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Boris Jung, Jean-Michel Constantin, Nans Rossel, Charlotte Le Goff, Mustapha Sebbane, et al.. Adaptive Support Ventilation Prevents Ventilator-induced Diaphragmatic Dysfunction in Piglet:: an in vivo and in vitro study.. *Anesthesiology*, 2010, 112, pp.1435-1443. 10.1097/ALN.0b013e3181d7b036 . hal-02546644

HAL Id: hal-02546644

<https://hal.umontpellier.fr/hal-02546644>

Submitted on 18 Apr 2020

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Adaptive Support Ventilation Prevents Ventilator-induced Diaphragmatic Dysfunction in Piglet

An In Vivo and In Vitro Study

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ABSTRACT

Background: Contrary to adaptive support ventilation (ASV), prolonged totally controlled mechanical ventilation (CMV) results in the absence of diaphragm activity and causes ventilator-induced diaphragmatic dysfunction. Because maintaining respiratory muscles at rest is likely a major cause of ventilator-induced diaphragmatic dysfunction, ASV may prevent

its occurrence in comparison with CMV. The aim of our study was to compare the effects of ASV with those of CMV on both *in vivo* and *in vitro* diaphragmatic properties.

Methods: Two groups of six anesthetized piglets were ventilated during a 72-h period. Piglets in the CMV group (n = 6) were ventilated without spontaneous ventilation, and piglets in the ASV group (n = 6) were ventilated with spontaneous breaths. Transdiaphragmatic pressure was measured after bilateral, supramaximal transjugular stimulation of the two phrenic nerves. A pressure–frequency curve was drawn after stimulation from 20 to 120 Hz of the phrenic nerves. Diaphragm fiber proportions and mean sectional area were evaluated.

Results: After 72 h of ventilation, transdiaphragmatic pressure decreased by 30% of its baseline value in the CMV group, whereas it did not decrease in the ASV group. Although CMV was associated with an atrophy of the diaphragm (evaluated by mean cross-sectional area of both the slow and fast myosin chains), atrophy was not detected in the ASV group.

Conclusion: Maintaining diaphragmatic contractile activity by using the ASV mode may protect the diaphragm against the deleterious effect of prolonged CMV, as demonstrated both *in vitro* and *in vivo*, in healthy piglets.

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What We Already Know about This Topic

- ❖ Controlled mechanical ventilation leads to diaphragmatic dysfunction and atrophy
- ❖ Adaptive support ventilation in which patient effort is included to trigger breathing hastens respiratory weaning in postoperative patients

What This Article Tells Us That Is New

- ❖ In piglets, phrenic nerve-stimulated diaphragmatic strength was greater and histologic atrophy was less if they were ventilated for 72 h with adaptive support rather than controlled mechanical ventilation

PROLONGED totally controlled mechanical ventilation (CMV) results in the complete absence of neural activation and mechanical activity of the diaphragm and has been shown to induce muscle atrophy, proteolysis, and reactive oxygen species liberation, leading to rapid losses in diaphragmatic function, a syndrome known as ventilator-induced diaphragmatic dysfunction (VIDD).¹⁻⁴ Few countermeasures to prevent VIDD have been evaluated. Continuous positive airway pressure,³ intermittent spontaneous breathing,¹ or assist-control mechanical ventilation⁴ has been shown to protect the diaphragm against the deleterious effects of CMV in animals. Our group demonstrated that maintaining spontaneous breathing activity with pressure support ventilation (PSV) reduced mechanical ventilation-induced proteolysis and inhibition of protein synthesis in comparison with CMV in an *in vitro* rat model.⁵ Adaptive support ventilation (ASV), a complex mode recently approved by the Food and Drug Administration, is a new automatic ventilation mode, allowing assisted ventilation, in which minute ventilation is controlled by a combination of tidal volume (V_T) and respiratory rate (RR) based on respiratory mechanics. In spontaneously breathing patients who are able to trigger a breath, the ventilator generates pressure support breaths, automatically adjusting inspiratory pressure to achieve the target V_T , and it delivers additional pressure-controlled breaths if the RR of the patient is less than the target RR.⁶⁻⁸ It has recently been shown that ASV may accelerate respiratory weaning after cardiac surgery in comparison with pressure-regulated, volume-controlled ventilation with an automode,⁹ synchronized intermittent ventilation,¹⁰ and pressure-controlled or pressure-support ventilation.¹¹ It has also been reported that ASV is feasible in the more severely ill patients of an intensive

care unit¹² and reduces the work of breathing in comparison with PSV when a dead space is added to the ventilator circuit (*i.e.*, increase ventilatory demand) of critically ill patients.¹³

Therefore, we developed a model to examine whether ASV may protect the diaphragm against the detrimental effects of CMV, both *in vivo* and *in vitro*, in the same animal. We hypothesized that ASV would induce, in a healthy piglet model, less diaphragmatic dysfunction than CMV after 72 h of mechanical ventilation.

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 Helsinki Declaration. Therefore, these experiments were conducted in an authorized laboratory and under the supervision of authorized researchers (S.J., X.C., and S.M.).

Animal Preparation

We used the same experimental design described in our previous studies (fig. 1).^{14,15} In brief, 14 piglets (15–20 kg) were anesthetized with intravenous pentobarbital sodium (5–6 mg/kg), intubated with a cuffed endotracheal tube, and mechanically ventilated (Galileo®; Hamilton Medical AG, Ruzhans, Switzerland), with an inspired fraction of oxygen of 0.35, a V_T between 10 and 12 ml/kg, an RR from 15 to 30 cycles/min to obtain normocapnia, and 5 cm H₂O of positive end-expiratory pressure. Neuromuscular blocking agents were not used. The piglets were anesthetized with continuous intravenous propofol (15–20 mg · kg⁻¹ · h⁻¹), midazo-

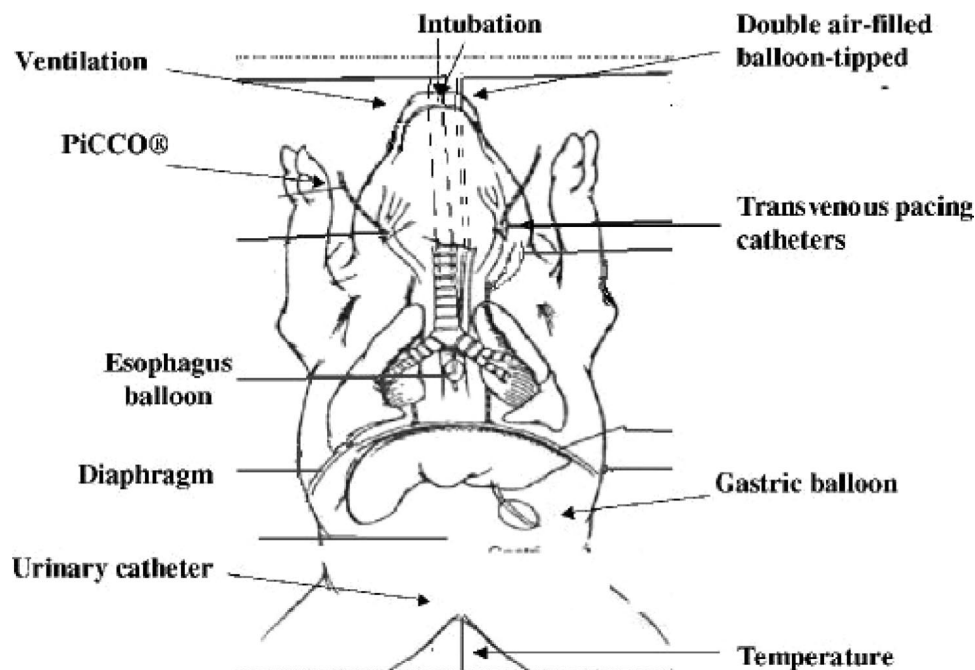


Fig. 1. Diagram showing jugular, carotid catheter preparation and double air-filled balloon with transvenous jugular phrenic pacing. PiCCO (Pulsion, Munich, Germany) = pulse-induced contour cardiac output.

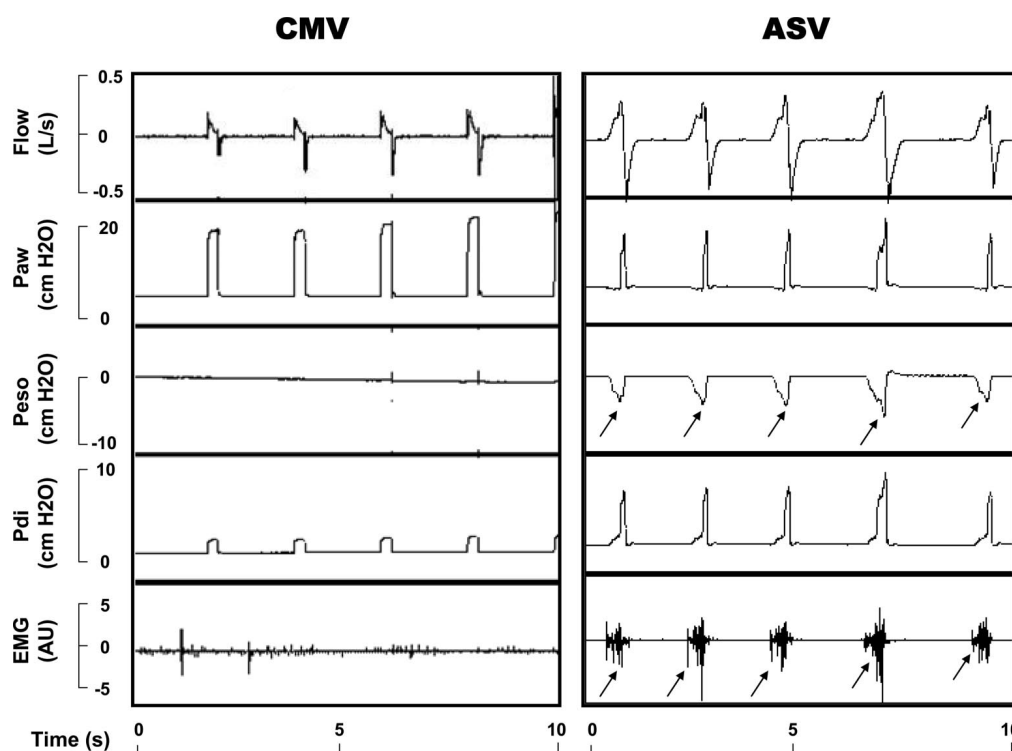


Fig. 2. Diagram of mechanical ventilation parameters in the controlled mechanical ventilation (CMV) and adaptive support ventilation (ASV) groups. From top to bottom airway flow (flow, l/s), airway pressure (Paw, cm H₂O), esophageal pressure (Peso, cm H₂O), transdiaphragmatic pressure (Pdi, cm H₂O), and diaphragmatic electrical activity measured with an electromyogram (EMG) are reported. Although the diaphragm was at rest without any spontaneous breathing activity in the CMV group, the diaphragm remains active, and spontaneous ventilation cycles occur in the ASV group. Arrows show spontaneous diaphragmatic activity on both electrical and mechanical signals.

lam (0.1–0.3 mg/h), and ketamine (3–4 mg · kg⁻¹ · h⁻¹). The level of sedation was monitored with bispectral index (BIS[®]; Aspect, Norwood, MA).¹⁶ An oral gastric tube was placed. A vesicostomy was performed, and a urine catheter was placed for urine collection. A rectal probe was used for frequent temperature measurements, and the animals were rested on soft cushions. Heating pads were used as needed to maintain a normal body temperature of 38.5°–39.5°C. A carotid arterial catheter (PiCCO[®]; Pulsion, Munich, Germany) was inserted for the monitoring of heart rate, arterial blood pressure, and cardiac output. Arterial pressure of carbon dioxide levels was checked by using a capnograph (Deltatrac[®]; Datex-Ohmeda, Helsinki, Finland) and then verified by arterial blood gases (iSTAT[®]; Abbott, Abbott Park, IL). Parenteral nutrition was given from the first day (10% glucose solution and 20% amino acids solution, and Hyperamine 20%[®]; Braun, Boulogne Billancourt, France) providing 30–35 kcal · kg⁻¹ · day⁻¹. All procedures were performed aseptically. The animals received prophylactic intravenous antibiotics three times daily (amoxicillin–clavulanate, 100 mg · kg⁻¹ · day⁻¹). At the end of the procedure, costal region diaphragm samples were removed and then animals were killed by intravenous injection of pentobarbital sodium and potassium chloride. A physician provided round-the-clock supervision and animal care for the entire duration of the study. The two groups received the same care, except for the ventilatory mode.

Ventilatory Care

Seven piglets were ventilated for 72 consecutive hours in a totally controlled (CMV group) mode, and seven piglets were ventilated for 72 consecutive hours in a partially spontaneous mode (ASV group; fig. 2). In the CMV group, ventilatory settings were as follows: V_T at 10–12 ml/kg of the ideal body weight, RR from 15 to 30 min⁻¹, and positive end-expiratory pressure level at 5 cm H₂O. The absence of spontaneous breathing was verified on the ventilator trend graphs, and electromyographic activity of the diaphragm was measured in a few animals to ensure that no electrical activity was present in the diaphragm. In the ASV group, ventilatory settings were pediatric mode setting in phase with ideal body weight of the piglet, inspiratory flow trigger at 0.3 l/min, percentage of mechanical ventilation between 100 and 150%, positive end-expiratory pressure level at 5 cm H₂O, and expiratory trigger at 25% of peak inspiratory flow. Active piglets were allowed to breathe spontaneously, and the ratio of pressure-assisted cycles was verified on the ventilator trend graphs. In both groups, oxygenation was maintained with F_{IO}₂ from 25 to 35% and minute ventilation to assess normocapnia. The deepness of anesthesia was adjusted to blow out the respiratory drive in the CMV group and to allow spontaneous breathing without awakening in the ASV group.

Assessment of Diaphragm Muscle Activity during Mechanical Ventilation

First Part of the Study: *In Vivo* Assessment of Transdiaphragmatic Pressure. We measured transdiaphragmatic pressure (P_{di}) for every 12 h to assess *in vivo* diaphragmatic contractile force in both groups as described in previous studies.^{14,15} In brief, double air-filled balloon-tipped catheters were placed transorally into the distal third of the esophagus and in the stomach for measurement of P_{di}. 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. P_{di} was produced by supramaximal stimulation at frequencies of 20, 40, 60, 80, 100, and 120 Hz in a serial manner. Each train of impulses lasted for 2,000 ms, and each pulse had duration of 150 ms. A pressure–frequency curve was obtained for both groups at each 12-h period and then compared.

Second Part of the Study: *In Vitro* Assessment of Diaphragm Histology. Biopsies (1 cm³) from the apposition zone of the costal diaphragm from the entire midcostal muscle spanning were removed just before euthanasia. Each biopsy was partitioned, frozen in liquid nitrogen-cooled isopentane after 3–5 min for length equilibrium, and stored at –80°C. Immunohistochemistry was assessed on 10- μ m unfixed serial transverse cryostat sections according to a well-described procedure.¹⁰ In brief, histologic analysis was performed on six cross-sections from each muscle. Stained sections were visualized under a Nikon optiphot-2 microscope (Nikon Instruments Europe, B.V. Amstelveen, The Netherlands). All diaphragm images were obtained under identical conditions and with the same objective lens. The shape of each muscle fiber was accurately defined (on images $\times 400$), and a schematic drawing of each stained diaphragm section was recorded. Schematic representations of each muscle section were analyzed by Histolab program (version 5-13-1, Microvision Instrument, license number; 2497, Evry, France), and data were averaged per approximately 500 muscle fibers from the dissected diaphragms of piglets from each group.

Ten-micrometer unfixed cryostat sections of CMV and ASV group diaphragms were stained by hematoxylin and eosin. On each schematic drawing, the fiber cross-sectional areas were measured. To assess relative fiber percentages, immunohistochemistry was assessed on 10- μ m unfixed serial transverse cryostat sections. Two adjacent sections were incubated for 1 h in 1% bovine serum albumin with the respective primary antibodies for both the mouth anti–slow myosin heavy chain (M-8421; Sigma, Saint Louis, MO; dilution 1/8,000) and the mouth anti–fast myosin heavy chain (M-4276; Sigma; dilution 1/8,000). In addition, polyclonal antibodies against dystrophin (H4; dilution 1/5,000), produced and characterized in our laboratory, were included in each of these stains to visualize fiber membranes. Immunoreactions were detected with Cy3 and fluorescein isothiocyanate-conjugated goat anti-piglet. After washing with phos-

phate-buffered saline solution, sections were incubated 1 h at room temperature with Cy3-goat anti-piglet IgG (Chemicon International, Molsheim, France; dilution 1/5,000) to detect myosin heavy chain, and with fluorescein isothiocyanate (Chemicon International, AP 132F; dilution 1/1,000) to detect dystrophin. Unbound antibodies were removed by washing the sections in phosphate-buffered saline. For negative controls, only the second antibody was applied. Sections were then visualized under adapted fluorescence. By using these stains, the myosin heavy chains that react with the chosen antibody on each section appeared orange, whereas the dystrophin at the membrane appeared green. We compared fiber type proportion measurements between the groups.

Statistical Analysis

Values are means and SD or medians and quartiles (25–75th), as required. Normality of the distribution was assessed with the Kolmogorov–Smirnov test. A two-way analysis of variance with time (H0, H12, H24, . . . , H72) as one factor and modality (CMV *vs.* ASV) as the other factor was used. When appropriate, a Newman–Keuls test was used. Non-parametric paired Wilcoxon tests were used to compare data from days 1 and 3 for each animal in both the CMV and ASV groups. All *P* values were two tailed, and a *P* value less than 0.05 was considered significant (StatView[®], version 5.0; SAS Institute Inc., Berkeley, CA).

Results

Systemic and Biologic Response to Mechanical Ventilation

Among the 14 piglets, two died before the end of the study: one because of myocardial infarction at H10 (CMV group) and one because of septic shock (peritonitis after bladder catheterization resulting in small bowel perforation in the ASV group) after 28 h of ventilation. These two piglets were subsequently excluded from the analyses.

No significant differences were observed between the CMV group and the ASV group for all the studied baseline variables. Long-term mechanical ventilation, either in CMV or ASV, did not have consequences on body weight, intestinal transit, diuresis (data not shown), or hemodynamic variables (table 1). BIS values were significantly different between the CMV and ASV groups (72 ± 8 *vs.* 42 ± 12 , *P* = 0.02). Mean midazolam level administration remained at a higher level in the CMV group (3.8 ± 0.7 mg/h) than that in the ASV group (1.5 ± 0.7 mg/h) during the study (*P* < 0.05 between CMV *vs.* ASV after 12 h of ventilation). Doses of propofol did not significantly differ between the groups during the study (102 ± 8 and 90 ± 10 mg/h in the CMV and ASV groups, respectively).

Ventilatory Parameters and Spontaneous Breathing Data

In the CMV group, spontaneous activity of the diaphragm was present for no more than 5% of the delivered breaths, as

Table 1. Hemodynamic and Respiratory Variables for the Seven Steps of Measures between the CMV (n = 6) and the ASV (n = 6) Groups

	H0	H12	H24	H36	H48	H60	H72
Minute ventilation (l/min)							
CMV	5.9 ± 0.8	4.4 ± 0.6	5.1 ± 0.6	4.9 ± 0.5	4.7 ± 0.4	4.8 ± 0.6	4.7 ± 0.8
ASV	5.4 ± 0.1	5.4 ± 0.1	5.9 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	5.7 ± 0.1	5.7 ± 0.2
Tidal volume (ml)							
CMV	240 ± 40	220 ± 20	230 ± 20	230 ± 25	225 ± 25	230 ± 25	205 ± 10
ASV	180 ± 17	192 ± 17	185 ± 28	215 ± 35	225 ± 35	220 ± 29	220 ± 29
Respiratory rate (c/min)							
CMV	24 ± 3	20 ± 3	22 ± 2	22 ± 2	21 ± 1	21 ± 1	20 ± 1
ASV	30 ± 6	28 ± 5	30 ± 6	28 ± 4	26 ± 6	26 ± 7	26 ± 5
pH							
CMV	7.52 ± 0.02	7.47 ± 0.06	7.46 ± 0.04	7.45 ± 0.01	7.44 ± 0.04	7.47 ± 0.04	7.44 ± 0.05
ASV	7.48 ± 0.14	7.46 ± 0.11	7.46 ± 0.08	7.46 ± 0.12	7.45 ± 0.16	7.46 ± 0.11	7.48 ± 0.11
Pao ₂ (mmHg)							
CMV	99 ± 8	97 ± 6	95 ± 5	98 ± 3	95 ± 6	97 ± 5	101 ± 6
ASV	102 ± 10	99 ± 8	98 ± 9	101 ± 5	97 ± 8	96 ± 8	98 ± 9
Paco ₂ (mmHg)							
CMV	33 ± 6	39 ± 3	41 ± 3	40 ± 3	39 ± 3	38 ± 2	37 ± 3
ASV	38 ± 9	35 ± 8	36 ± 7	36 ± 9	38 ± 12	37 ± 7	33 ± 5
Sao ₂ (%)							
CMV	98 ± 2	99 ± 2	97 ± 3	100 ± 2	98 ± 2	97 ± 3	96 ± 4
ASV	99 ± 3	98 ± 4	97 ± 4	97 ± 3	98 ± 4	98 ± 2	97 ± 3
Plasma bicarbonate (mM)							
CMV	27 ± 4	29 ± 4	29 ± 5	29 ± 3	27 ± 3	30 ± 2	26 ± 2
ASV	28 ± 5	25 ± 5	23 ± 4	26 ± 7	23 ± 1	25 ± 3	23 ± 6
HR (c/min)							
CMV	101 ± 10	106 ± 12	107 ± 12	103 ± 10	97 ± 8	101 ± 14	101 ± 15
ASV	104 ± 26	100 ± 32	90 ± 22	95 ± 23	89 ± 14	103 ± 14	99 ± 18
MBP (mmHg)							
CMV	75 ± 7	80 ± 10	88 ± 7	86 ± 9	84 ± 13	83 ± 13	88 ± 14
ASV	75 ± 17	84 ± 17	85 ± 15	79 ± 22	76 ± 27	80 ± 24	76 ± 12
CO (l/min)							
CMV	2.2 ± 0.3	2.7 ± 0.3	2.8 ± 0.5	2.8 ± 0.2	2.7 ± 0.2	2.7 ± 0.2	2.7 ± 0.3
ASV	2.7 ± 0.9	2.9 ± 0.9	2.7 ± 0.8	2.5 ± 0.9	2.6 ± 0.9	2.6 ± 1.2	2.4 ± 0.9

Data are presented as mean ± SD. There were no significant intergroup or intragroup differences for hemodynamic and for respiratory variables and arterial blood gases in both groups.

ASV = adaptative support ventilation; CMV = controlled mechanical ventilation; CO = cardiac output; HR = heart rate; MBP = mean blood pressure; Pao₂ = arterial pressure of oxygen; Paco₂ = arterial pressure of carbon dioxide; Sao₂ = oxygen saturation of arterial blood.

determined by analysis of the trend graphs and the Pdi tracings. In the ASV group, active piglets triggered 80% of the delivered breaths, as assessed by the Galileo[®] trend graphs of ventilator and, for a few animals, the surface electromyogram activity (fig. 2). Once normal gas exchanges were obtained, ventilatory settings were not modified throughout the study (table 1).

In Vivo Assessment of Diaphragmatic Force

The baseline pressure–frequency curves of the two groups were not significantly different (Pdi = 23.4 ± 2.6 vs. 24.6 ± 4.8 cm H₂O at 20 Hz and 45.8 ± 3.0 vs. 46.8 ± 1.5 cm H₂O at 100 Hz for the CMV and ASV groups, respectively; fig. 3, A and B).

Although Pdi decreased significantly in the CMV group between baseline and H72 at all frequencies except 20 Hz

($P < 0.05$; fig. 3A), it did not change significantly in the ASV group (fig. 3B).

Furthermore, the decrease in the Pdi between H0 and H72 in the CMV group (−26% [−17 to −32]) was significantly higher than that in the ASV group (−2% [−8 to +3]; $P < 0.05$) at all frequencies of stimulation, except at 40 and 60 Hz at which the difference did not reach the statistical significance (fig. 3). Figure 4 shows the evolution of Pdi over time at a stimulation frequency of 100 Hz. In the CMV group, Pdi decreased significantly after 48 h of ventilation ($P < 0.05$). In the ASV group, Pdi did not significantly differ during the whole study period.

In Vitro Assessment of Diaphragmatic Histology

Figure 5A shows photomicrographs of typical diaphragm muscle cross-sections obtained in one animal of each group.

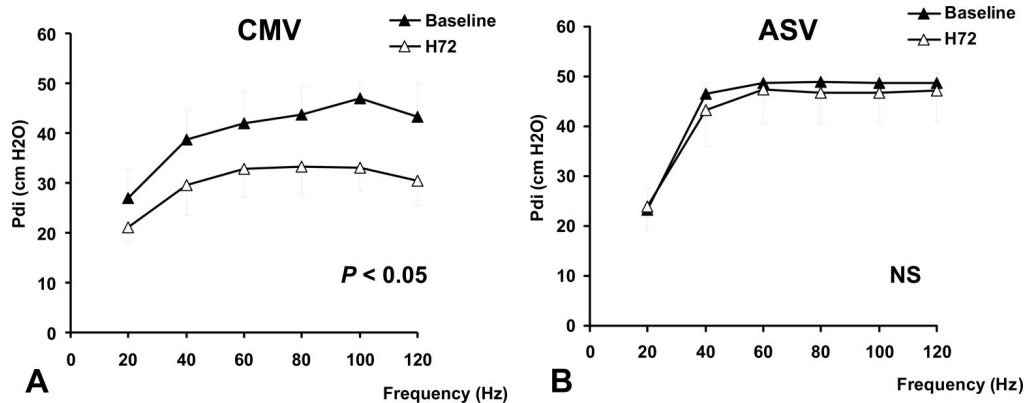


Fig. 3. Diaphragmatic pressure–frequency curves obtained in the controlled mechanical ventilation (CMV) group (A) ($n = 6$) and in the adaptive support ventilation (ASV) group (B) ($n = 6$). Transdiaphragmatic pressure (Pdi) after supramaximal phrenic nerve stimulation at 20, 40, 60, 80, 100, and 120 Hz over 72 h of mechanical ventilation obtained in both groups. In the CMV group, Pdi significantly decreased between baseline and H72 at all frequencies (except at 20 Hz) ($P < 0.05$). Data are mean \pm SD. P values refer to between H72 vs. baseline. NS = not significant. No significant differences were observed for the values obtained at all frequencies in the ASV group between H72 and baseline.

Fibers that react with the antibody appear orange in figure 5B, whereas fibers not reacting with the antibody appear black. A representative slow-twitch fiber is indicated by an open square and a fast-twitch fiber by an open circle. In the CMV group, we observed a marked atrophy of both slow- and fast-twitch fibers after 72 h of totally controlled ventilation. Indeed, after 72 h of CMV, fiber cross-sectional area was decreased by 30–40% for both slow- and fast-twitch fibers ($P < 0.05$; fig. 5C). The atrophy reported in the CMV group contrasts with that reported in the ASV group, which was not associated with fiber cross-sectional area variation after 72 consecutive hours of ventilation (fig. 5C). Atrophy in the CMV group concerned both slow- and fast-twitch fibers, and fiber proportion at H72 was not different in comparison with baseline in both groups (fig. 5D).

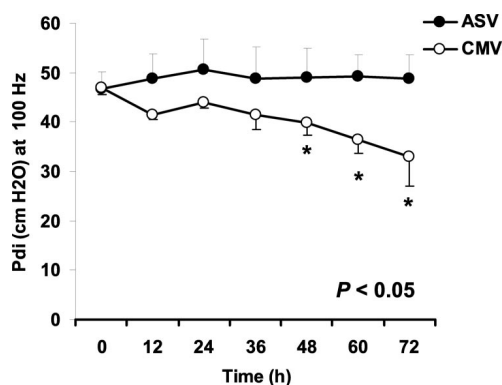


Fig. 4. Transdiaphragmatic pressure (Pdi) differences evaluated at a frequency of 100 Hz between the adaptive support ventilation (ASV) group ($n = 6$) and controlled mechanical ventilation (CMV) ($n = 6$) group during the whole study period. Pdi in the CMV group was lower than that in the ASV group after 48 h of ventilation. Data are mean \pm SD. P values refer to between-group differences. * $P < 0.05$ vs. baseline values.

Discussion

This study demonstrates that maintaining spontaneous ventilation with ASV is efficient to protect the diaphragm against the occurrence of VIDD, both *in vivo* and *in vitro*, in the same piglet model. In addition, we report that *in vivo*, both diaphragmatic contractility and diaphragmatic atrophy occur when a totally controlled ventilation mode is applied but are prevented when spontaneous cycles with ASV are maintained.

By using *in vitro* animal models, several studies have reported that CMV-induced diaphragm inactivity decreases protein synthesis and increases degradation of key contractile proteins, resulting in diaphragmatic force loss.^{3,15,17–21} Protein degradation is mediated *via* oxidative stress,^{15,22,23} apoptosis,²⁴ and proteasome proteolysis.^{5,25–27} Few studies evaluating VIDD *in vivo* mainly with animals have been published^{3,15,21}, although one was with humans.² Some other acute situations (*i.e.*, myorelaxants, sepsis, acute hypercapnic acidosis, and others) seem to induce VIDD in animal studies.^{14,28,29} Sepsis may be associated with VIDD in the intensive care unit, although mainly explored through experimental studies. Several studies described the mechanisms leading to the VIDD, showing inflammation, proteolysis, or nitric oxide pathways.^{28,30–32} Although one study demonstrated that CMV decreases VIDD compared with spontaneous breathing in septic rats, to the best of our knowledge,³³ none compared assisted *versus* CMV.

In this study, we confirmed that prolonged CMV decreased diaphragmatic contractile force *in vivo* in a healthy piglet model. In comparison with baseline, diaphragmatic force-generating capacity decreased by 25% after 72 h of ventilation in the CMV group (figs. 3A and 4).

Although it is widely accepted that VIDD occurs after several days of CMV, few countermeasures have been tested. Recently, it has been shown that administration of Trolox,³⁴ an antioxidant, glutamine,³⁵ or leupeptin,²⁵ which decreases

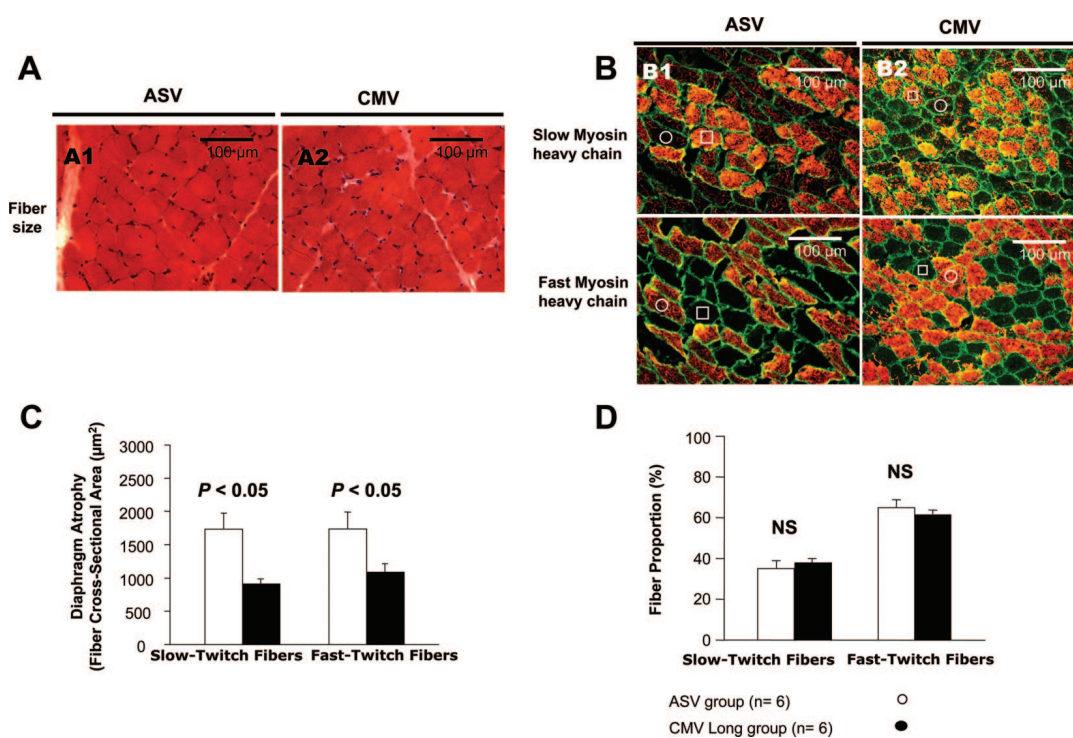


Fig. 5. Comparison of representative adaptive support ventilation (ASV) (A1, B1) and controlled mechanical ventilation (CMV) (A2, B2) diaphragm-biopsy specimen with respect to fiber size or phenotype. Histologic analysis was performed on six cross-sections from each muscle under a Nikon optiphot-2 microscope (Nikon Instruments Europe, B.V. Amstelveen, The Netherlands). (A) Hematoxylin and eosin coloration shows that neither inflammatory infiltrate nor necrosis is present in the CMV and ASV group. (B) To assess for the slow- and fast-twitch fiber type cross-sectional area (CSA) and relative fiber percentages, immunochemistry was assessed on 10- μm unfixed serial transverse cryostat sections. Two adjacent sections were incubated in 1% bovine serum albumin with the primary antibody 1 h for mouse anti-slow myosin heavy chain. The myosin heavy chains that react with the chosen antibody on each section appeared orange, whereas the dystrophin at the membrane appeared green. Schematic representation (on images $\times 400$) of each muscle section was analyzed by Histolab program (version 5-13-1; Microvision Instrument, Evry, France; license number: 2497), and data were averaged per approximately 500 muscle fibers from each dissected diaphragm of both the CMV and ASV groups. (C) Both the slow- and the fast-twitch fibers in the CMV group are statistically smaller than those in the ASV group. (D) Fiber proportion (%) was not different between the CMV and ASV group. NS = not significant.

calpain and cathepsin activity (proteases), prevents VIDD during mechanical ventilation in a rat model. Although medications to prevent or treat VIDD may be a point of interest in the future, ventilator strategies that allow diaphragmatic contractions seem to protect against VIDD.

Indeed, Sassoon *et al.*⁴ reported the beneficial effect of assisted controlled ventilation on *in vitro* contractile properties of the diaphragm and the decrease in the muscle atrophy factor-box messenger RNA, a marker of gene atrophy in comparison with total CMV in rabbits. Gayan-Ramirez *et al.*¹ also reported the beneficial effect of intermittent spontaneous breathing on *in vitro* contractility and myogenesis regulatory factors in the diaphragm. Recently, we reported that PSV was effective in reducing diaphragm proteolysis and inhibition of protein synthesis compared with continuous mechanical ventilation.⁵ This study is the first one performed in an *in vivo* model of piglet, a large animal whose physiology is similar to human beings, to report that maintaining spontaneous activity of the diaphragm with ASV may protect diaphragm against VIDD evaluated both *in vivo* (contractility) and *in vitro* (atrophy and fibers repartition).

With the ASV mode, the ventilator software uses a modified version of the equation derived by Otis *et al.*⁷ to minimize the work of breathing.^{8,12,13,36,37} For subjects who are unable to trigger a breath, the ventilator generates a pressure-controlled cycle, automatically adjusting inspiratory pressure and time to achieve a target V_T and a target RR. When the spontaneous activity of the diaphragm is sufficient to trigger a breath, the ventilator generates pressure-support breaths and, if necessary, delivers additional pressure-controlled breaths. In the ASV mode, similar to PSV, when an apnea occurs, the ventilator switches to the CMV mode but with an option that allows the patient to return to an assisted mode and triggers breaths.

In this 72 consecutive-hour study, the ASV mode allowed for a high proportion of pressure-supported cycles but ensured initiation of the apnea-induced CMV mode when necessary, with an optional return to PSV. In the ASV group, piglets were ventilated on pressure support more than 80% of the time, although they were ventilated in CMV mode the remainder of the time. On the contrary, in the CMV group, we could identify diaphragmatic activity

during lesser than 5% of all breaths on the trend graphs of the Galileo[®] ventilator.

In this study, the piglets ventilated in ASV mode did not show any decrease in Pdi during the study period, contrary to those ventilated with CMV mode in whom Pdi decreased by 25% after 60 h of ventilation. We speculate that this protective effect is related to the spontaneous activity of the diaphragm because we did not compare ASV with pressure support or any other spontaneous mode.

Some study limitations must be pointed out. First, we did not compare our results with a control group without anesthesia, or mechanical ventilation, because large animals must be anesthetized for procedures such as phrenic stimulation and Pdi recording. Second, we did not compare ASV with PSV mode and therefore cannot conclude on the relative effects of spontaneous ventilation *versus* the specificity of the ASV mode. However, ASV may have beneficial effects compared with PSV because of the theoretical decrease in the work of breathing with the equation software derived by Otis *et al.* and the possibility of an automated backup from the apnea ventilation to ASV (*i.e.*, spontaneous ventilation mode) and *vice versa*, which allowed piglets to breathe spontaneously as much as possible. Third, although piglet respiratory muscles are close to those of humans, this study was limited because we compared the effect of CMV with that of ASV on healthy diaphragm muscles. Nevertheless, we can speculate that the reported alteration of Pdi would be even worse in pathologic situations, such as sepsis, that induce VIDD *per se*.^{28,38,39} In the CMV group, sedation level was more important than that in the ASV group to neutralize the centers of breathing. Although we cannot eliminate a direct effect of sedation on Pdi, a recent review on VIDD stated that the effect of sedation on diaphragm function was clearly lower than the direct effect of mechanical ventilation.⁴⁰ Furthermore, in the clinical situation, sedation is frequently necessary to ensure patient-ventilator synchrony with the CMV mode and may lengthen the ventilation weaning process, thereby promoting VIDD.^{41,42} Although this point represents clearly a methodological limit of our study, it reflects the clinical interaction among sedation, mechanical ventilation, and weaning.^{42,43}

In conclusion, this study reports that maintaining diaphragmatic contractile activity during 72 consecutive hours with the ASV mode may protect the diaphragm against the deleterious effect of total CMV in healthy piglets. ASV prevented the *in vivo* alteration of diaphragmatic contractility and *in vitro* diaphragmatic atrophy, contrary to total CMV, which was associated with both diaphragmatic atrophy and *in vivo* alteration of the diaphragm contractility. Further studies, including physiologic human studies, are required to more fully understand the effect of ASV on diaphragmatic function.

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