Diaphragm neurostimulation during mechanical ventilation reduces atelectasis and transpulmonary plateau pressure, preserving lung homogeneity and PaO2/FiO2

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Abstract

Tidal volume delivered by mechanical ventilation to a sedated patient is distributed in a non-physiological pattern, causing atelectasis (underinflation) and overdistension (overinflation). Activation of the diaphragm during controlled mechanical ventilation in these sedated patients may provide a method to reduce atelectasis and alveolar inhomogeneity, protecting the lungs from ventilator-induced lung injury while also protecting the diaphragm by preventing ventilator-induced diaphragm dysfunction. We studied the hypothesis that diaphragm contractions elicited by transvenous phrenic nerve stimulation, delivered in synchrony with volume-control ventilation, would reduce atelectasis and lung inhomogeneity in a healthy, normal-lung pig model. Twenty-five large pigs were ventilated for 50 hours with lung-protective volume-control ventilation combined with synchronous transvenous phrenic-nerve neurostimulation on every breath, or every second breath. This was compared to lung-protective ventilation alone. Lung mechanics and ventilation pressures were measured using esophageal pressure manometry and electrical impedance tomography. Alveolar homogeneity was measured using alveolar chord length of preserved lung tissue. Lung injury was measured using inflammatory cytokine concentration in bronchoalveolar lavage fluid and serum. We found that diaphragm neurostimulation on every breath preserved PaO2/FiO2 and significantly reduced the loss of end-expiratory lung volume after 50 hours of mechanical ventilation. Neurostimulation on every breath reduced plateau and driving pressures, improved both static and dynamic compliance and resulted in less alveolar inhomogeneity. These findings support that temporary transvenous diaphragm neurostimulation during volume-controlled, lung-protective ventilation may offer a potential method to provide both lung- and diaphragm-protective ventilation.

New and Noteworthy:

Temporary transvenous diaphragm neurostimulation has been shown to mitigate diaphragm atrophy in a preclinical model. This study contributes to this work by demonstrating that diaphragm neurostimulation can also offer lung protection from ventilator injury, providing a potential solution to the dilemma of lung- vs. diaphragm-protective ventilation. Our findings show that neurostimulation on every breath preserved PaO2/FiO2, end-expiratory lung volume, alveolar homogeneity and required lower pressures than lung-protective ventilation over 50 hours in healthy pigs.
**Background:**

Delivery of tidal volume by mechanical ventilation to a deeply sedated patient does not follow normal physiological transpulmonary pressure gradients as there are no associated respiratory muscle contractions. In the supine position, tidal volume is distributed more to non-dependent, ventral alveoli, causing overdistention, and alveolar collapse is promoted in dependent, dorsal alveoli as they receive less volume, resulting in atelectasis. Atelectasis causes injury to lung tissue through the shearing forces created during cycles of alveolar collapse and expansion during mechanical ventilation. Atelectasis is further encouraged by the use of low-volume, lung-protective ventilation strategies, gravitational forces and increased hydrostatic pressure from abdominal contents due to patient position. Increased atelectasis-related pulmonary shunt exacerbates hypoxia and hypercarbia, which has been shown to be a potent inducer of inflammation and lung epithelial injury. The proportion of tidal volume is increased in open alveoli when atelectasis forms, which increases respiratory system driving pressure, leading to worsening overdistension and injury. Decreased driving pressure is strongly associated with increased survival in acute respiratory distress syndrome (ARDS) patients.

Many critically ill, mechanically ventilated patients are administered deep sedation in order to either facilitate achieving therapeutic goals, or to prevent asynchronous interaction with the mechanical ventilator. Prolonged deep sedation worsens ventilator-induced lung injury (VILI) and puts the patient at risk for ventilator-induced diaphragm atrophy. The PLUG group has identified a need to combine both lung- and diaphragm-protective ventilation in a single strategy.

Concepts from the field of functional electrical nerve stimulation provide a mechanism to combat both atelectasis-driven lung injury and diaphragm atrophy in sedated patients unable to breathe.
spontaneously, by delivering a targeted stimulus to the phrenic nerves. (26-29) This neurostimulation contracts the diaphragm, preserving muscle strength and counteracting the compressive pressure from abdominal contents. (26, 29) It also promotes more physiologically normal distribution of tidal volume, which helps to keep dorsal alveoli open as they receive more tidal volume. (26) Electrically stimulated diaphragmatic activity that prevented atelectasis was shown in a series of 12 patients undergoing intraabdominal surgery who received right-sided percutaneous electrical stimulation of the phrenic nerve in the neck. (26) A 9.5 French central venous catheter equipped with stimulation electrodes has been developed recently (LIVE Catheter, Lungpacer Medical Inc.). (30) The catheter is temporarily and percutaneously inserted in the left subclavian vein and stimulation current is delivered transvenously to capture the phrenic nerves for diaphragm activation. This activation is delivered adjunctively, and in synchrony with, traditional mechanical ventilation. This ventilatory adjunct has been shown to mitigate ventilator-induced diaphragm atrophy and dysfunction in a pig model and has potential for preventing compressive atelectasis and atelectrauma. (29) It has been shown to effectively elicit diaphragm contractions in humans and is presently under evaluation in clinical trials (ClinicalTrials.gov: NCT03783884). (28) Diaphragm neurostimulation offers a potential solution for a protective ventilation strategy that protects both the diaphragm and the lungs at the same time, but it is not self-evident that elicited respiratory efforts are similar to diaphragm contractions during spontaneous breaths. Therefore, it is imperative to carefully study this promising intervention to ensure that it provides some of the benefits associated with the diaphragm contractions that occur during normal physiological spontaneous breathing.

The purpose of this study is to investigate the effects of phrenic nerve paced diaphragm contractions on atelectasis formation and alveolar homogeneity in a preclinical model, which may apply to heavily sedated patients receiving positive-pressure ventilation. We aim to show that TTDN can restore some of
the benefits of diaphragm contraction to preclinical subjects that are deeply sedated, thereby offering a potential mechanism to prevent the development of all the aspects that contribute to VILI and VIDD during the acute sedation phase of their ICU course in humans. Our primary hypothesis is that temporary, transvenous diaphragm neurostimulation (TTDN), in combination with mechanical ventilation, will restore a more physiological distribution of tidal volume and reduce end-expiratory lung volume loss. This will improve PaO\textsubscript{2}/FiO\textsubscript{2} and reduce the amount of atelectasis formation, reducing alveolar inhomogeneity, compared to mechanical ventilation alone.

**Methods:**

Twenty-five healthy female Yorkshire pigs (43-71 kg) underwent 50 hours of ventilation in ICU conditions. One group received volume-control mechanical ventilation only (MV group, n=10). A second group received synchronous TTDN during volume-control ventilation on every second breath (MV+TTDN50% group, n=8). A third group received synchronous TTDN during volume-control ventilation on every breath (MV+TTDN100% group, n=7). All animals were ventilated in the supine position. Six pigs, never intubated or ventilated, served as histology controls (NV Group, n=6). The study was conducted in accordance with Canadian Council for Animal Care guidelines and approved by the University of British Columbia and Simon Fraser University Animal Ethics Committees. Detailed descriptions of protocols are provided in the Online Data Supplement (https://doi.org/10.6084/m9.figshare.14077430). Animals were weighed at the start of the experiment and animal length was measured from the tip of the snout to the base of the tail.

Both of the MV+TTDN groups received a neurostimulation catheter inserted percutaneously into the left subclavian vein. TTDN was delivered in synchrony with the inspiratory phase (Ti) of ventilator-delivered breaths as previously published.(29) Synchrony was achieved using a flow sensor inline at the ventilator
circuit-endotracheal tube junction. Stimulation was initiated when the start of inspiration was detected. The duration of stimulation was set to be the same length as the ventilator’s Ti. TTDN was delivered at 25 Hz with a stimulation intensity level set to target a reduction in ventilator (Paw) pressure-time product (PTP) of 15-20% when compared to MV-only PTP. Refer to Figure E2 in the Online Data Supplement for an example of PTP reduction (https://doi.org/10.6084/m9.figshare.14077430). MV and MV+TTDN groups were deeply sedated with intravenous anesthesia to ablate respiratory drive and were monitored with Bispectral Index Monitoring, validated for use in pigs, to measure the level of sedation under anesthesia. Neuromuscular blocking agent was not used in this study as this blocks nerve conduction to the diaphragm, which precludes diaphragm neurostimulation. We have chosen this model in order to investigate whether the positive effects of diaphragm contractions during spontaneous breathing can be restored to subjects who cannot breathe spontaneously due to a treatment requirement for deep sedation. NV animals were lightly sedated briefly with inhaled anesthetic via mask to facilitate gathering relevant data, prior to euthanasia.

The MV and MV+TTDN groups were ventilated using a lung-protective strategy with tidal volume of 8 ml/kg in volume control, PEEP of 5 cmH2O and FiO2 titrated to SpO2 > 94%. We used volume-control and not pressure-control because the combination of TTDN with pressure-control ventilation would have resulted in large variations in tidal volume, preventing a consistent lung-protective ventilation protocol based on tidal volume. Lung mechanics were measured every 2 hours by electrical impedance tomography (EIT; Dräger PulmoVista 500). The EIT band was placed at the 6th intercostal space and was left in place for the duration of the experiment. Esophageal balloons were used to measure transpulmonary pressures in all subjects. Transpulmonary pressures were measured for both MV and MV+TTDN breaths at end-inspiration and end-expiration. Inflammatory cytokines were obtained from the right cranial lobe via bronchoscopy. This is the only lobe accessible with commercially available
bronchoscopes due to the length of the trachea in pigs of this size. At necropsy, lung tissue samples were
taken from the left lung only, at five specific locations (Figure 3 details sample locations) and fixed in
formalin and embedded in paraffin. Lungs were not reinflated to standard transpulmonary pressure as
this posed a distinct threat of re-recruitment of atelectatic alveoli. Alveolar expansion at the time of
tissue fixation was quantified by measuring alveolar chord length.(32) Refer to the Online Data
Supplement for detailed protocols (https://doi.org/10.6084/m9.figshare.14077430).

A power calculation with an alpha of 0.05 and a beta of 0.80 based on the PaO2/FiO2 ratio changes seen
in the experiment by Grasso et al. with negative-pressure ventilation in lung-injured rabbits (350 mmHg
vs. 75 mmHg, SD 75) yields two animals per arm.(33) A minimum of six animals per group was used to
ensure that groups were large enough to be able to use nonparametric statistical tests. Additional
animals were added to the MV, MV+TTDN50% and MV+TTDN100% groups to replace lost tissue samples
from a freezer failure, procedural challenges during the 2020 COVID-19 pandemic, and as part of an
effort to balance experimental pairs. Statistical analysis was performed using GraphPad Prism version
8.0.0 (GraphPad Software, San Diego, California USA, www.graphpad.com). All measurements are
reported as median (IQR), 95% confidence interval of the median and were tested using nonparametric
statistical tests. Medians among all groups were compared using Kruskal-Wallis one-way analysis of
variance. A post-hoc Dunn’s Test of Multiple Comparisons was used to test which specific groups were
different from each other if Kruskal-Wallis analysis showed a significant result. As repeated-measures
ANOVA cannot handle missing values, we analyzed the data by fitting a mixed model as implemented in
GraphPad Prism 8.0. This mixed model uses a compound symmetry covariance matrix and is fit using
Restricted Maximum Likelihood (REML). Fixed effects were time, TTDN dose and TTDN dose interaction
with time. Random effect was subjects. A Geisser-Greenhouse correction was used to correct for lack of
sphericity. Longitudinal data are graphed as median (IQR) with simple linear regression for line of best fit.

**Results:**

**Subject Groups:**

There was a difference between subject weights in all groups in the analysis of variance, with MV+TTDN100% being larger than the MV+TTDN50% group as per the post-hoc analysis, however the length-to-weight ratio was the same for all four groups (Table 1). There was no difference in fluid balance, anesthetic drug dose, ventilator settings or blood gas values between ventilated groups (Table 2). Controlled mechanical ventilation resulting in normal arterial blood gas values was achieved in all ventilated groups, for the duration of the 50-hours. The mixed-effects analysis identified that PaO\textsubscript{2} and PaCO\textsubscript{2} were both affected by time but not by TTDN dose or the interaction of TTDN dose with time.

Spontaneous breathing was evaluated by manually reviewing pressure and flow waveforms, and was detected on an average of 8 episodes for 36,917 breaths (50 hours) per experiment, for each ventilated animal.

**Table 1: Group weight, drug dosage and fluid balance comparisons**

**Table 2: Ventilation comparisons for the groups that received mechanical ventilation**

**Alterations in Distribution of Tidal Volume by TTDN:**

Ventilator-triggered TTDN diaphragm contractions, in synchrony with the inspiratory phase of a volume-control breath changed the distribution of tidal volume. Dorsal distribution of tidal volume was different between the groups, p<0.0001 (Table 3, Figure 1A). Tidal volume in the ventral lung region was also different between all groups, p<0.0001 (Table 3, Figure 1A). Dorsal tidal volume changed from 47% (45-
53) for breaths with no diaphragm contraction (passive) to 53% (50-57) for breaths when the diaphragm was contracted (active) in the same animal of the MV+TTDN50% group, with a concurrent shift in ventral tidal volume, 53% (47-55) to 47% (44-51) in the same animal of the MV+TTDN50% group, p=0.0499.

Figure 1B shows an example of an EIT image documenting the redistribution of tidal volume from ventral to dorsal lung regions between passive and active breaths in the same animal.

Table 3: Distribution of tidal volume in dorsal and ventral lung regions

Table 4: Net loss of end-expiratory lung volume as measured by EIT, at the end of 50 hours of mechanical ventilation.

Figure 2: A: Change in end-expiratory lung volume over 50 hours, as measured by EIT, in all three ventilated groups. B: Example EIT images showing the gain (blue) or loss (orange) of end-expiratory lung volume in animals from each of the ventilated groups.

Alveolar Expansion and Tissue Homogeneity:

The alveolar chord length is different between all groups for each tissue sample location (p<0.0001 location 1, p=0.0045 location 2, p<0.0001 location 3, p<0.0001 location 4, p<0.0001 location 5). Samples taken from the lower lobe (sample locations 1-3) were not different between the NV and MV+TTDN100%
group in the post-hoc analysis. Refer to Table 5. Figure 3 shows hematoxylin and eosin-stained sections of lung tissue from each sample location and group.

Table 5: Alveolar chord length for each tissue sample location.

Figure 3: Examples of H&E-stained lung tissue at five sampled locations showing the different patterns of alveolar expansion between the groups at each sample site.

Ventilation Pressures and Dynamic Compliance:

Table 6: Esophageal pressures, ventilation pressures, compliance and $\text{PaO}_2/\text{FiO}_2$ Ratio at baseline and at the end of 50 hours of mechanical ventilation.

Esophageal pressure measured at end-inspiration was not different at baseline or study end, between groups or for the interaction of TTDN dose with time. Esophageal pressure was affected by time, $p=0.0214$ (Table 6). Plateau pressure was different between the three groups at study end, $p=0.0258$. The mixed-effect analysis shows that plateau pressure was affected by time ($p=0.0054$) and TTDN dose ($p=0.0203$). The interaction of TTDN dose with time was not significant. Driving pressure (plateau pressure minus PEEP) was different at study end between the three groups, $p=0.0258$. The mixed-effect analysis shows that driving pressure was affected by time ($p=0.0118$) and TTDN dose ($p=0.0251$). The interaction of TTDN dose with time was not significant. Transpulmonary plateau pressure was different between all three groups at both baseline ($p=0.0015$) and study end ($p=0.0015$). The mixed-effect analysis shows that transpulmonary plateau pressure was affected by time ($p=0.0189$) and TTDN dose ($p=0.0016$). The interaction of TTDN dose with time was not significant. Refer to Table 6 and Figure 4.

Measurements were taken for all breaths (MV or MV+TTDN as dictated by the study group).

EIT-measured dynamic compliance was different between all three groups at both baseline ($p=0.0006$) and study end ($p<0.0001$). The mixed-effect analysis shows that dynamic compliance was not affected by time but the effect of TTDN dose was significant ($p<0.0001$). The interaction of TTDN and time was also significant, $p=0.0048$. The slope of the linear regression was different between all three groups ($p<0.0001$). Static compliance was different between the three groups at both baseline ($p=0.0257$) and
study end (p=0.0035). The mixed-effect analysis shows that static compliance was not affected by time but the effect of TTDN dose was significant (p<0.0001). The interaction of TTDN dose and time was not significant. Measurements were taken for all breaths (MV or MV+TTDN as dictated by the study group).

Refer to Table 6 and Figure 4.

Figure 4: A: Plateau pressure, transpulmonary plateau pressure and driving pressure over 50 hours of mechanical ventilation. B: Dynamic and static compliance over 50 hours of mechanical ventilation.

Change in PaO₂/FiO₂:

The mixed-effect analysis shows that PaO₂/FiO₂ was affected by time (p<0.0001) and TTDN dose (p=0.0393). The interaction of TTDN dose and time was not significant (Table 6). The longitudinal change in PaO₂/FiO₂ over 50 hours was not different, however the slope of the linear regression was different between all three groups (p=0.0044).

Figure 5: PaO₂/FiO₂ Ratio Over 50 Hours of Mechanical Ventilation

Inflammatory Cytokines in Bronchoalveolar Lavage Fluid and Serum, and Modified Lung Injury Score:

Inflammatory cytokines in the BALF were the lowest in the MV+TTDN100% group but did not reach statistical significance. There were also no statistical differences between inflammatory cytokine concentrations measured in serum or in the modified lung injury scores performed on histology samples.

Refer to Table E2 in the Online Data Supplement for details.

Adverse Events:

No instances of ventilator asynchrony, such as double-triggered breaths or breath stacking, were observed. We manually reviewed pressure and flow waveforms recorded at the termination of the ventilator circuit, as well as esophageal pressure tracings, and no evidence of reverse triggering was observed. There were no adverse hemodynamic effects and no dangerous cardiac arrhythmias recorded.
We had two instances where mucus plugs were mobilized by diaphragm contractions that plugged-off an area of the lung in the MV+TTDN50% group in the last 6-8 hours of the experiment. These episodes resolved over 2-4 hours with suctioning but required a temporary increase in FiO₂ for a period. This did not occur in the other groups; however, all groups were suctioned every 6 hours routinely to ensure that any derecruitment due to suctioning was similar between groups. Extravascular lung water was not significantly different between groups (Figure E4 in the Online Data Supplement).

Discussion:

TTDN combined with lung-protective mechanical ventilation distributed tidal volume in a more physiological pattern, mitigated the loss of EELV, improved alveolar homogeneity and preserved PaO₂/FiO₂ when provided on every breath. These results indicate that TTDN on every breath protects the lungs against potential ventilator-induced lung injury by keeping them “open.” Improved lung compliance and the reduction of both transpulmonary plateau and driving pressures support the biological plausibility and experimental validity of this work. The positive findings in this study, as well as the absence of negative interactions such as asynchrony, attest that it is reasonable to evaluate the impact of TTDN in human patients.

The significant mitigation of end-expiratory lung-volume loss with the use of adjunctive TTDN on every breath is an important finding and establishes a biological foundation for the potential clinical benefit of TTDN in critically ill patients. There was twice as much end-expiratory lung volume lost in the MV group compared to the MV+TTDN100% group, with the MV+TTDN50% group being closer to the MV group. This demonstrates that TTDN on every breath preserves more end-expiratory lung volume than it does on every second breath, thus the dose of TTDN is important. The more alveoli that are preserved for tidal respiration, the greater the reduction in the overall stress on individual units, thereby reducing...
atelectrauma and cyclic strain. This is further supported by the improved PaO2/FiO2, lower transpulmonary plateau pressure and respiratory system driving pressures in the MV+TTDN100% group versus the MV+TTDN50% group. TTDN on every breath reduced driving pressure in comparison to the gold-standard lung-protective ventilation strategy alone. We have previously shown that TTDN on every second breath, targeting the same ventilator PTP reduction as this study, mitigates ventilator-induced diaphragm atrophy. This combined with the potential for the reduction of atelectrauma, when delivered on every breath, suggests that diaphragm neurostimulation may be a promising candidate for a combined lung- and diaphragm-protective ventilation strategy.

Lung inhomogeneity results in abrupt changes in the configuration and internal pressures of alveoli. Increased inhomogeneity leads to stress amplification in the walls of the neighboring lung units. Atelectasis increases lung inhomogeneity, causing an up to four-fold increase in stress and strain in the areas adjacent to areas of collapse, driving lung injury. Once injury is initiated in an alveolus, less stress and strain are required to further damage it, described as the two-hit hypothesis. TTDN preserves the range of alveolar expansion when delivered both on every breath and every second breath, however the MV+TTDN100% group had a range closer to the NV group, with the lower lobe (location 1-3) not different in post-hoc analysis. The MV group showed the largest loss of end-expiratory lung volume and the largest range of alveolar chord length and thus alveolar inhomogeneity (57.85-109.60 µm). The smallest measurements in the MV group were in the base of the lower lobe (sample site 1), the area with the most atelectasis, while the largest were in the upper regions of the upper lobe, the area most prone to overdistension and injury. The MV+TTDN100% group was the most similar to the NV group (74.67-88.12 µm vs. 80.28-86.54 µm), having the least inhomogeneity of the ventilated groups and the lowest loss of end-expiratory lung volume. The impact of reduced lung compliance on alveolar chord length cannot be excluded, however the MV and MV+TTDN50% groups had similar compliance,
but their alveolar chord length ranges were different. These alveolar chord length findings are supported by the pattern of EELV loss, alterations in driving pressure and gross histology observation. The reduction in inhomogeneity by TTDN is a key finding of this study as it elucidates how this emerging intervention may encourage alveolar homogeneity while preserving end-expiratory lung volume thereby promoting an “open lung.”

PaO2/FiO2, an indicator of pulmonary shunt, is well correlated with atelectasis. (36-38) While not reaching the level of mild lung injury (<300) in any group, PaO2/FiO2 was better preserved in the MV+TTDN100% group compared to the other groups. The MV+TTDN50% group had two out of eight animals experience significant mucus plugging episodes in the last 8 hours of the experiment that reduced PaO2/FiO2 ratios to a 95% CI of 72-497 for study end, whilst remaining clinically stable. This phenomenon has also been described in a case report from a human clinical trial using this technology for diaphragm rehabilitation. (39) This did not occur in the group receiving TTDN on every breath and is possibly related to the intermittent pattern of diaphragm contractions being more effective at mobilizing mucus plugs in the same way as a cough-assist procedure. (40) While lung-protective ventilation over 50 hours did not have a serious impact on PaO2/FiO2 in this healthy lung model, TTDN on every breath maintained an “open lung”, leading to more alveoli participating in gas exchange, thereby leading to better PaO2/FiO2 over time. This was likely not pronounced due to the significant physiological reserves in a healthy lung.

There was a systematic reduction in the transpulmonary plateau pressure readings in the MV+TTDN100% group over time. This pattern is echoed in both the dynamic and static compliance measurements, suggesting that TTDN on every breath changes compliance in ways other than recruitment of collapsed alveoli, as it is evident at the start of the experiment. Pellegrini et al. have postulated that the diaphragm acts like a “brake” during expiration in spontaneous breathing by
maintaining diaphragm tonicity even after a phasic contraction. They describe that a decrease in end-expiratory lung volume is associated with an electromechanical coupling that slows down the decrease of the phasic electrical diaphragm activity during expiration. They also demonstrated a residue of inspiratory electrical diaphragm activity that lasts for some time during inspiration. Lower end-expiratory lung volumes resulted in an increased residual diaphragm electrical activity for longer periods during expiration. Perhaps the changes in transpulmonary pressure and dynamic compliance seen in the MV+TTDN100% group are due to reinstating this diaphragm tonicity in expiration that reduces the transmission of abdominal pressure to the lungs. TTDN on every second breath does not have the same effects on transpulmonary plateau pressures and dynamic compliance, suggesting that the dose of diaphragm stimulation may be important in this phenomenon. Due to limitations in equipment acquisition, we have transdiaphragmatic pressure measurements for the MV+TTDN100% group only, and thus cannot explore this theory in this data set.

Uncontrolled changes in tidal volume, pendelluft, increased lung perfusion and patient-ventilator asynchrony may result from uncontrolled spontaneous patient efforts while on a controlled mode of mechanical ventilation and are hypothesized to have an adverse effect on lung injury. TTDN may be seen to mimic these efforts as the diaphragm is contracting during a ventilator-delivered breath. However, a TTDN contraction is completely synchronous as it follows the ventilator during inspiration and the strength of the contraction is adjusted to reach a targeted PTP reduction. This study demonstrated no evidence of increased injury due to TTDN as lung injury scores were the same for all groups and inflammatory markers were not significantly different for any group. Extravascular lung water was also not significantly different, therefore TTDN did not cause an adverse transcapillary pressure gradient. This is relevant as strong respiratory efforts may lead to an increase in extravascular
lungs such as is the case with negative-pressure pulmonary edema. Variation in tidal volume was prevented by the use of volume-controlled breaths and there was no observed ventilator asynchrony or reverse triggering. The lack of ventilator asynchrony and absence of increased signs of lung injury in the TTDN groups support that TTDN does not increase the risk of lung injury and that it is reasonable to investigate the potential benefits of TTDN in patients with acute lung injury.

Our study has certain limitations, as it uses a preclinical model with healthy lungs and only a small number of subjects. We recognize that many ICU patients do not fit this model, but the patients who require extended sedation and mechanical ventilation, and are the most at risk for VIDD, do fit this model, and we have previously established that TTDN can offer protection from VIDD in healthy pigs. (29) The goal of this study was to investigate the effects that TTDN can have on ventilator-induced lung injury, the results of which may apply to heavily sedated, critically ill patients receiving controlled mechanical ventilation. Whether the results are the same in human ICU subjects who are not healthy remains to be seen. This study was limited in its ability to measure transpulmonary driving pressure and transdiaphragmatic pressure as we did not obtain a full data set due to late equipment acquisition. While pressure and compliance data are encouraging, more transpulmonary and transdiaphragmatic pressure data are needed to be conclusive. Critically ill patients that are affected by VILI are typically ventilated for more than 50 hours, however a longer duration of this experiment would have been technically difficult. The ability to do caudal lobe bronchial washings was inhibited by the length of bronchosopes available, thus we could not fully investigate the level of lung inflammation and possible injury. Future investigation would benefit from using an injured-lung model to adequately evaluate the effect of TTDN on VILI in a nonhomogeneous, injured lung, to better reflect commonly encountered clinical scenarios. While the MV+TTDN100% group subjects were heavier than the other groups, their bodies were
proportionally larger and not just heavier, as the length-to-weight ratio was the same in all groups. We normalized tidal volume to 8 ml/kg.

Not only is TTDN beneficial but the manner in which it is delivered provides a specific benefit beyond simply a dose-dependent improvement. The MV+TTDN50% group received neurostimulated diaphragm contractions on every second breath, creating an environment where alveoli were constantly subjected to fluctuating stress and strain between active and passive breaths. The distribution of tidal volume in both the active and passive breaths in this group suggests that a single diaphragm contraction after a passive breath does not fully restore physiological tidal distribution. This, along with variable stress, may potentiate the positive effects seen from TTDN provided with every breath. Our previous work demonstrated a clear benefit to the diaphragm when TTDN was offered every second breath and this study shows that the benefit to the lungs is greater when TTDN is provided on every breath. Work is underway to evaluate the effect on diaphragm atrophy in this group. This will help to elucidate whether TTDN on every breath is the best candidate for a lung- and diaphragm-protective ventilation protocol, informing future experimental directions.

This study is important with findings that will inform the application of a promising new therapeutic modality. Our findings show that TTDN on every breath preserved PaO₂/FiO₂ and required lower driving pressure than the reference-standard lung-protective mechanical ventilation strategy over 50 hours in healthy lungs. There was less end-expiratory lung volume loss and better alveolar homogeneity with TTDN on every breath. Furthermore, TTDN resulted in reduced atelectasis and the accompanying potential for lung injury. This offers insights into the impact that atelectrauma has on VILI in non-injured lungs and its associated alveolar inhomogeneity. This study supports the hypothesis that synchronous diaphragm contraction on every breath during controlled mechanical ventilation is beneficial and is no
more harmful than stand-alone lung-protective mechanical ventilation in healthy, non-injured lungs.
TTDN may offer a tool to provide both lung- and diaphragm-protective ventilation in humans.

Conclusion:
TTDN in synchrony with volume-controlled, lung-protective ventilation for 50 hours preserved PaO\textsubscript{2}/FiO\textsubscript{2}, required lower driving pressure and resulted in less end-expiratory lung volume loss, with improved alveolar homogeneity. This knowledge has important translational implications, and this new technology introduces an innovative and exciting way to protect the lungs of ventilated patients at the same time as it protects the diaphragm.

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References:

1. Steinberg J, Schiller HJ, Halter JM, Gatto LA, Dasilva M, Amato M, McCann UG and Nieman GF. Tidal volume increases do not affect alveolar mechanics in normal lung but cause alveolar overdistension and exacerbate alveolar instability after surfactant deactivation. *Crit.Care Med.* 30: 12: 2675-2683, 2002.

2. Mauri T, Guérin C and Hubmayr R. The ten pressures of the respiratory system during assisted breathing. *Intensive Care Med.* 43: 10: 1504-1506, 2017.

3. Roussos CS, Fukuchi Y, Macklem PT and Engel LA. Influence of diaphragmatic contraction on ventilation distribution in horizontal man. *J.Appl.Physiol.* 40: 3: 417-424, 1976.

4. Schiller HJ, McCann UG,2nd, Carney DE, Gatto LA, Steinberg JM and Nieman GF. Altered alveolar mechanics in the acutely injured lung. *Crit.Care Med.* 29: 5: 1049-1055, 2001.

5. Mead J, Takishima T and Leith D. Stress distribution in lungs: a model of pulmonary elasticity. *J.Appl.Physiol.* 28: 5: 596-608, 1970.

6. Tonetti T, Cressoni M, Collino F, Maiolo G, Rapetti F, Quintel M and Gattinoni L. Volutrauma, Atelectrauma, and Mechanical Power. *Crit.Care Med.* 45: 3: 2017.

7. Ma B and Bates JH. Modeling the complex dynamics of derecruitment in the lung. *Ann.Biomed.Eng.* 38: 11: 3466-3477, 2010.

8. Vlahakis NE, Schroeder MA, Limper AH and Hubmayr RD. Stretch induces cytokine release by alveolar epithelial cells in vitro. *Am.J.Physiol.* 277: 1: 167, 1999.

9. Meessen NE, van der Grinten, C. P., Luijendijk SC and Folgering HT. Continuous negative airway pressure increases tonic activity in diaphragm and intercostal muscles in humans. *J.Appl.Physiol.(1985)* 77: 3: 1256-1262, 1994.

10. Reber A, Nylund U and Hedenstierna G. Position and shape of the diaphragm: implications for atelectasis formation. 53: 11: 1054-1061, 1998.
11. Muller N, Volgyesi G, Becker L, Bryan MH and Bryan AC. Diaphragmatic muscle tone. *J.Appl.Physiol.* 47: 2: 279-284, 1979.

12. Tojo K, Nagamine Y, Yazawa T, Mihara T, Baba Y, Ota S, Goto T and Kurahashi K. Atelectasis causes alveolar hypoxia-induced inflammation during uneven mechanical ventilation in rats. 3: 18, 2015.

13. Mead J, Takishima T and Leith D. Stress distribution in lungs: a model of pulmonary elasticity. *J.Appl.Physiol.* 28: 5: 596-608, 1970.

14. Umbrello, M., Chiumello, D. Interpretation of the transpulmonary pressure in the critically ill patient. 6: 19: 2018.

15. Neto AS, Hemmes SN, Barbas CS, Beiderlinden M, Fernandez-Bustamante A, Futier E, Gajic O, El-Tahan MR, Ghamdi AA, Günay E, Jaber S, Kokulu S, Koizian A, Licker M, Lin WQ, Maslow AD, Memtsoudis SG, Reis Miranda D, Moine P, Ng T, Paparella D, Ranieri VM, Scavonetto F, Schilling T, Selmo G, Severgnini P, Sprung J, Sundar S, Talmor D, Treschan T, Unzueta C, Weingarten TN, Wolthuis EK, Wrigge H, Amato MB, Costa EL, de Abreu MG, Pelosi P, Schultz MJ and PROVE Network Investigators. Association between driving pressure and development of postoperative pulmonary complications in patients undergoing mechanical ventilation for general anaesthesia: a meta-analysis of individual patient data. *Lancet Respir.Med.* 4: 4: 272-280, 2016.

16. Kacmarek RM, Villar J, Sulemanji D, Montiel R, Ferrando C, Blanco J, Koh Y, Soler JA, Martínez D, Hernández M, Tucci M, Borges JB, Lubillo S, Santos A, Araujo JB, Amato MBP, Suárez-Sipmann F and the Open Lung, Approach Network. Open Lung Approach for the Acute Respiratory Distress Syndrome: A Pilot, Randomized Controlled Trial*. *Crit.Care Med.* 44: 1: 2016.

17. Cinnella G, Grasso S, Raimondo P, D’Antini D, Mirabella L, Rauseo M and Dambrosio M. Physiological Effects of the Open Lung Approach in Patients with Early, Mild, Diffuse Acute Respiratory Distress Syndrome: An Electrical Impedance Tomography Study. 123: 5: 1113-1121, 2015.
18. Amato MBP, Meade MO, Slutsky AS, Brochard L, Costa ELV, Schoenfeld DA, Stewart TE, Briel M, Talmor D, Mercat A, Richard JM, Carvalho CRR and Brower RG. Driving Pressure and Survival in the Acute Respiratory Distress Syndrome. *N. Engl. J. Med.* 372: 8: 747-755, 2015.

19. Acute Respiratory Distress Syndrome Network, Brower RG, Matthay MA, Morris A, Schoenfeld D, Thompson BT and Wheeler A. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. *N. Engl. J. Med.* 342: 18: 1301-1308, 2000.

20. Pohlman MC, McCallister KE, Schweickert WD, Pohlman AS, Nigos CP, Krishnan JA, Charbeneau JT, Gehlbach BK, Kress JP and Hall JB. Excessive tidal volume from breath stacking during lung-protective ventilation for acute lung injury. *Crit. Care Med.* 36: 11: 3019-3023, 2008.

21. Dres M, Demoule A. Beyond Ventilator-induced Diaphragm Dysfunction: New Evidence for Critical Illness-associated Diaphragm Weakness. 131: 3: 462-463, 2019.

22. Vivier EMD, Roussey AMD, Doroszewski F, Rosselli SMD, Pommier CMD, Carteaux GMD, Ph.D., Mekontso Dessap, Armand M. D. and Ph.D. Atrophy of Diaphragm and Pectoral Muscles in Critically Ill Patients. 131: 3: 569-579, 2019.

23. Jaber S, Jung B, Matecki S and Petrof BJ. Clinical review: ventilator-induced diaphragmatic dysfunction--human studies confirm animal model findings! *Crit. Care* 15: 2: 206, 2011.

24. Yoshida T, Fujino Y, Amato MBP and Kavanagh BP. Fifty Years of Research in ARDS. Spontaneous Breathing during Mechanical Ventilation. Risks, Mechanisms, and Management. *Am. J. Respir. Crit. Care Med.* 195: 8: 985-992, 2017.

25. Goligher EC, Dres M, Patel BK, Sahetya SK, Beitzler JR, Telias I, Yoshida T, Vaporidi K, Grieco DL, Schepens T, Grasselli G, Spadaro S, Dianti J, Amato M, Bellani G, Demoule A, Fan E, Ferguson ND, Georgopoulos D, Guérin C, Khemani RG, Laghi F, Mercat A, Mojoli F, Ottenheijm CAC, Jaber S, Heunks
L, Mancebo J, Mauri T, Pesenti A and Brochard L. Lung- and Diaphragm-Protective Ventilation. Am. J. Respir. Crit. Care Med. 202: 7: 950-961, 2020.

26. Hedenstierna G, Tokics L, Lundquist H, Andersson T, Strandberg A and Brismar B. Phrenic nerve stimulation during halothane anesthesia. Effects of atelectasis. 80: 4: 751-760, 1994.

27. Hirschfeld S, Exner G, Luukkaala T and Baer GA. Mechanical ventilation or phrenic nerve stimulation for treatment of spinal cord injury-induced respiratory insufficiency. Spinal Cord 46: 11: 738-742, 2008.

28. Reynolds S, Ebner A, Meffen T, Thakkar V, Gani M, Taylor K, Clark L, Sadarangani G, Meyyappan R, Sandoval R, Rohrs E and Hoffer JA. Diaphragm Activation in Ventilated Patients Using a Novel Transvenous Phrenic Nerve Pacing Catheter. Crit. Care Med. 45: 7: e691-e694, 2017.

29. Reynolds SC, Meyyappan R, Thakkar V, Tran BD, Nolette MA, Sadarangani G, Sandoval RA, Bruulsema L, Hannigan B, Li JW, Rohrs E, Zurba J and Hoffer JA. Mitigation of Ventilator-induced Diaphragm Atrophy by Transvenous Phrenic Nerve Stimulation. Am. J. Respir. Crit. Care Med. 195: 3: 339-348, 2017.

30. HOFFER JA, TRAN BD, NASH JE, EVANS DG and THAKKAR VS. APPARATUS AND METHODS FOR ASSISTED BREATHING BY TRANSVASCULAR NERVE STIMULATION. 2018.

31. Haga HA, Tevik A and Moerch H. Bispectral index as an indicator of anaesthetic depth during isoflurane anaesthesia in the pig. 26: 1: 3-7, 1999.

32. Hsia CC, Hyde DM, Ochs M, Weibel ER and ATS/ERS Joint Task Force on Quantitative Assessment of Lung Structure. An official research policy statement of the American Thoracic Society/European Respiratory Society: standards for quantitative assessment of lung structure. Am. J. Respir. Crit. Care Med. 181: 4: 394-418, 2010.
33. Grasso F, Engelberts D, Helm E, Frndova H, Jarvis S, Talakoub O, McKerlie C, Babyn P, Post M and Kavanagh BP. Negative-pressure ventilation: better oxygenation and less lung injury. *Am.J.Respir.Crit.Care Med.* 177: 4: 412-418, 2008.

34. Gattinoni L, Carlesso E, Cadringher P, Valenza F, Vagginelli F and Chiumello D. Physical and biological triggers of ventilator-induced lung injury and its prevention. 22: Supplement 47: 15s-25s, 2003.

35. Hernandez LA, Coker PJ, May S, Thompson AL and Parker JC. Mechanical ventilation increases microvascular permeability in oleic acid-injured lungs. *J.Appl.Physiol.(1985)* 69: 6: 2057-2061, 1990.

36. Feiner JR and Weiskopf RB. Evaluating Pulmonary Function: An Assessment of PaO2/FIO2. *Crit.Care Med.* 45: 1: e40-e48, 2017.

37. Radermacher P, Maggiore SM and Mercat A. Fifty Years of Research in ARDS. Gas Exchange in Acute Respiratory Distress Syndrome. *Am.J.Respir.Crit.Care Med.* 196: 8: 964-984, 2017.

38. Szakmany T, Heigl P and Molnar Z. Correlation between extravascular lung water and oxygenation in ALI/ARDS patients in septic shock: possible role in the development of atelectasis? *Anaesth.Intensive Care* 32: 2: 196-201, 2004.

39. Ataya A and Silverman EP. Clearance of Large Pulmonary Mucus Plug Allowing Liberation from Mechanical Ventilation Facilitated by Novel Transvenous Phrenic Nerve Pacing Catheter. In: *A47. CRITICAL CARE CASE REPORTS: MECHANICAL VENTILATION FROM NIV TO ECMO* Anonymous American Thoracic Society, 2019, p. A1750.

40. Auger C, Hernando V and Galmiche H. Use of Mechanical Insufflation-Exsufflation Devices for Airway Clearance in Subjects With Neuromuscular Disease. *Respir.Care* 62: 2: 236-245, 2017.

41. Pellegrini M, Hedenstierna G, Roneus A, Segelsjö M, Larsson A and Perchiazi G. The Diaphragm Acts as a Brake during Expiration to Prevent Lung Collapse. *Am.J.Respir.Crit.Care Med.* 195: 12: 1608-1616, 2017.
42. Li H, Chen L and Brochard L. Protecting lungs during spontaneous breathing: what can we do? 9: 9: 2777-2781, 2017.

43. Yoshida T, Uchiyama A, Matsuura N, Mashimo T and Fujino Y. Spontaneous breathing during lung-protective ventilation in an experimental acute lung injury model: high transpulmonary pressure associated with strong spontaneous breathing effort may worsen lung injury. Crit. Care Med. 40: 5: 1578-1585, 2012.

44. Yoshida T, Grieco DL, Brochard L and Fujino Y. Patient self-inflicted lung injury and positive end-expiratory pressure for safe spontaneous breathing. Curr. Opin. Crit. Care 2019.

45. Kallet RH, Alonso JA, Luce JM and Matthay MA. Exacerbation of acute pulmonary edema during assisted mechanical ventilation using a low-tidal volume, lung-protective ventilator strategy. 116: 6: 1826-1832, 1999.

46. Yoshida T, Amato Marcelo, Gricco Domenico, Chen Lu, Lima Cristhiano, Roldan Rollin, Morais Caio, Gomes Susimeire, Costa Eduardo, Cardoso Paulo, Charbonney Emmanuel, Richard Jean-Chrisophe, Brochard Laurent and Kavanaugh Brian. Esophageal Manometry and Regional Transpulmonary Pressure in Lung Injury. ePUB ahead of print: 2018.

47. Akoumianaki E, Maggiore SM, Valenza F, Bellani G, Jubran A, Loring SH, Pelosi P, Talmor D, Grasso S, Chiumello D, Guerin C, Patroniti N, Ranieri VM, Gattinoni L, Nava S, Terragni PP, Pesenti A, Tobin M, Mancebo J, Brochard L and PLUG Working Group (Acute Respiratory Failure Section of the European Society of Intensive Care Medicine). The application of esophageal pressure measurement in patients with respiratory failure. Am. J. Respir. Crit. Care Med. 189: 5: 520-531, 2014.
**Figure Legend:**

**Figure 1:**
- A: Distribution of tidal volume measured by EIT and how it differs in the dorsal and ventral lung regions in the four experimental groups.
- B: Example of an EIT image showing the redistribution tidal volume from the ventral region (orange=loss) into the dorsal region (blue) from a passive breath to an active breath where the diaphragm was contracted.

**Figure 2:**
- A: Change in end-expiratory lung volume over 50 hours, as measured by EIT, in all three ventilated groups.
- B: Example EIT images showing the gain (blue) or loss (orange) of end-expiratory lung volume in animals from each of the ventilated groups.

**Figure 3:**
- Examples of H&E-stained lung tissue at five sampled locations showing the different patterns of alveolar expansion between the groups at each sample site.

**Figure 4:**
- A: Plateau pressure, transpulmonary plateau pressure and driving pressure over 50 hours of mechanical ventilation.
- B: Dynamic and static compliance over 50 hours of mechanical ventilation.

**Figure 5:**
- $\text{PaO}_2/\text{FiO}_2$ Ratio Over 50 Hours of Mechanical Ventilation.
Example of the change in distribution of tidal volume as measured with EIT. Passive breath compared to neurostimulated (active) breath in the same subject in the MV+TTDN 50% group. This shows the gain of tidal volume distribution in the dorsal lung region (blue) and the loss in the ventral lung region (orange).
Change in Global End-Expiratory Lung Volume as Measured by EIT, over 50 hours of Mechanical Ventilation

- MV
- MV + TTDN 50%
- MV + TTDN 100%

p = 0.0034
Length of Alveolar Expansion at Time of Tissue Fixation (Chord Length)

Group and sample location

MV 5
MV 4
MV 3
MV 2
MV 1
MV+TTDN 50% 5
MV+TTDN 50% 4
MV+TTDN 50% 3
MV+TTDN 50% 2
MV+TTDN 50% 1
MV+TTDN 100% 5
MV+TTDN 100% 4
MV+TTDN 100% 3
MV+TTDN 100% 2
MV+TTDN 100% 1
NV 5
NV 4
NV 3
NV 2
NV 1

p<0.0001
p<0.0001
p<0.0001
ns
PaO$_2$/FiO$_2$ Ratio Over 50 Hours

- **MV**
- **MV+TTDN50%**
- **MV+TTDN100%**

Slope $p=0.0044$
Table 1: Group weight, drug dosage and fluid balance comparisons

| Group Comparisons | No of Subjects | Parameter | Weight | Length/Weight | Fluid Balance | Midazolam | Fentanyl | Propofol | Ketamine |
|-------------------|----------------|-----------|--------|---------------|--------------|-----------|----------|----------|----------|
|                   |                | Units     | kg     | cm/kg         | ml/kg/hour   | mg/hour   | mcg/hour | mcg/kg/min | mcg      |
| NV                | 6              | Median    | 54.00  | -             | -             | -         | -        | -         | -        |
|                   |                | IQR       | 52.25-56.00 | -            | -             | -         | -        | -         | -        |
|                   |                | 95% CI    | 50.00-56.00 | -            | -             | -         | -        | -         | -        |
| MV                | 10             | Median    | 57.25  | 1.94          | 0.93          | 52.45     | 481.30   | 108.80    | 1356.00  |
|                   |                | IQR       | 53.48-64.13 | 1.93-1.98   | 0.40-1.41    | 43.48-80.35 | 393.00-803.50 | 66.45-118.80 | 856.40-3425.00 |
|                   |                | 95% CI    | 47.70-64.50 | 1.93-1.99   | 0.18-1.67    | 40.00-80.36 | 372.10-803.60 | 59.69-126.10 | 785.00-4700.00 |
| MV+TTDN 50%       | 8              | Median    | 55.20  | 1.97          | 1.10          | 63.84     | 638.40   | 86.44     | 1236.00  |
|                   |                | IQR       | 52.23-60.98 | 1.95-1.99   | 0.59-1.39    | 35.91-76.79 | 359.10-767.90 | 66.10-93.87 | 465.10-5875.00 |
|                   |                | 95% CI    | 48.50-63.00 | 1.94-1.99   | 0.47-2.13    | 27.00-80.36 | 270.00-803.60 | 53.80-97.41 | 142.90-8560.00 |
| MV+TTDN 100%      | 7              | Median    | 69.00  | 1.92          | 0.43          | 57.60     | 358.40   | 67.70     | 1020.00  |
|                   |                | IQR       | 65.70-70.30 | 1.91-1.93   | 0.24-0.62    | 50.52-71.04 | 330.40-422.40 | 62.40-83.70 | 820.00-1271.00 |
|                   |                | 95% CI    | 60.80-71.70 | 1.91-1.94   | 0.21-0.89    | 49.56-75.70 | 302.40-473.20 | 61.40-90.00 | 810.00-1470.00 |

Kruskal-Wallis Test

| Dunn's Multiple Comparisons Test |
|----------------------------------|
| NV vs. MV - - - - - - ns         |
| NV vs. MV+TTDN50% - - - - - - ns |
| NV vs. MV+TTDN100% 0.0032 - - - - |
| MV vs. MV+TTDN50% - - - - - - ns |
| MV vs. MV+TTDN100% - - - - - - ns |
| MV+TTDN50% vs. MV+TTDN100% 0.0059 - - - - |

"-" = not applicable, "ns" = not significant
Table 2: Ventilation comparisons for the groups that received mechanical ventilation

| Ventilation and Blood Gas Results | Parameter | pH | PaO2 | PaCO2 | RR | FiO2 | Tidal Volume/kg |
|----------------------------------|-----------|----|------|-------|----|------|-----------------|
|                                  | Units     | -  | mmHg | mmHg  | -  | -    | ml/kg           |
| MV                               | Median    | 7.43 | 128.00 | 46.00 | 21.00 | 0.30 | 8.00 |
| IQR                              | 7.42-7.44 | 121-144 | 44-48 | 21-22 | 0.30-0.30 | 7.75-8.32 |
| 95% CI                           | 7.42-7.44 | 117-144 | 44-48 | 21-22 | 0.30-0.30 | 7.68-8.76 |
| MV+TTDN 50%                      | Median    | 7.45 | 121.00 | 45.00 | 22.00 | 0.30 | 7.99 |
| IQR                              | 7.45-7.48 | 106-139 | 42-46 | 21-22 | 0.30-0.30 | 7.89-8.06 |
| 95% CI                           | 7.45-7.49 | 104-143 | 42-46 | 22-22 | 0.30-0.30 | 7.61-8.07 |
| MV+TTDN 100%                     | Median    | 7.46 | 135.00 | 45.00 | 19.00 | 0.30 | 7.97 |
| IQR                              | 7.44-7.47 | 130-146 | 43-46 | 19-20 | 0.30-0.30 | 7.90-8.09 |
| 95% CI                           | 7.44-7.48 | 130-147 | 43-46 | 20-20 | 0.30-0.30 | 7.83-8.09 |
| Mixed-effects Analysis (p value)  | Time      | ns  | <0.0001 | 0.0245 | ns  | ns   | ns             |
|                                  | Group     | ns  | ns   | ns   | ns  | ns   | ns             |
|                                  | Interaction | ns  | ns   | ns   | ns  | ns   | ns             |

"-" = not applicable, "ns" = not significant
| Tidal Volume Distribution | Parameter          | Dorsal Tidal Volume | Ventral Tidal Volume |
|--------------------------|--------------------|---------------------|----------------------|
|                          | Units              | %                   | %                    |
| NV                       | Median             | 61                  | 39                   |
|                          | IQR                | 49-70               | 30-51                |
|                          | 95% CI             | 48-73               | 27-52                |
| MV                       | Median             | 37                  | 63                   |
|                          | IQR                | 32-45               | 55-68                |
|                          | 95% CI             | 15-46               | 54-85                |
| MV+TTDN 50% (Passive)    | Median             | 47                  | 53                   |
|                          | IQR                | 45-53               | 47-55                |
|                          | 95% CI             | 44-54               | 45-56                |
| MV+TTDN 50% (Active)     | Median             | 53                  | 47                   |
|                          | IQR                | 50-57               | 44-51                |
|                          | 95% CI             | 48-68               | 32-52                |
| MV+TTDN 100%             | Median             | 59                  | 41                   |
|                          | IQR                | 57-70               | 30-43                |
|                          | 95% CI             | 53-77               | 23-48                |

Kruskal-Wallis Test (p value) <0.0001 <0.0001

Dunn’s Multiple Comparisons Test (p value)

| Test                          | NV vs. MV | NV vs. MV+TTDN50% Passive | NV vs. MV+TTDN50% Active | NV vs. MV+TTDN100% | MV vs. MV+TTDN50% Passive | MV vs. MV+TTDN50% Active | MV vs. MV+TTDN100% | MV+TTDN50% Passive vs. MV+TTDN100% | MV+TTDN50% Active vs. MV+TTDN100% |
|-------------------------------|-----------|---------------------------|-------------------------|-------------------|---------------------------|-------------------------|-------------------|--------------------------------------|-----------------------------------|
| NV vs. MV                     | 0.0029    |                           |                         |                   |                           |                         |                   |                                      |                                   |
| NV vs. MV+TTDN50% Passive     | ns        |                           |                         |                   |                           |                         |                   |                                      |                                   |
| NV vs. MV+TTDN50% Active      | ns        |                           |                         |                   |                           |                         |                   |                                      |                                   |
| NV vs. MV+TTDN100%            | ns        |                           |                         |                   |                           |                         |                   |                                      |                                   |
| MV vs. MV+TTDN50% Passive     | ns        |                           |                         |                   |                           |                         |                   |                                      |                                   |
| MV vs. MV+TTDN50% Active      | 0.0182    |                           |                         |                   |                           |                         |                   |                                      |                                   |
| MV vs. MV+TTDN100%            | 0.0029    |                           |                         |                   |                           |                         |                   | 0.0001                               |                                   |
| MV+TTDN50% Passive vs. MV+TTDN100% | ns     |                           |                         |                   |                           |                         |                   |                                      |                                   |
| MV+TTDN50% Active vs. MV+TTDN100% | ns     |                           |                         |                   |                           |                         |                   |                                      |                                   |

*"* = not applicable, "ns" = not significant
Table 4: Net loss of end-expiratory lung volume as measured by EIT, at the end of 50 hours of mechanical ventilation.

| Net Loss of End-expiratory Lung Volume | Parameter | dEELV Global |
|----------------------------------------|-----------|--------------|
| MV                                     | Median    | 1,203        |
|                                        | IQR       | 985-1554     |
|                                        | 95% CI    | 984-1638     |
| MV+TTDN 50%                            | Median    | 1,038        |
|                                        | IQR       | 845-1259     |
|                                        | 95% CI    | 461-1423     |
| MV+TTDN 100%                           | Median    | 677          |
|                                        | IQR       | 398-804      |
|                                        | 95% CI    | 372-848      |

Kruskal-Wallis Test (p value): 0.0034

Dunn’s Multiple Comparisons Test (p value):
- MV vs. MV+TTDN50%: ns
- MV vs. MV+TTDN100%: 0.0028
- MV+TTDN50% vs. MV+TTDN100%: ns

Mixed-effects Analysis (p value):
- Time: <0.0001
- Group: 0.0043
- Interaction: 0.0034

“-“ = not applicable, “ns” = not significant
Table 5: Alveolar chord length for each tissue sample location.

| Alveolar Chord Length Measurement | Parameter          | Sample Location 5 | Sample Location 4 | Sample Location 3 | Sample Location 2 | Sample Location 1 |
|----------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
|                                  | Units             | µm                | µm                | µm                | µm                | µm                |
| NV                               | Median            | 83.99             | 82.71             | 86.54             | 80.28             | 84.66             |
|                                  | IQR               | 59.10-117.90      | 56.67-120.30      | 59.95-123.60      | 53.64-118.00      | 57.67-119.00      |
|                                  | 95% CI            | 81.04-86.72       | 80.18-85.79       | 83.51-89.82       | 76.36-84.78       | 81.44-87.01       |
| MV                               | Median            | 109.6             | 108.4             | 93.51             | 78.95             | 57.85             |
|                                  | IQR               | 77.69-163.60      | 76.68-166.80      | 63.90-142.40      | 55.84-122.10      | 46.41-76.02       |
|                                  | 95% CI            | 104.90-113.70     | 105.30-114.20     | 89.47-96.19       | 77.03-81.36       | 53.82-60.54       |
| MV+TTDN 50%                      | Median            | 92.04             | 97.67             | 73.7              | 76.73             | 73.81             |
|                                  | IQR               | 64.63-138.90      | 64.45-150.10      | 52.38-112.90      | 51.07-109.00      | 50.37-105.60      |
|                                  | 95% CI            | 88.57-95.65       | 94.07-101.70      | 71.45-76.37       | 74.91-79.39       | 69.90-77.19       |
| MV+TTDN 100%                     | Median            | 85.94             | 88.12             | 80.72             | 76.68             | 74.67             |
|                                  | IQR               | 64.58-118.10      | 67.60-123.80      | 58.53-117.00      | 59.20-108.3       | 55.84-104.30      |
|                                  | 95% CI            | 84.08-88.12       | 84.78-90.81       | 82.74-78.70       | 74.67-78.71       | 71.98-78.03       |

| Dunn's Multiple Comparisons Test (p value) | <0.0001 | <0.0001 | <0.0001 | 0.0045 | <0.0001 |

| Kruskal-Wallis Test (p value) | <0.0001 | <0.0001 | <0.0001 | ns     | <0.0001 |

| NV vs. MV                      | <0.0001 | <0.0001 | <0.0001 | ns     | <0.0001 |
| NV vs. MV+TTDN50%              | <0.0001 | <0.0001 | <0.0001 | ns     | <0.0001 |
| NV vs. MV+TTDN100%             | 0.0133  | <0.0001 | <0.0001 | ns     | ns     |
| MV vs. MV+TTDN50%              | <0.0001 | <0.0001 | <0.0001 | 0.0095 | <0.0001 |
| MV vs. MV+TTDN100%             | <0.0001 | <0.0001 | <0.0001 | ns     | <0.0001 |
| MV+TTDN50% vs. MV+TTDN100%     | 0.0082  | 0.0023  | <0.0001 | 0.0055 | ns     |

"-" = not applicable, "ns" = not significant
Table 6: Esophageal pressures, ventilation pressures, compliance and PaO2/FiO2 Ratio at baseline and at the end of 50 hours of mechanical ventilation.

|                         | Pressures, Compliance and P/F Ratio |Esophageal Pressure cmH2O| Plateau Pressure cmH2O| Driving Pressure cmH2O| Transpulmonary Plateau Pressure cmH2O| Dynamic Compliance ml/cmH2O| Static Compliance ml/cmH2O| PaO2/FiO2 Ratio |
|-------------------------|-------------------------------------|--------------------------|-----------------------|------------------------|--------------------------------------|-----------------------------|---------------------------|-------------------|
|                         |                                     | Med | 95% CI | Med | 95% CI | Med | 95% CI | Med | 95% CI | Med | 95% CI | Med | 95% CI | Med | 95% CI | Med | 95% CI | Med | 95% CI | Med | 95% CI | Med | 95% CI |
| MV                      | Baseline                            | 9   | 14     | 15   | 16     | 14-17 | 11-12 | 9-12 | 9-12 | 9-12 | 7-11 | 9-12 | 7-11 | 39  | 41     | 41   | 44   | 48   | 52   | 52   | 52   | 455  | 455  |
|                         | Study End                           | 7   | 10     | 10   | 19     | 21-22 | 14-17 | 14   | 16   | 14   | 16-17 | 17   | 16-17 | 48  | 53     | 53   | 30   | 36   | 38   | 38   | 403  | 444  | 407  |
| MV+ TTDN 50%            | Baseline                            | 9   | 13     | 15   | 16     | 15-18 | 15-20 | 10   | 13   | 10   | 13-15 | 14-17 | 14-17 | 39  | 35     | 34   | 32   | 32   | 26   | 26   | 436  | 435  | 433  |
|                         | Study End                           | 7   | 10     | 4    | 10     | 20-22 | 15-17 | 14-17 | 14-17 | 12-17 | 14-17 | 14-17 | 30  | 24     | 23   | 35   | 35   | 26   | 26   | 462  | 498  | 547  |
| MV+ TTDN 100%           | Baseline                            | 10  | 12     | 13   | 16     | 17-19 | 17-19 | 6    | 5.7  | 6    | 5-7   | 5-8  | 6     | 5.7 | 6     | 5-7  | 6     | 5-7  | 34   | 41   | 41   | 37-41 | 336-415 | 497  |
|                         | Study End                           | 10  | 12     | 13   | 18     | 19-19 | 13-16 | 7    | 6-13 | 5-15 | 5-13  | 5-15 | 5-15 | 87  | 79     | 81   | 64   | 64   | 55   | 55   | 433  | 470  | 540  |

Kruskal-Wallis Test (p value)  

MV vs.  
MV+  
TTDN 50%  
TTDN 100%  

Mixed-Effects Analysis (p value)  

TMK vs. MTK 50%  
TTDN 50%  
TTDN 100%  

Dunn's Multiple Comparisons Test (p value)  

<sup>*</sup> = not applicable, <sup>ns</sup> = not significant