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Impact of under-assisted ventilation on diaphragm function and structure in a porcine model

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Abstract

Background:

Long-term controlled mechanical ventilation (CMV) in intensive care unit (ICU) induces ventilatory-induced-diaphragm-dysfunction (VIDD). The transition from CMV to assisted mechanical ventilation is a challenge that requires clinicians to balance over-assistance and under-assistance. While the effects of over-assistance on the diaphragm are well known, we aimed to assess the impact of under-assistance on diaphragm function and structure in piglet model with pre-existing VIDD (after long-term CMV) or without VIDD (short-term CMV).

Methods:

Twenty-two Large-White female piglets were anesthetized, ventilated, and separated into two groups: a VIDD group (n=10) with long-term 72-hour CMV, and a no-VIDD group (n=12) with short-term 2-hour CMV. After sedation reduction at the end of CMV period, each piglet was switched to under-assisted ventilation for 2 hours. Diaphragm function (supramaximal diaphragm pressure-generating capacity assessed by negative tracheal pressure after transvenous phrenic nerve stimulation) and diaphragm structure (mini-invasive in vivo biopsies) were assessed before and after under-assisted ventilation.

Results:

In VIDD group, supramaximal diaphragm pressure-generating capacity decreased by 22% from 69.9 ± 12.7 to 54.9 ± 19.7 cmH₂O ($p=0.04$) after 72 hours of CMV evidencing VIDD, then dropped by a further 29% from 54.9 ± 19.7 to 38.9 ± 15.5 cmH₂O ($p<0.01$) after 2 hours of under-assisted ventilation. Diaphragm pressure-generating capacity remains stable from 55.3 ± 22.7 to 58.2 ± 24 cmH₂O ($p=0.24$) in no-VIDD group. Diaphragm structure showed sarcomeric injuries increase from $13 \pm 10\%$ to $24 \pm 19\%$ ($p<0.01$) and lipid droplets decrease from $14 \pm 8\%$ to $11 \pm 6\%$ ($p=0.03$) of the total micrograph area after 2 hours of under-assisted ventilation in the VIDD group. Sarcomeric injuries and lipid droplets accounted respectively for $17 \pm 16\%$ and $2 \pm 3\%$ of the total micrograph area after under-assisted ventilation in the no-VIDD group.

Conclusions:

In this porcine model, a short two-hour exposure of under-assisted ventilation induces impairment of diaphragm function with damage to the diaphragm structure in ICU condition with pre-existing VIDD.

What We Already Know about This Topic

- Ventilator-induced diaphragm dysfunction (VIDD) is associated with prolonged weaning and morbidity.
- It is most commonly associated with overassistance during ventilation, but the effects of underassistance are less well studied.

What This Article Tells Us That Is New

- Using a piglet model, the authors evaluated the impact of under-assisted ventilation during the transition phase from continuous to assisted ventilation in subjects with or without preexisting VIDD.
- After a 2-h period of underassisted ventilation, supramaximal diaphragm pressure-generating capacity decreased significantly in the group with preexisting VIDD relative to those without. This was accompanied by histologic evidence suggesting worsened damage.

Mechanical ventilation is a life-saving intervention and the main organ support in the intensive care unit (ICU). However, it is a two-edged sword. Inappropriate ventilator settings can induce ventilator-induced lung injury¹ and ventilator-induced diaphragm dysfunction (VIDD),^{2,3} which could persist beyond extubation.⁴ After controlled mechanical ventilation, reducing sedation and achieving the transition from controlled mechanical ventilation to assisted mechanical ventilation is a challenging process with a delicate balance between over-assistance and underassistance.⁵ VIDD due to over-assistance (diaphragm injury due to insufficient muscle loading) is well established,^{3,6–11} but the impact of under-assistance (diaphragm injury due to excess muscle loading) on diaphragm structure and function is not as well documented and remains controversial and speculative. In some experimental studies, it appears that excessive muscle loading was associated with diaphragm injury with sarcomere disruption,^{12–15} but the direct effect on diaphragm function is unknown. The impact of preexisting VIDD, common in ICU patients after prolonged controlled mechanical ventilation,^{16,17} also remains to be determined.

In this experimental study, we aimed to explore the impact of under-assisted ventilation during the transition phase between controlled to assisted ventilation in a pragmatic piglet model reflecting ICU conditions with VIDD induced by prolonged controlled mechanical ventilation. We hypothesized that under-assisted ventilation induces impairment of diaphragm function and damage to diaphragm structure, especially on a diaphragm previously primed with prolonged controlled mechanical ventilation (preexisting VIDD). The objective was to assess the impact of under-assistance on diaphragm function and structure in these piglets with preexisting VIDD (after long-term controlled mechanical ventilation) in comparison to piglets without VIDD (after short-term controlled mechanical ventilation).

Materials and Methods

Study Design

We performed an experimental study in a piglet model of long-term (72 h) *versus* short-term (2 h) controlled mechanical ventilation followed by an additional 2 h of underassisted ventilation. The institutional (University of Montpellier, Montpellier, France) and the regional ethics committees for animal research and care (CEEA-LR-36) and the French ministry of agriculture (Paris, France) approved the study protocol (Autorisation de Projets utilisant des Animaux à des Fins Scientifiques, Authorization of Projects Using Animals for Scientific Purposes, APAFIS No. 15866 2018070509081002). All experiments were conducted in an authorized laboratory under the supervision of authorized researchers (S.J., S.M., B.J.), complied with the Animal Research Reporting of *In Vivo* Experiments (ARRIVE) guidelines (Supplemental Digital Content, <https://links.lww.com/ALN/D842>), and conformed to the Guide for the Care and Use of Laboratory Animals (National Academy of Sciences, Washington, D.C.). Detailed methods are described in the Supplemental Digital Content (<https://links.lww.com/ALN/D842>).

Experimental Protocol

The experimental protocol with timeline is summarized in figure 1. Piglets were under controlled mechanical ventilation for 72 h (VIDD group) *versus* 2 h (no-VIDD group). The animals were then exposed to 2 h of underassisted mechanical ventilation. Thus, the total duration of invasive mechanical ventilation was 74 h *versus* 4 h. A diaphragm function assessment was performed at baseline (after 2 h of animal preparation) for the preexisting VIDD group to prove VIDD with before/after prolonged mechanical ventilation comparison. A complete assessment of diaphragm function and structure was performed before (*in vivo* diaphragm biopsy only for the VIDD group) and after the underassisted ventilation.

There was no randomization, each piglet was its own control regarding underassistance (before and after underassistance), and for the two groups, the experiments were carried out in two distinct periods (2019 and 2020). All researchers involved in the study were aware of the group allocation. Two piglets were anesthetized at the same time in each session. Doctor and nurse tandems trained in critical care medicine and animal experiments provided round-the-clock supervision, monitoring, and animal care, alternating 12-h shifts for the entire duration of the study.

Piglet Model

Twenty-two healthy Large White piglets, *Sus scrofa domesticus* (29 ± 2 kg), were sedated with propofol, midazolam, ketamine, and sufentanil to ensure proper anesthesia, adequate ventilation without dyssynchrony, and stable hemodynamics. No neuromuscular blocking agent were used. After orotracheal intubation with a cuffed endotracheal tube, the piglets were mechanically ventilated in volume-controlled mode with tidal volume (VT) of 10 ± 2 ml/kg and respiratory rate of 20 to 30 min⁻¹ to achieve physiologic hematosis and to avoid dyssynchrony.

The positive end-expiratory pressure (PEEP) was initially set at 5 cm H₂O, and the inspired oxygen fraction was adjusted to obtain an arterial partial pressure of oxygen (Pao₂) from 60 to 90 mmHg.

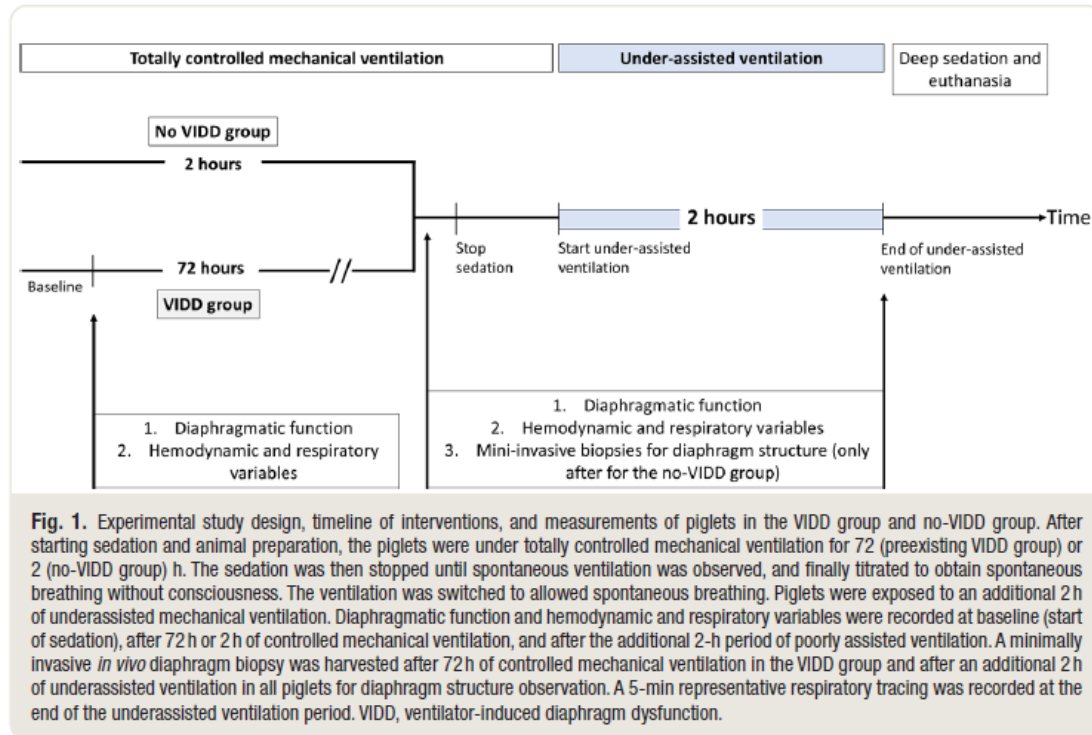


Fig. 1. Experimental study design, timeline of interventions, and measurements of piglets in the VIDD group and no-VIDD group. After starting sedation and animal preparation, the piglets were under totally controlled mechanical ventilation for 72 (preexisting VIDD group) or 2 (no-VIDD group) h. The sedation was then stopped until spontaneous ventilation was observed, and finally titrated to obtain spontaneous breathing without consciousness. The ventilation was switched to allowed spontaneous breathing. Piglets were exposed to an additional 2 h of underassisted mechanical ventilation. Diaphragmatic function and hemodynamic and respiratory variables were recorded at baseline (start of sedation), after 72 h or 2 h of controlled mechanical ventilation, and after the additional 2-h period of poorly assisted ventilation. A minimally invasive *in vivo* diaphragm biopsy was harvested after 72 h of controlled mechanical ventilation in the VIDD group and after an additional 2 h of underassisted ventilation in all piglets for diaphragm structure observation. A 5-min representative respiratory tracing was recorded at the end of the underassisted ventilation period. VIDD, ventilator-induced diaphragm dysfunction.

The experimental design was similar to previously reported experimental studies.^{7–9,18} Briefly, the right femoral artery, femoral vein, and urinary bladder were catheterized. Piglets were all females to facilitate bladder catheterization. Cardiac output was monitored by a pulse contour cardiac output system (PiCCO, Pulsion Medical System, Germany). An oral gastric tube was inserted for enteral nutrition. An air-filled balloon-tipped esophageal catheter (Marquat, France) was placed trans-orally in the distal third of the esophagus. Bipolar transvenous pacing catheters (Pacel, Abbott, USA) were introduced *via* each internal jugular vein and adjusted to achieve stimulation of the phrenic nerve and subsequent contraction of the diaphragm (fig. S1, <https://links.lww.com/ALN/D842>). These contractions produce a negative pressure that reflects diaphragmatic function. At the end of the experiment, piglets were euthanized with barbiturate overdose.

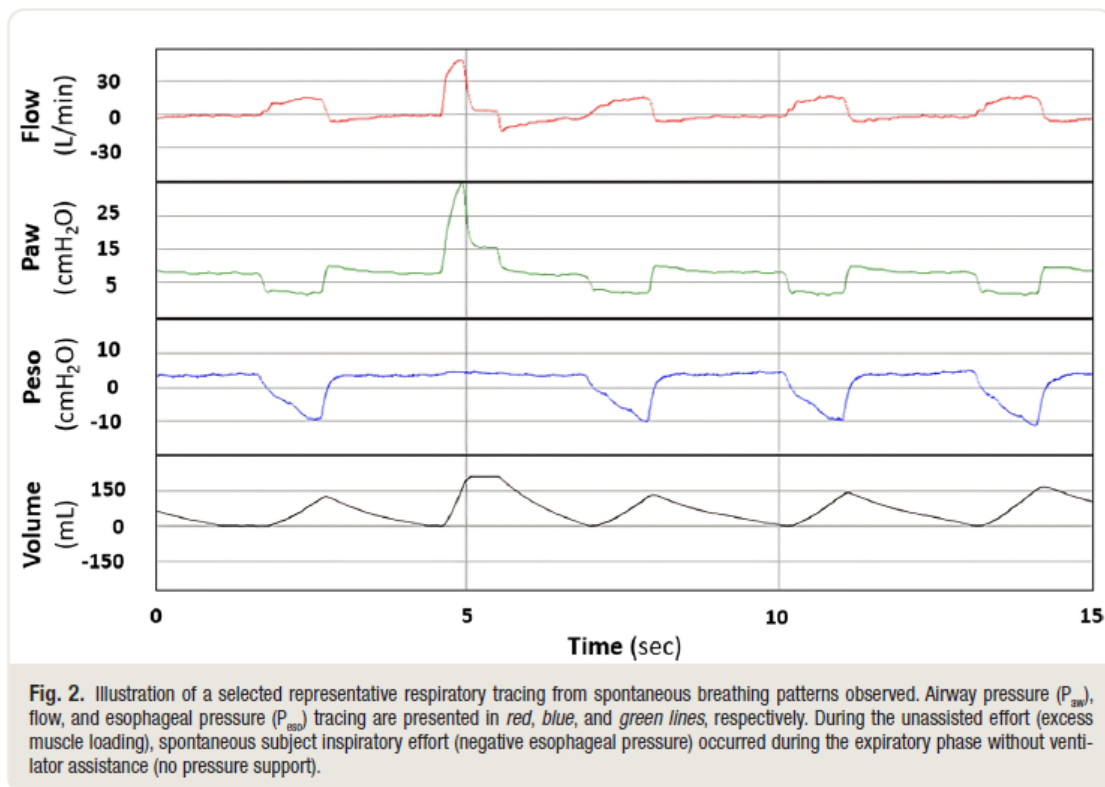
Heart rate; systolic, diastolic, and mean arterial pressure; cardiac output; and oxygen saturation measured by pulse oximetry were continuously monitored. VT and minute volume ventilation were also measured continuously. Blood gases with serum creatinine were measured several times a day using a point-of-care analyzer (Epoc, Siemens, Germany).

Induction and Assessment of Underassisted Mechanical Ventilation

After the 72 h or 2 h of controlled mechanical ventilation, the sedation was stopped until spontaneous ventilation was observed, and then titrated to obtain spontaneous breathing without consciousness. Piglets were switched to ventilation modes that allowed spontaneous breathing and then experienced 2 h of underassistance, defined by inadequate and insufficient

respiratory support (inducing excessive muscle loading in capable patient/subject and/or hematosis disorders).

The ventilator was set in asynchronous intermittent mandatory ventilation mode (volume-controlled mode with spontaneous breathing cycles without trigger between mandatory cycles) with gradual decrease of respiratory rate (mandatory cycles) to the lowest tolerated by the piglets with a target of five per minute (fig. 2). To induce and to maximize the underassistance diaphragm injury, between mandatory volume-controlled cycles, the spontaneous cycles (*i.e.*, unassisted respiratory cycle) were totally unsupported.



The aim of maintaining mandatory cycles was to ensure correct hematosis. The other respiratory parameters such as mandatory VT or PEEP were not modified. The ventilator was alternatively switched to pressure support mode with a pressure support level optimized to obtain a VT of 8 to 10 ml/kg to allow better tolerance of the experiment. In this pragmatic model, there were no artificial inspiratory efforts created by stimulation. The inspiratory effort during the underassisted ventilation period could differ among piglets, with a potentially different impact on diaphragm impairment. 19 To assess this, piglets were ranked by the level of breathing effort using mean swing esophageal pressure and divided into three groups according to this ranking. Flow, airway pressure, and esophageal pressure were recorded using a pneumotachograph and a differential pressure transducer (TSD 160, Biopac Systems, USA). We selected a representative 5-min tracing¹⁹ at the end of the 2 h of underassisted mechanical ventilation period to collect data on physiologic variables, ventilatory characteristics, and respiratory mechanics.

Diaphragm Function

To assess diaphragm function, we used bilateral phrenic nerve stimulation to obtain a negative tracheal pressure after airway occlusion.^{7–9,18} A Stimpex Neurostimulator (Axonic, France)

was connected to the pacing catheters for stimulation. We stimulated at 10 mA with gradually increasing frequency until we obtained diaphragmatic contraction resulting in supramaximal negative pressure, reflecting diaphragm function. All measurements were obtained from sedated piglets in a supine position, with end expiratory pressure of 0 cm H₂O to ensure relaxation of the diaphragm in its original state and conformation at functional residual capacity lung volume.²⁰

As previously described,^{7–9,21} a force frequency curve was obtained by stimulating end-expiratory diaphragm contractions with occluded airways using supramaximal phrenic nerve stimulation with a stimulus duration of 0.15 ms and a train duration of 2,000 ms. Contractions were obtained at fixed frequencies of 10, 20, 30, 40, 60, 80, and 100 Hz. We ensured a 2-min rest period between each frequency.

Diaphragm Structure

Mini-invasive muscle biopsies were harvested *in vivo* by a surgical team from the right subcostal diaphragm after 72 h of controlled mechanical ventilation in the VIDD group, and after the 2 h of underassisted ventilation period for each piglet. We chose to perform biopsies before the 2 h of underassisted ventilation in the VIDD group to be aware of lesions already induced by prolonged ventilation. Samples of dissected diaphragm strips were immediately fixed for electron microscopy examination.

Detailed methods are described in the Supplemental Digital Content (<https://links.lww.com/ALN/D842>). Computer images captured from randomly selected microscopic fields were analyzed by two independent observers blinded to group allocation. A total of 12 micrographs per piglet were examined in the VIDD group, 6 from diaphragm before underassisted ventilation and 6 from diaphragm after underassisted ventilation. Six micrographs per piglet were examined from diaphragm after underassisted ventilation in the no-VIDD group. The percentage of area including sarcomeric injuries and lipid droplets was determined by a standardized stereology grid method²² using ImageJ software (Wayne Rasband and contributors, National Institutes of Health, USA). As described in a previous study,³ disrupted sarcomeres were defined as a zone with distinct distortion of the usual sarcomeric architecture.

Statistical Analysis

According to previous studies,^{7,9,19} we expected a diaphragm supramaximal pressure-generating capacity of 60 cm H₂O before underassistance, and a drop of 35% to 40 cm H₂O after, with an SD of 8 cm H₂O. The required sample size was four to six subjects (two-sided alternative or not) with an alpha risk of 5% and a power of 80%. Taking account of potential deaths, we decided to include 10 subjects in the VIDD group. We expected a smaller decline in the no-VIDD group, so we decided to include 12 subjects.

All statistical analyses were performed using R software 4.4.2 (R Project for Statistical Computing, <https://cran.r-project.org/bin/windows/base/old/4.4.2/>; accessed November 2024). All *P* values were two-tailed, and a *P* value of less than 0.05 was required to rule out the null hypothesis. After performing a Shapiro–Wilk test to ensure the normality of the

variable distribution, we expressed data as mean \pm SD. The comparison of diaphragm pressure-generating capacity along frequencies between, before, and after underassistance was performed using repeated-measures ANOVA.

An *ad hoc* test for each frequency was performed then using a paired Student *t* test. Regarding the diaphragm structure, comparisons of means of sarcomeric injuries or lipid droplets accumulation between, before, and after underassistance were performed using a paired Student *t* test.

Results

Piglet Model

Of all the piglets, initially healthy, one died of sudden cardiac arrest in each group, and they were therefore excluded. The other 20 piglets completed the experiment. All characteristics with hemodynamic, respiratory, and metabolic values and sedation dose of the VIDD group during the 72 h of controlled mechanical ventilation are presented in figure S2 (<https://links.lww.com/ALN/D842>). There were no significant dyssynchrony events detected or recorded during the controlled mechanical ventilation period. The VIDD was proven by a decrease of diaphragm pressure-generating capacity by 22% from 69.9 ± 12.7 to 54.9 ± 19.7 cm H₂O ($P = 0.04$) after 72 h of controlled mechanical ventilation (fig. S3, <https://links.lww.com/ALN/D842>).

Table 1. Individual Values and Mean Values of Unassisted Respiratory Cycles Rate and Inspiratory Efforts

Piglet	Unassisted Respiratory Cycle Rate (%)	Swing P _{esd} (cm H ₂ O)*
Piglet V1	73	2.3 \pm 0.4
Piglet V2	78	10.1 \pm 0.8
Piglet V3	85	6.3 \pm 1.2
Piglet V4	95	9.1 \pm 0.8
Piglet V5	93	3.5 \pm 0.6
Piglet V6	86	3.1 \pm 0.2
Piglet V7	66	6.0 \pm 2.4
Piglet V8	83	12.4 \pm 1.6
Piglet V9	80	8.0 \pm 1
Total V piglets	82 \pm 9	6.7 \pm 3.4
Piglet C1	52	6.8 \pm 1.2
Piglet C2	51	8.9 \pm 0.5
Piglet C3	68	8.7 \pm 1.1
Piglet C4	73	15.4 \pm 2.9
Piglet C5	73	17.2 \pm 2.0
Piglet C6	80	16.3 \pm 3.7
Piglet C7	81	10.0 \pm 1.2
Piglet C8	82	24.7 \pm 1.5
Piglet C9	81	28.3 \pm 2.6
Piglet C10	57	11.5 \pm 2.1
Piglet C11	74	7.9 \pm 1.1
Total C piglets	70 \pm 12	14.1 \pm 1.8

*Data are presented as mean \pm SD.

C piglets, piglets with no preexisting ventilatory-induced diaphragm dysfunction; P_{esd}, esophageal pressure; V piglets, piglets with preexisting ventilatory-induced diaphragm dysfunction.

Underassisted Ventilation

The 5-min selected tracing from the end of the 2 h of the underassistance period revealed a rate of unassisted respiratory cycles (over all cycles, spontaneous/unassisted and mandatory cycles) of $82 \pm 9\%$ for the VIDD group and $70 \pm 12\%$ for the no-VIDD group. The inspiratory effort per breath was 6.7 ± 3.4 cm H₂O for the VIDD group and 14.1 ± 1.8 cm H₂O for the no-VIDD group (table 1). Detailed ventilatory characteristics and respiratory mechanics are presented in table S1 (<https://links.lww.com/ALN/D842>). In the VIDD group, four piglets presented a significant dyssynchrony rate (30 to 62%) with flow starvation during pressure support ventilation after the underassisted ventilation. Piglets were divided into three groups according to inspiratory effort (fig. S4, <https://links.lww.com/ALN/D842>).

Hematois disorders were reported after underassistance with a significant difference compared to before underassistance in both groups (tables S2 and S3, <https://links.lww.com/ALN/D842>). Except for heart rate, there was no other significant difference in hemodynamic or respiratory values and sedation in the VIDD group. There was a significant difference in mean arterial pressure, heart rate, and respiratory rate in the no-VIDD group.

Diaphragm Function

Supramaximal diaphragm pressure-generating capacity decreased significantly from 56 ± 19.2 before to 39.5 ± 15.6 cm H₂O ($P < 0.01$) after 2 h of underassisted ventilation in the VIDD group and varied without significant difference from 55.3 ± 22.7 to 58.2 ± 24 cm H₂O ($P = 0.24$) in the no-VIDD group (fig. 3; fig. S5, <https://links.lww.com/ALN/D842>). These results did not correlate with the level of inspiratory effort (figs. S6 and S7, <https://links.lww.com/ALN/D842>).

Additional statistical results are presented in the Supplemental Digital Content (<https://links.lww.com/ALN/D842>).

Diaphragm Structure

Diaphragm structure showed an increase in sarcomeric injuries from $13 \pm 10\%$ to $24 \pm 19\%$ ($P < 0.01$) of the total micrograph area after 2 h of underassisted ventilation, and a decrease of lipid droplets from $14 \pm 8\%$ to $11 \pm 6\%$ ($P = 0.03$) in the VIDD group. Sarcomeric injuries and lipid droplets accounted, respectively, for $17 \pm 16\%$ and $2 \pm 3\%$ of the total micrograph area after underassisted ventilation in the no-VIDD group (fig. 4; fig. S8, <https://links.lww.com/ALN/D842>). This alteration of diaphragm structure did not correlate with the level of inspiratory effort (fig. S5, <https://links.lww.com/ALN/D842>). The diaphragm samples were analyzed with excellent agreement between the two observers (intraclass correlation coefficient greater than 0.9).

Discussion

Two hours of underassisted ventilation induces impairment of diaphragm function with damage to the diaphragm structure in ICU condition with preexisting VIDD in this experimental piglet model. Despite diaphragm structure injuries, there was no diaphragm dysfunction in the no-VIDD group. There was no significant difference according to level of inspiratory effort.

To our knowledge, we have shown for the first time the harmful effect of combining overassistance diaphragm injury with underassistance diaphragm injury over a short period of exposure.

Piglet Model

The piglet model of long-term controlled mechanical ventilation, with transvenous phrenic nerve stimulation for diaphragm function assessment, was developed in previous experiments.^{7–9,21} This time we added a 2-h period of underassisted mechanical ventilation reducing sedation and using simple unsuitable respiratory parameters. Our aim was to amplify what could happen in clinical practice. After a period of sedation and controlled mechanical ventilation, the ICU patient is awakened. Then underassistance may appear and persist without being detected, without ventilatory parameters being adapted, with potential damage to the diaphragm, as described in the current study. The no-VIDD group, conceived as a control, appears to be of interest because in clinical practice, even after short-term controlled mechanical ventilation (postoperative or post-emergency settings), clinicians wonder about the balance between overassistance and underassistance during transition from controlled mechanical ventilation to assisted ventilation.²³

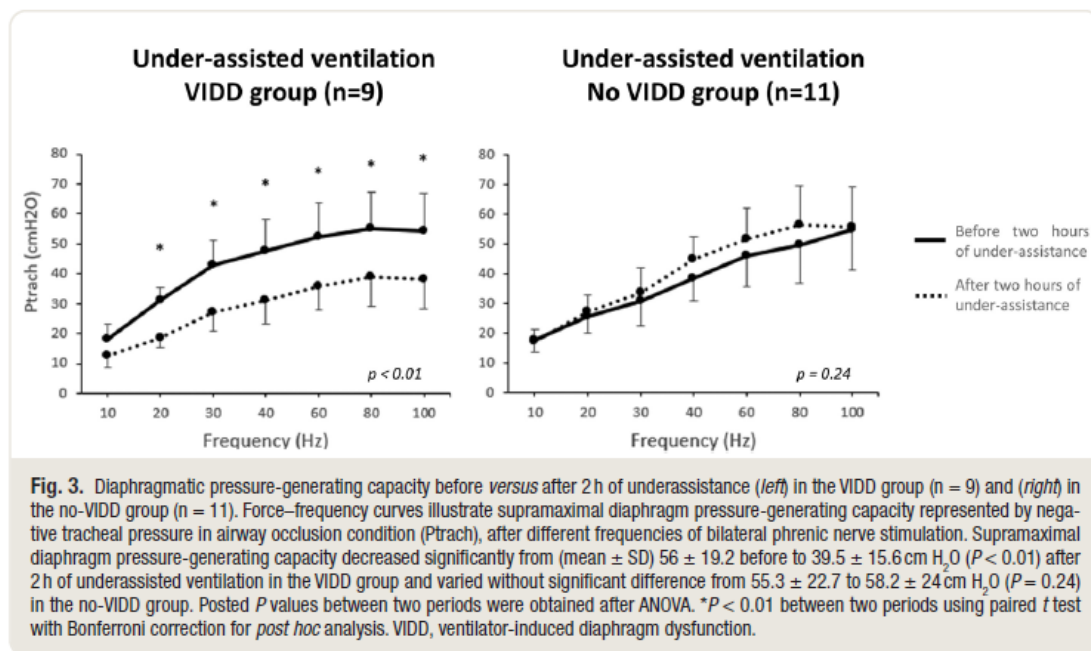
The underassistance in this model is very close to ineffective effort, and there is a fine line between underassistance diaphragm injury and eccentric diaphragm injury. Ineffective effort is the most frequent type of dyssynchrony in patients under pressure support ventilation²⁴ and is associated with extended ICU length of stay,^{25,26} with more extubation failure²⁷ and higher ICU and hospital mortality.^{25,28}

Further, recent similar experimental studies with piglet models have not shown diaphragm impairment after 12 h of ineffective efforts and double triggering,²⁹ or after 3 h of reverse triggering¹⁹ except for piglets with high breathing effort. Dyssynchrony index was high in these two studies, but the severe subject–ventilator dyssynchrony period started directly after animal preparation. The absence of prolonged controlled mechanical ventilation before probably explains the difference with our results. A diaphragm impaired by preexisting VIDD appears more prone to be affected by additional diaphragm injury.

Diaphragm Impairment

There are four distinct types of diaphragmatic injury.³⁰ First is overassistance diaphragm injury, disuse atrophy due to insufficient muscle loading. Second is underassistance diaphragm injury, sarcomeric injuries and contractile fatigue due to excessive muscle loading in response to inadequate and insufficient respiratory support. Third is eccentric diaphragm

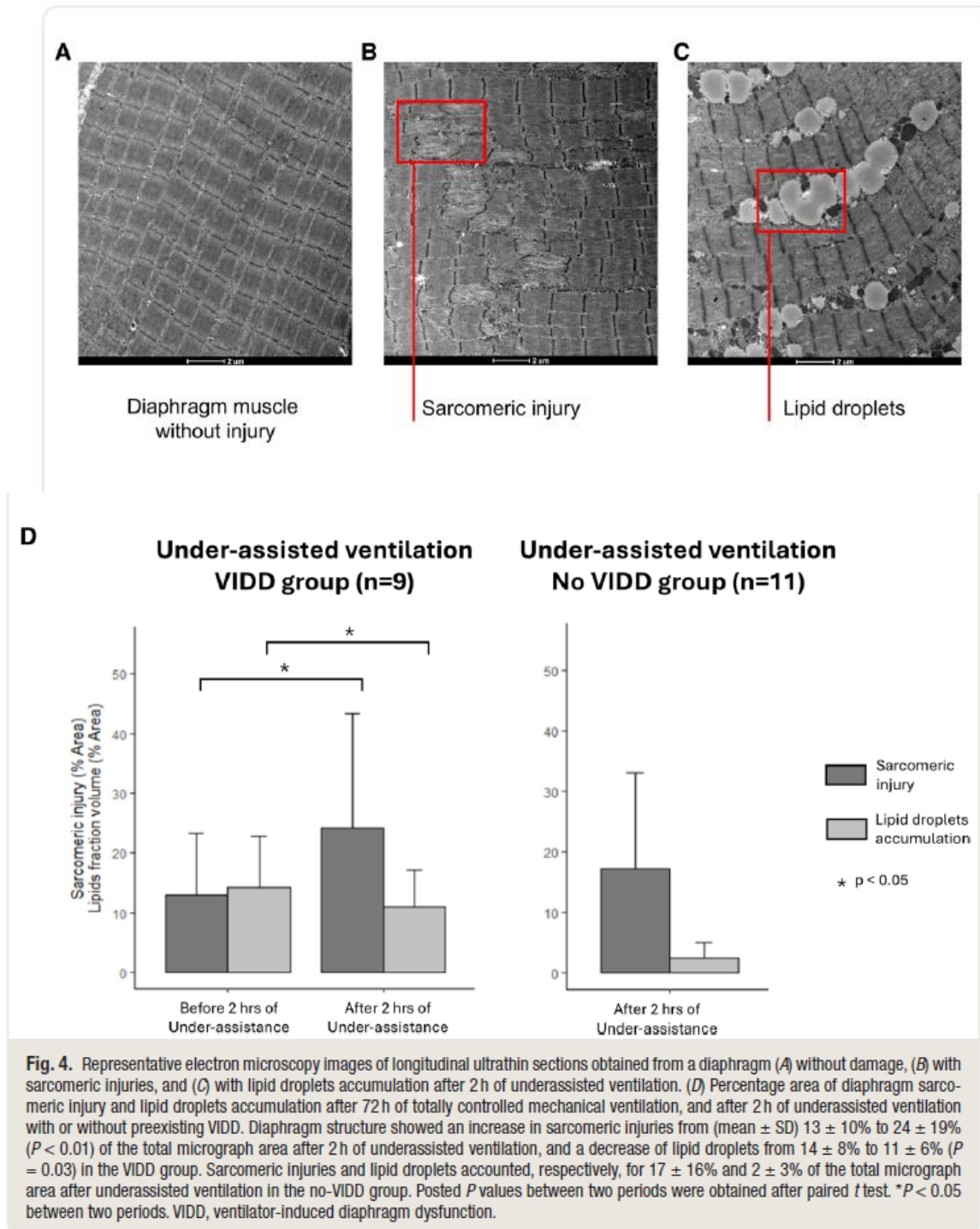
injury, contractile stress delivered to the muscle when it is lengthening (eccentric loading), induced by dyssynchrony (ineffective effort, premature cycling, reverse triggering). Fourth is expiratory diaphragm injury, contractile stress delivered after PEEP withdrawal on a reduced diaphragm (longitudinal atrophy) due to prolonged excessive PEEP. The concept of underassistance and eccentric diaphragm injury is rather recent,^{30–33} and the direct impact on the diaphragm is not clearly established. The current study provides substantial data on the topic with a focus on diaphragm with preexisting VID D due to prolonged controlled mechanical ventilation. To our knowledge, this is the first study that evaluated the effect of overassistance and underassistance on diaphragm function and structure in both population of healthy piglets ventilated for a short-term period (2 h) and piglets ventilated for a long-term period (72 h) who developed VID D.



We first confirm previous findings indicating that diaphragms exhibiting VID D related to overassistance present sarcomeric injuries and an increased intramuscular lipid content related to relative energetic oversupply.^{6,18,34} Second, we observed for the first time sarcomeric injuries due to underassistance with distortion of the usual sarcomeric architecture, sarcolemmal rupture, and sarcomeric disarray. In primed diaphragms (preexisting VID D), injuries were added to those already present after prolonged controlled mechanical ventilation. Concurrently, there was a slight but significant decrease in lipid droplets, which we can only speculate may be related to an increase energy demand associated with active diaphragm contraction and huge efforts due to underassistance.

A substantial level of sarcomeric injuries was also observed in the no-VID D group after the sole application of 2 h of underassisted ventilation, without inducing significant diaphragm dysfunction. There may be a threshold of sarcomeric injuries before diaphragm dysfunction explaining the difference between the no-VID D and VID D groups. Lipid droplets accumulation could also have an impact here. Other mechanisms may be involved but were not explored in this study such as inflammation, proteolysis, oxidative stress, or metabolic disorders. The absence of correlation between diaphragm impairment and level of breathing effort in the VID D group could be explained by the alteration of diaphragm force due to

prolonged controlled mechanical ventilation (major difference with the study by Damiani *et al.*19). Low breathing effort could be due to VIDD with potentially increased susceptibility to injuries. There was a difference in breathing effort between groups that could be a bias for a comparison. However, in the current study, we wanted a pragmatic model of two clinical situations (ICU model with prolonged controlled mechanical ventilation and operating room or emergency ward model with short-term controlled mechanical ventilation). A difference in breathing effort was expected between these two groups.



Limitations

This study has several limitations. First, there are four potential confounding factors of diaphragm impairment. To start with, the severe hypercapnic acidosis, result of inadequate assisted ventilation, whose effect on altering the piglet diaphragm contractility has already been demonstrated.⁸ However, it cannot be responsible for the additional damage to the diaphragm structure. It should be remembered that moderate hypercapnia has a protective effect.^{21,35} Then the impact of the minimally invasive *in vivo* diaphragm biopsy could also affect the results. However, we found sarcomeric injury in the no-VIDD group who did not undergo biopsies. After, the cumulative doses of sedatives and fluid balance in the VIDD group. Last, the presence of flow starvation in the VIDD group, but less than half presented a significant dyssynchrony rate. There was no flow starvation in the no-VIDD group. Second, no biopsies were harvested at baseline, and thus before the underassistance period in the no-VIDD group. As piglets were initially healthy, we considered it unnecessary to perform biopsies at baseline (either for the VIDD or the no-VIDD group). The biopsies performed before the underassistance period in the VIDD group were conducted to discern the impact of prolonged invasive mechanical ventilation on the underassistance that followed. Third, there was no proper control group. We already demonstrated in a previous study⁷ the absence of diaphragm function impairment after 72 h of assisted ventilation. Thus, we considered that for 2 h, it would be more relevant and more ethical to involve animals in a control group without VIDD to study another prevalent clinical situation rather than a proper control group of simple assisted ventilation after the controlled ventilation period. Fourth, we recorded only a 5-min representative tracing at the end of the 2 h of underassisted ventilation period.

We considered a certain stability of the underassistance over this period. Fifth, some piglets were ventilated with high VT during the controlled mechanical ventilation period that did not reflect the standard of care. Our aim was to avoid dyssynchrony during this period, which has previously been shown to be difficult with low VT.¹⁹ Sixth, there was no measurement of end-expiratory lung volume. This has never been demonstrated, but it could affect the diaphragm conformation and thus the impact of eccentric contractions.

Clinical Implications and Perspectives

This study provides additional data on the impact of the underassistance and arguments to encourage lung and diaphragm protective ventilation.³⁶ After prolonged controlled mechanical ventilation, the transition to assisted ventilation should be made with caution, with adequate pressure support and parameters to avoid underassistance. In case of fast weaning process, the same precautions should be taken when performing a spontaneous breathing trial. It is possible that 2 h of underassistance was too short to induce diaphragm injury in the no-VIDD group. Another study evaluating the time effect of underassistance would be interesting. To go further, it might be interesting to explore different pathways such as muscle inflammation, oxidative stress, and proteolysis after underassistance. We may look at whether diaphragm function is affected in the same way by eccentric diaphragm injury. Wittenstein *et al.*, in their experiment of acute respiratory distress syndrome piglets with dyssynchrony after a short passive ventilation,²⁹ did not assess the diaphragm function. Evaluating the impact of dyssynchrony after prolonged controlled mechanical ventilation with a piglet model of acute respiratory distress syndrome, sepsis, trauma, and postoperative status could be of interest. With varying dyssynchrony rate levels, we could assess the effects of various dyssynchrony patterns alone. The effect of diaphragm conformation might be assessed using different PEEP

and end-expiratory lung volume levels. By using minimally invasive extracorporeal carbon dioxide removal, as previously described,³⁷ we could enhance the experimental model, get rid of hypercapnic acidosis as a confounding factor, and confirm our findings.

Conclusions

In this experimental study with a piglet model, we reported that 2 h of underassisted ventilation induces impairment of diaphragm function with damage to the diaphragm structure in ICU condition with preexisting VIDD. Further research is needed to confirm these findings and assess the clinical impact in ICU patients.

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Competing Interests

Dr. Jaber reports receiving consulting fees from Drager (Lubeck, Germany), Mindray (Shenzhen, China), Medtronic (Dublin, Ireland), Baxter (Deerfield, Illinois), Fresenius-Xenios (Bad Homburg vor der Höhe, Germany), and Fisher & Paykel (Auckland, New Zealand). Dr. De Jong reports receiving consulting fees from Viatrix (Canonsburg, Pennsylvania), Medtronic, and Sanofi (Gentilly, France). Dr. Brochard's laboratory has received research grants from Medtronic, Drager, and Stimit (Bienne, Switzerland) and equipment from Sentec (Therwil, Switzerland), Fisher & Paykel, and Philips (Amsterdam, The Netherlands). Dr. Brochard has received lecture fees from Fisher & Paykel. The other authors declare no competing interests.

References

1. Slutsky AS, Ranieri VM: Ventilator-induced lung injury. *N Engl J Med* 2013; 369:2126–36
2. Vassilakopoulos T, Petrof BJ: Ventilator-induced diaphragmatic dysfunction. *Am J Respir Crit Care Med* 2004; 169:336–41
3. Jaber S, Petrof BJ, Jung B, et al.: Rapidly progressive diaphragmatic weakness and injury during mechanical ventilation in humans. *Am J Respir Crit Care Med* 2011; 183:364–71

4. Dridi H, Jung B, Yehya M, et al.: Late ventilator-induced diaphragmatic dysfunction after extubation. *Crit Care Med* 2020; 48:e1300–5
5. Schepens T, Goligher EC: Lung- and diaphragm-protective ventilation in acute respiratory distress syndrome: Rationale and challenges. *Anesthesiology* 2019; 130:620–33
6. Mrozek S, Jung B, Petrof BJ, et al.: Rapid onset of specific diaphragm weakness in a healthy murine model of ventilator-induced diaphragmatic dysfunction. *Anesthesiology* 2012; 117:560–7
7. Jung B, Constantin JM, Rossel N, et al.: Adaptive support ventilation prevents ventilator-induced diaphragmatic dysfunction in piglet. *Anesthesiology* 2010; 112:1435–43
8. Jaber S, Jung B, Sebbane M, et al.: Alteration of the piglet diaphragm contractility in vivo and its recovery after acute hypercapnia. *Anesthesiology* 2008; 108:651–8
9. Jaber S, Sebbane M, Koechlin C, et al.: Effects of short vs. prolonged mechanical ventilation on antioxidant systems in piglet diaphragm. *Intensive Care Med* 2005; 31:1427–33
10. Radell PJ, Remahl S, Nichols DG, Eriksson LI: Effects of prolonged mechanical ventilation and inactivity on piglet diaphragm function. *Intensive Care Med* 2002; 28:358–64
11. Powers SK, Kavazis AN, Levine S: Prolonged mechanical ventilation alters diaphragmatic structure and function. *Crit Care Med* 2009; 37:S347–53
12. Reid WD, Huang J, Bryson S, Walker DC, Belcastro AN: Diaphragm injury and myofibrillar structure induced by resistive loading. *J Appl Physiol* (1985) 1994; 76:176–84
13. Reid WD, Belcastro AN: Time course of diaphragm injury and calpain activity during resistive loading. *Am J Respir Crit Care Med* 2000; 162:1801–6
14. Orozco-Levi M, Lloreta J, Minguella J, Serrano S, Broquetas JM, Gea J: Injury of the human diaphragm associated with exertion and chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2001; 164:1734–9
15. Reid WD, Belcastro AN: Chronic resistive loading induces diaphragm injury and ventilatory failure in the hamster. *Respir Physiol* 1999; 118:203–18
16. Jung B, Moury PH, Mahul M, et al.: Diaphragmatic dysfunction in patients with ICU-acquired weakness and its impact on extubation failure. *Intensive Care Med* 2016; 42:853–61
17. Demoule A, Molinari N, Jung B, et al.: Patterns of diaphragm function in critically ill patients receiving prolonged mechanical ventilation: A prospective longitudinal study. *Ann Intensive Care* 2016; 6:75
18. Aarab Y, Flatres A, Garnier F, et al.: Shear wave elastography, a new tool for diaphragmatic qualitative assessment. A translational study. *Am J Respir Crit Care Med* 2021; 204:797–806
19. Damiani LF, Engelberts D, Bastia L, et al.: Impact of reverse triggering dyssynchrony during lung protective ventilation on diaphragm function: An experimental model. *Am J Respir Crit Care Med* 2022; 205:663–73
20. Green M, Road J, Sieck GC, et al.: ATS/ERS statement on respiratory muscle testing. *Am J Respir Crit Care Med* 2002; 166:518–624
21. Jung B, Sebbane M, Goff C, et al.: Moderate and prolonged hypercapnic acidosis may protect against ventilator-induced diaphragmatic dysfunction in healthy piglet: An in vivo study. *Crit Care* 2013; 17:R15
22. Broskey NT, Daraspe J, Humbel BM, Amati F: Skeletal muscle mitochondrial and lipid droplet content assessed with standardized grid sizes for stereology. *J Appl Physiol* (1985) 2013; 115:765–70
23. Jeong H, Tanatporn P, Ahn HJ, et al.: Pressure support versus spontaneous ventilation during anesthetic emergence— Effect on postoperative atelectasis: A randomized controlled trial. *Anesthesiology* 2021; 135:1004–14
24. Thille AW, Rodriguez P, Cabello B, Lellouche F, Brochard L: Patient-ventilator asynchrony during assisted mechanical ventilation. *Intensive Care Med* 2006; 32:1515–22

25. Blanch L, Villagra A, Sales B, et al.: Asynchronies during mechanical ventilation are associated with mortality. *Intensive Care Med* 2015; 41:633–41
26. de Wit M, Miller KB, Green DA, Ostman HE, Gennings C, Epstein SK: Ineffective triggering predicts increased duration of mechanical ventilation. *Crit Care Med* 2009; 37:2740–5
27. Sousa ML de A, Magrans R, Hayashi FK, Blanch L, Kacmarek RM, Ferreira JC: Predictors of asynchronies during assisted ventilation and its impact on clinical outcomes: The EPISYNC cohort study. *J Crit Care* 2020; 57:30–5
28. Kyo M, Shimatani T, Hosokawa K, et al.: Patient–ventilator asynchrony, impact on clinical outcomes and effectiveness of interventions: A systematic review and meta-analysis. *J Intensive Care* 2021; 9:50
29. Wittenstein J, Huhle R, Leiderman M, et al.: Effect of patient–ventilator asynchrony on lung and diaphragmatic injury in experimental acute respiratory distress syndrome in a porcine model. *Br J Anaesth* 2021; 130:e169–78
30. Goligher EC, Brochard LJ, Reid WD, et al.: Diaphragmatic myotrauma: A mediator of prolonged ventilation and poor patient outcomes in acute respiratory failure. *Lancet Respir Med* 2019; 7:90–8
31. Goligher EC: Myotrauma in mechanically ventilated patients. *Intensive Care Med* 2019; 45:881–4
32. Bertoni M, Spadaro S, Goligher EC: Monitoring patient respiratory effort during mechanical ventilation: Lung and diaphragm-protective ventilation. *Crit Care* 2020; 24:106
33. Goligher EC, Dres M, Patel BK, et al.: Lung- and diaphragm-protective ventilation. *Am J Respir Crit Care Med* 2020; 202:950–61
34. Picard M, Jung B, Liang F, et al.: Mitochondrial dysfunction and lipid accumulation in the human diaphragm during mechanical ventilation. *Am J Respir Crit Care Med* 2012; 186:1140–9
35. Schellekens WJM, van Hees HW, Kox M, et al.: Hypercapnia attenuates ventilator-induced diaphragm atrophy and modulates dysfunction. *Crit Care* 2014; 18:R28
36. Dianti J, Fard S, Wong J, et al.: Strategies for lung- and diaphragm-protective ventilation in acute hypoxemic respiratory failure: A physiological trial. *Crit Care* 2022; 26:259
37. Duscio E, Cipulli F, Vasques F, et al.: Extracorporeal CO₂ removal: The minimally invasive approach, theory, and practice. *Crit Care Med* 2019; 47:33–40

