control	142 ± 23	164 ± 27	151 ± 19	160 ± 22	146 ± 31		141 ± 22	154 ± 24	152 ± 21	
Hypercapnia	123 ± 32	151 ± 55	148 ± 63	145 ± 51	120 ± 69†		122 ± 31	144 ± 36	141 ± 39	
Sao_2, %						NS				NS
Control	99 ± 1	99 ± 1	99 ± 1	99 ± 1	99 ± 2		98 ± 1	99 ± 1	99 ± 1	
Hypercapnia	98 ± 1	99 ± 2	99 ± 1	99 ± 1	99 ± 2		98 ± 1	99 ± 1	99 ± 2	
Bicarbonate, mm						NS				NS
Control	28 ± 2	29 ± 3	28 ± 1	29 ± 2	29 ± 2		28 ± 1	28 ± 3	28 ± 2	
Hypercapnia	30 ± 1	30 ± 3	31 ± 2	31 ± 3	30 ± 4		29 ± 1	29 ± 5	30 ± 5	
$ETco_2$, mmHg						$P < 0.05$				NS
Control	39 ± 5	38 ± 4	39 ± 3	38 ± 5	37 ± 5		38 ± 4	39 ± 4	38 ± 5	
Hypercapnia	39 ± 6	56 ± 8†	77 ± 4†	90 ± 7†	105 ± 13†		39 ± 5	44 ± 4	43 ± 5	
Lung compliance, ml/cm H_2O						NS				NS
Control	79 ± 7	82 ± 7	77 ± 9	79 ± 7	77 ± 6		78 ± 6	78 ± 7	83 ± 5	
Hypercapnia	74 ± 4	74 ± 5	74 ± 4	72 ± 5	73 ± 4		73 ± 3	74 ± 5	73 ± 4	

Data are expressed as mean ± SD. Targeted ranges of capnia were reached for the induction phase as well as for the recovery phase for the eight piglets in the hypercapnia group. Exposure to a short and an acute respiratory acidosis resulted in a significant increase of heart rate (HR), blood pressure, and cardiac output (CO), which returned to initial values after the return to normocapnia. The recovery phase was attained in only five piglets. There were not any significant differences for hemodynamic as well as for respiratory parameters in the control group (five piglets). Statistical comparison was not performed for arterial carbon dioxide tension ($Paco_2$). *P* values for between group comparisons refer to interaction between time and group.

* $P < 0.05$ *vs.* baseline value; † $P < 0.05$ *vs.* control group.

$ETco_2$ = end-tidal carbon dioxide; MBP = mean blood pressure; NS = not significant; Pao_2 = arterial oxygen tension; R1 = first recovery step at 30 min after return to normocapnia; R2 = second recovery step 60 min after return to normocapnia; Sao_2 = arterial oxygen saturation; SBP = systolic blood pressure; T0 = first level of $Paco_2$ (baseline–normocapnia); T1–T4 = successive levels of increase in $Paco_2$.

Discussion

Our main results are that, first, a short exposure to acute hypercapnic acidosis decreases contractile force (25–30%) of the diaphragm proportionally to the cumulative effects of increasing levels of hypercapnia and, second, the recovery of this alteration is incomplete 60 min after the return to normocapnia.

In accord with similar findings reported in the literature, respiratory acidosis seems to alter diaphragmatic contractile force.[23,24,27] In the current study, we found that Pdi decreased significantly from 41 ± 3 cm H_2O to 29 ± 3 cm H_2O between the first (40 mmHg) and fifth (116 mmHg) stages of capnia at the supramaximal frequency of stimulation (120 Hz) (figs. 3 and 4). To our knowledge, Schnader *et al.*[23] were the first to evaluate the effect of different ranges of hypercapnia on diaphragm force in dogs. They evaluated seven levels of capnia by reducing the tidal volume and respiratory rate in six mechanically ventilated dogs breathing various inspiratory gas mixtures. After direct stimulation of the phrenic nerve, they showed an alteration of the contractile force proportional to the level of capnia, as we found in the current study for similar levels of capnia (fig. 3). However, the reduction of tidal volume, which resulted in a decreased functional residual capacity in the trial of Schnader *et al.*,[23] may have contributed to the deterioration to the length–force relation of the diaphragm because variations of pulmonary volume change the orientation of the diaphragmatic fibers, thereby changing their contractile properties.[28] To prevent any change of the length–force relation, we used only different inspiratory gas mixtures of capnia to induce hypercapnia. Moreover, the stability of transpulmonary pressures suggests that lung volumes were similar throughout the study.

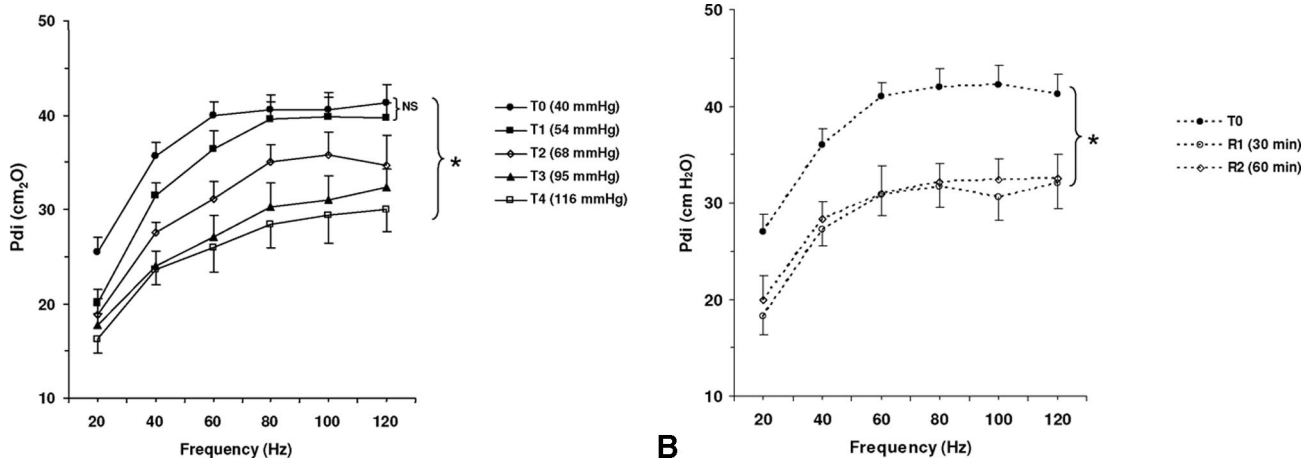

Fig. 3. Diaphragmatic pressure–frequency curves obtained in the hypercapnia group. (*A*) Induction of hypercapnia phase (n = 8). Transdiaphragmatic pressure (Pdi) after supramaximal phrenic nerve stimulation at 20, 40, 60, 80, 100, and 120 Hz for each level of arterial carbon dioxide tension ($Paco_2$) obtained in the hypercapnia group. Pdi was significantly lower (* $P < 0.05$ *vs.* baseline) between the third (T2), fourth (T3), and fifth (T4) levels of capnia and the initial value of Pdi (T0: normocapnia–baseline). (*B*) Recovery phase (n = 5). Pdi obtained after supramaximal phrenic nerve stimulation at 20, 40, 60, 80, 100, and 120 Hz at baseline ($Paco_2$ = 40 mmHg) and at 30 and 60 min after return to normocapnia in the five piglets in which recovery phase has been performed. Pdi was significantly lower (* $P < 0.05$ *vs.* baseline) between the recovery phase and the initial value of Pdi (T0: normocapnia–baseline). Data are expressed as mean ± SD. NS = not significant.

Indeed, the obtained lung compliance values throughout the study varied from 72 ± 5 to 83 ± 5 ml/cm H_2O, with no significant difference between steps (table 1).

Yanos *et al.*[24] compared the effects of an acute metabolic and respiratory acidosis (at the same level: pH = 7.05) on diaphragmatic contractile properties in ventilated dogs. As in the study of Schnader *et al.*[23] and our current study, the authors found a decrease in diaphragmatic force due to respiratory acidosis, whereas they did not observe a significant alteration with a metabolic acidosis. They explained the discrepancy between the results observed with the two types of acidosis[24] by a faster decrease in intracellular pH when hypercapnia was the cause of acidosis.

The results of our trial confirm that even a short and acute exposure to hypercapnia acidosis alters the con-

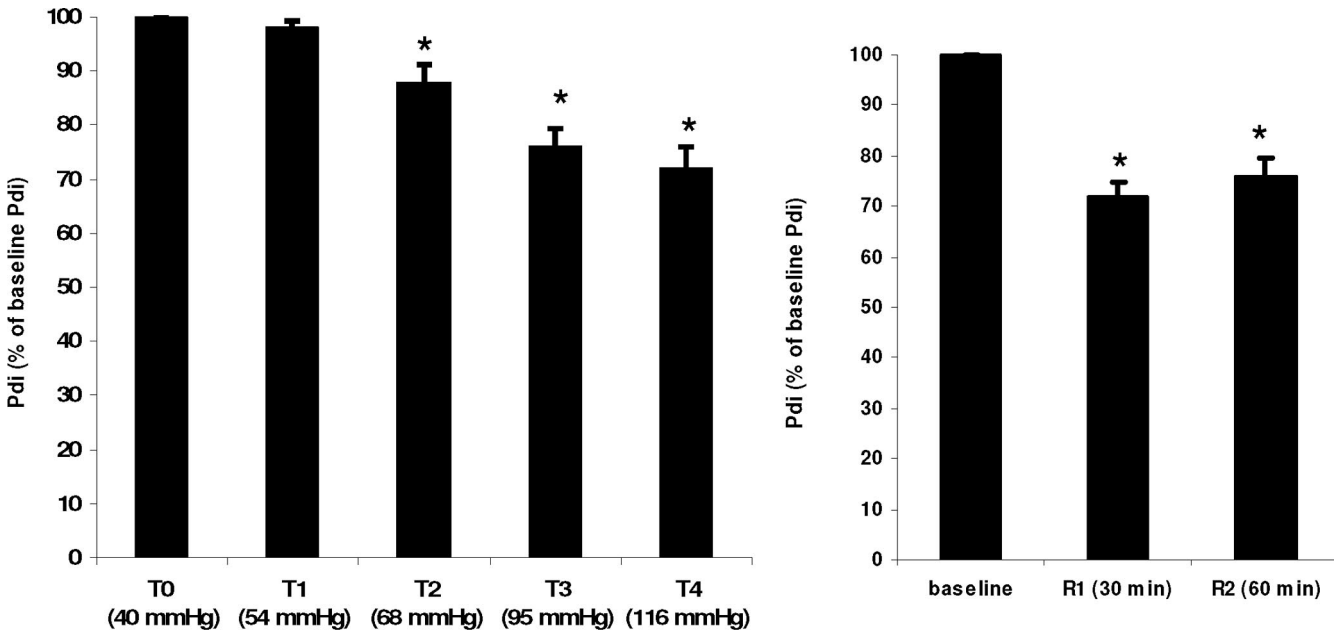

Fig. 4. Transdiaphragmatic pressure (Pdi) expressed as percentage of Pdi at normocapnia (baseline) obtained at 100 Hz stimulation at each level of capnia. (*A*) Induction of hypercapnia phase (n = 8). Pdi was significantly lower (* $P < 0.05$) at 100 Hz between the third (T2), fourth (T3), and fifth (T4) levels of capnia and the initial value of Pdi (T0: normocapnia–baseline). (*B*) Recovery phase (n = 5 piglets). Pdi was significantly lower (* $P < 0.05$) at 100 Hz stimulation between the recovery points (R1 and R2: 30 and 60 min after the return to normocapnia, respectively) and the baseline point (normocapnia). Data are expressed as percentage (mean ± SD).

tractile property of the diaphragm and thus increases the respiratory muscle work. Therefore, this diaphragm contractile property alteration may potentiate the rapid deterioration that occurs during acute exacerbation of respiratory status in these patients.

In addition, four human trials[29–32] studied the effect of hypercapnia on diaphragmatic force in healthy subjects. They all conserved spontaneous ventilation and performed physical exercise in a carbon dioxide–added atmosphere. They found conflicting results about the effect of carbon dioxide on the diaphragm, but these works are difficult to transpose to intensive care practice because the conditions of the studies were not similar (studied population, lower level of hypercapnia, duration of the procedure, hyperventilation as a consequence of the inhalation of carbon dioxide level of neuromuscular drive).

Reduced muscle contractility during hypercapnia is thought to be a result of the decrease in intracellular pH,[6] which has been demonstrated using ^{31}phosphorus nuclear magnetic resonance.[33] Increased binding of calcium to the sarcoplasmic reticulum, decreased affinity of troponin for calcium, and reduction in the rate of glycolysis, and hence adenosine triphosphate resynthesis, have been suggested as possible mechanisms underlying the reduction in contractility. Some authors[23,30] postulated that decreased force production during hypercapnia was caused by a secondary decrease in intracellular pH, which would decrease the binding of calcium to troponin. However, neither of these two experimental studies[23,24] evaluated recovery upon return to normocapnia.

Our results show that after a short and acute exposure to hypercapnic acidosis, the Pdi does not recover its baseline value 1 h after discontinuation of the carbon dioxide insufflation (figs. 3 and 4). This alteration was not completely reversed when normocapnia was restored, the recovery of the diaphragmatic contractile force reaching only 80% of its initial value 60 min after discontinuation of the carbon dioxide insufflation (figs. 3 and 4). This may play a role in the recovery of decompensated chronically obstructive or asthmatic patients who may not immediately recover their diaphragmatic force after an acute respiratory failure episode. We investigated the healthy piglet diaphragm, but caution must be used when extrapolating these results to an altered diaphragm.[34]

Only one human study[30] evaluated the recovery period after short (2-min) exposure to moderate hypercapnia (end-tidal carbon dioxide was increased from 5.5% to 8.9%) in seven healthy subjects who spontaneously hyperventilated. Although the model of hypercapnia used is different from ours, the results of the current study are in accord with those reported by Rafferty *et al.*[30] These authors[30] showed that hypercapnia induced a significant decrease in twitch Pdi and found that 60 min after the discontinuation of carbon dioxide, the Pdi reached only 87% of its baseline value. In contrast, Rafferty *et al.*[30] observed that the recovery of diaphragmatic force was complete 90 min after the discontinuation of carbon dioxide.

Diaphragm muscle activity is an important determinant of diaphragmatic blood flow. To our knowledge, the effects of increased cardiac output due to hypercapnia on the diaphragmatic blood flow and its consequences on the contractile properties have never been reported. We found an increase in cardiac output, heart rate, and blood pressure (table 1) with hypercapnia. Among the trials that studied the effects of hypercapnia on diaphragmatic function, only one[24] evaluated cardiac output variations. In their trial, Yanos *et al.*[24] studied only one level of acidosis and did not find a significant increase in cardiac output, for both metabolic and respiratory acidosis. Our results are in accord with those reported by Yanos *et al.*,[24] at the same level of pH (table 1), but cardiac output increased significantly for a $Paco_2$ greater than 95 mmHg. Kendrick *et al.*[33] demonstrated a linear relation between hypercapnia and diaphragmatic blood flow. Although we did not evaluate the pH or capnia intracellular and extracellular variations in the diaphragm, we can speculate that these mechanisms may in part explain our results. Greatly increasing blood flow acts to maintain diaphragm contractility by preventing the buildup of metabolic by-products in the intracellular or extracellular milieu.[34] Therefore, increasing the arterial level of carbon dioxide leads to an increase in cardiac output and hence diaphragm blood flow, which in turn acts to reduce any direct effects of capnia on diaphragm contractility, possibly explaining the absence of an effect of acute hypercapnia on the diaphragm in some human studies.[29–32] Despite having a stimulatory effect on either ventilation or cardiac output, hypercapnia may have a deleterious effect on diaphragmatic contractile function by a combinative effect of acidosis-induced calcium sequestration into the sarcoplasmic reticulum[23] and promoting oxidative stress in striated muscles.[35]

Moreover, although in the hypercapnia group the anesthesia level was adapted to blunt the respiratory stimulus induced by hypercapnia, we did not observe any significant decrease in cardiac output. In addition, the anesthetic drug dose did not differ significantly between the two groups through the study period. However, Fujii *et al.*[36] reported in dogs that either a low dose (1.5 $mg \cdot kg^{-1} \cdot h^{-1}$) or a high dose (6.0 $mg \cdot kg^{-1} \cdot h^{-1}$) of propofol infusion decreased Pdi at low frequency (20 Hz) but had no change at a higher frequency (120 Hz). Nishina *et al.*[37] reported that low or high concentrations of ketamine and of propofol had no effect on diaphragm contractility during nonfatigued and fatigued conditions. Regarding the hemodynamic literature related to hypercapnia[17,19,20,38] and the studies that evaluated the effects of anesthetic drugs on diaphragm function,[36,37,39] we can assume that the observed alteration of diaphragmatic func-

tion in the current study was mainly due to hypercapnia rather than hemodynamic variations or anesthetic drugs.

Some remarks must be included to assess the limitations of our study. First, although piglet respiratory muscles are similar to those of humans, this study was limited because we studied the effects of hypercapnia acidosis on healthy diaphragm muscles. Although the evidence for the capacity of hypercapnic acidosis to induce diaphragm dysfunction in animal models is convincing, it is considerably more difficult to obtain conclusive proof of diaphragmatic dysfunction in mechanically ventilated critically ill patients or exhausted patients in acute respiratory failure. It is known that prolonged mechanical ventilation induces diaphragm alteration.[25,40,41] The duration of experiments in the current study did not exceeded 6 h, which is not enough to induce a diaphragm dysfunction. Indeed, in a previous study by our team[25] and in the trial of Radell *et al.*,[42] the diaphragmatic pressure was preserved until 24 h after controlled mechanical ventilation in piglets. Second, the periods of hypercapnia were not randomized with a washout period of normocapnia, and so a cumulative effect cannot be discarded. However, in the clinical situation, the evolution of hypercapnia acidosis is generally a gradual process, similar to what we tried to reproduce in the design of our current study. Third, we evaluated the effect of "short and acute" hypercapnia acidosis, but we cannot extrapolate the effects of prolonged or moderate hypercapnia (>24 h) on diaphragmatic contractile function. Patients presenting an acute respiratory distress syndrome are frequently ventilated in moderate hypercapnia for several days. In case of septic origin, the diaphragmatic alteration is due to both the infection and the prolonged mechanical ventilation.

In conclusion, our study showed an alteration in diaphragmatic contractile properties proportional to the severity of the respiratory acidosis and the absence of total recovery 1 h after discontinuation of the carbon dioxide insufflation. This alteration may play a role in weak patients with acute respiratory failure whose diaphragm is frequently ineffective despite an intensive workload. Further studies are needed to better evaluate the evolution of the diaphragm function after a prolonged "protective ventilation" period with mild to moderate hypercapnia and its impact on clinical outcomes related to the weaning process.

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