High Pleural Pressure Prevents Alveolar Overdistension and Hemodynamic Collapse in ARDS with Class III Obesity

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Online Data Supplement

SUPPLEMENTARY Appendix

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The swine study was conducted at Faculdade de Medicina da Universidade de São Paulo, Brazil and at Massachusetts General Hospital, Thier Research Building, Boston, US, approved by the respective institution's Ethical Committee for Experimental Studies.

The clinical study was approved by the Partners HealthCare Institutional Review Board (Protocol #2015P001515) and a written consent was obtained from the subjects' surrogate. The study is registered at Clinical Trial: https://clinicaltrials.gov/ct2/show/NCT02503241 and NCT #02503241

Appendix 1. Clinical Study

Screening and consenting process

The Medical and Surgical intensive care units at Massachusetts General Hospital (Boston, USA) were screened daily for potential subjects.

Inclusion criteria:

- 18 years or older
- BMI ≥ 35 kg/m^2
- Matching the Berlin definition for the diagnosis of ARDS (1)
- Waist circumference > 88 cm (for women)
- Waist circumference > 102 cm (for men)
- Presence of an arterial line
- Presence of a central venous catheter

Exclusion criteria:

- Known presence of esophageal varices
- Recent esophageal trauma or surgery
- Severe thrombocytopenia (Platelets count ≤ 5,000/mm^3)
- Severe coagulopathy (INR ≥ 4)
- Presence or history of pneumothorax
- Pregnancy
- Patients with poor oxygenation index (PaO_2/F_IO_2< 100 mmHg with at least
- 10 cmH_2O of PEEP)
- Pacemaker and/or internal cardiac defibrillator
- Hemodynamic parameters: systolic blood pressure <100 mmHg and >180

mmHg, or if systolic blood pressure is between 100-180 mmHg on high dose of IV continuous infusion of norepinephrine (>20 µg per minute), or dobutamine (>10 µg per minute), or dopamine (>10 µg per kg per minute), or epinephrine (>10 µg per minute).

Once an eligible subject was identified, an attending physician approached the patient surrogate (court-appointed guardian, healthcare proxy/attorney, spouse, adult child or other close family member) and mentioned this clinical study. Then, if the patient surrogate was willing to participate, an investigator went through all the protocol details and answered questions regarding the research study. Then, written informed consent was obtained from the patient surrogate.

Preparation and procedures

Subjects were placed in the supine position, with the head of the bed at 30 degrees. They were deeply sedated (Richmond Agitation and Sedation Score between -4 and -5) and paralyzed (0.2 mg/kg of cisatracurium besylate - Nimbex, AbbVie). If, throughout the study protocol, any sign of breathing effort was noticed, an additional dose of paralytic was administered (0.05mg/kg ideal body weight -IBW). Subject were ventilated on VCV with Vt of 6 ml/kg of IBW. The F_IO_2 was 100% throughout the study protocol. A nasogastric catheter equipped with an air-filled balloon (Avea GS SmarthCath, Carefusion) was placed in the lower third of the esophagus. The esophageal balloon inflation volume was checked, and the position was validated by an occlusion test (2-4). As previously shown (5), esophageal pressure (P_{ES}) was used as a surrogate of pleural pressure (P_{PL}) in the middle of the most gravitationally dependent regions of the lungs.

Flow, airways pressure (P_{AW}) and P_{ES} were continuously recorded, including during end-inspiratory and end-expiratory pauses (zero flow). The tidal volume (Vt) was calculated as the integral of the expiratory flow-time waveform from a mean respiratory cycle (for each patient and at each study phase). The mean respiratory cycle was obtained by resampling and interpolating a group of cycles (5-10 cycles) using Pneumobench Analysis 9.0, developed with LabVIEW (National Instruments). The derived ventilation parameters were calculated as follow:

- Plateau pressure (Plateau P) = airway pressure in the end of an inspiratory pause
- PEEP = airway pressure in the end of an expiratory pause
- Driving pressure (DP)= Plateau pressure - PEEP
- Compliance respiratory system (C_{RS}) = Vt / DP
- End-expiratory P_{ES} = esophageal pressure in the end of an expiratory pause
- End-expiratory transpulmonary pressure ($P_L E$) = PEEP – Expiratory P_{ES} (6)

Electrical impedance tomography

Electrical Impedance Tomography (EIT) (Enlight 1800, Timpel) belt was positioned, after the measurement of thoracic circumference, between the 5^{th} to 6^{th} intercostal space and connected to the device (Figure E1, panel A).

EIT is a functional imaging method and is based on the impedance distribution within the chest. A harmless electrical current is injected, the potential difference is measured, and it is used to reconstruct two-dimensional images. Inside the chest, the lungs and blood content cyclic variations are the major contributors to changes in impedance (ΔZ).

The analysis of ΔZ can estimate aeration, distribution of ventilation, collapse/overdistension and ventilation/perfusion ratio (V/Q) (7). The generated ventilation and perfusion images were divided into three regions of interest (ROI): the anterior region, most non-dependent (ROI-1), the intermediate region (ROI-2) and the posterior region, the most dependent (ROI-3).

In this study, ΔZ was correlated to the variations in the absolute air content in ROI-1, ROI-2 and ROI-3 and divided by the driving pressure providing regional compliance values per each ROI (Table 2). Lung collapse was calculated for each electrical impedance tomography pixel by comparing pixel-compliance during the decremental PEEP titration. Each pixel-compliance was determined dividing tidal ΔZ by the variation in pressure during the respiratory cycle (Compliance $_{PIXEL}$ = ΔZ / ΔP). For a given pixel, if aeration increased and compliance improved, it meant collapse reversal compared to the measurement performed at the highest PEEP (8). The maximum compliance and aeration are depicted among all the PEEP steps and used as reference for the comparison.

The three ROIs also estimated local ventilation/perfusion ratios (V/Q) during the infusion of hypertonic saline solution (9, 10). Perfusion was measured as the change in electrical impedance in the thorax after a bolus of hypertonic saline solution (10 mL at 5%) during a 15 second apneic pause. Taking together ventilation and perfusion, a V/Q mapping was designed per each ROI and compared between the two PEEP levels (9) (Figure E1, panel A).

Regarding the variations in EELV, the volume at end of expiration was measured with EIT and from the PEEP=24 to PEEP=10 cmH_2O in patients with ARDS and obesity; and

for the patients with ARDS and without obesity, from PEEP =23 to PEEP=11 cmH_2O. The Variations in PEEP determined the changes in EELV, being “zero volume” the lowest PEEP and “maximum volume” the highest PEEP.

Cardiac and hemodynamic monitoring

TAPSE and S’ were measured by a portable ultrasound machine (Philips Ultrasound). The TTEs were performed by a board-certified critical care physician. TAPSE and S’ were used as indices of right heart systolic function. TAPSE was measured in a standard apical four-chamber view by placing the M-mode cursor at the tricuspid lateral annulus and measuring the longitudinal motion of the annulus at the peak of systole. S’ was measured in a standard apical four-chamber view using the tissue doppler mode, with the pulsed doppler sample volume placed at the tricuspid level of the right ventricular free wall (11).

Hemodynamic management

Fluids and vasoactive drugs administration were determined by the attending team without interference by the study team. During the first 4 hours after study completion, the amount of administered fluid, urinary output and vasoactive-inotropic score (VIS) were measured. In hospital follow-up was completed for every patient enrolled.

Vasoactive-inotropic score (VIS)

VIS was measured during this study protocol, this index provided comparable values of possible vasoactive agents administered per study phase. The formula used was:

dopamine dose (mcg/kg/min) + dobutamine dose (mcg/kg/min) + 100 x epinephrine dose (mcg/kg/min) + 10 x milrinone dose (mcg/kg/min) + 10,000 x vasopressin dose (U/kg/min) + 100 x norepinephrine (mcg/kg/min) + 10 x phenylephrine dose (mcg/kg/min) (12, 13). The weight used to calculate the VIS was 130% of the IBW.

Study protocol

Two steps were compared:

1) $Lung_{ARDSnet}$: The ventilator was set according to the ARDSnet recommendations and the PEEP guided by the lower PEEP/ higher F_IO_2 table (14, 15) and data was collected after 30 minutes (Figure E2, panel A).

2)$Lung_{RECRUITED}$: LRM was performed during PCV with delta pressure of 10 cmH_2O, RR of 20 bpm, PEEP was increased in a staircase fashion until a plateau pressure of 50 cmH_2O for 1 minute. After the LRM, we switched again the ventilator mode to VCV (5ml/kg of IBW) and dropped the PEEP 2 by 2 cmH_2O every 30 sec. The Optimal PEEP was determined by the PEEP value with the best C_{RS} plus 2 cmH_2O. Finally, a second LRM was performed and the selected Optimal PEEP was set.

Measurements were taken 30 minutes after optimal PEEP was set (Figure E2, panel A). During the first 4 hours after the completion of study procedures, the volume of fluid administered, urinary output, and the VIS (adjusted for subject weight) were measured. Subjects were followed for 28 days, and the following data were recorded: incidence of re-intubation, incidence of tracheostomy and pneumothorax, ventilator free days, intensive care unit and hospital length-of-stay (LOS), and 28-day mortality.

Patients with ARDS and without obesity

Between July 2013 and March 2014, at University of Sao Paulo, Sao Paulo, Brazil, five ARDS patient enrolled in a randomized clinical trial (16) were also monitored with EIT.

Statistical analysis of the clinical study

The primary aim of the clinical study was to assess the cardiovascular response to the transition from standard of care ventilation strategy ($Lung_{ARDSnet}$) to lung recruitment with best respiratory system compliance PEEP ($Lung_{RECRUITED}$) in patients with ARDS and obesity. Thus, we calculated the sample size with the goal of measuring significant lung recruitment after the $Lung_{RECRUITED}$ strategy was employed.

The sample size calculation was based on experimental data from our previous study in subjects without ARDS and with obesity (17). Assuming an average decrease in driving pressure of 2.1 cmH_2O after lung recruitment maneuver, a minimum of 11 subjects were necessary to detect a difference between $Lung_{ARDSnet}$ and $Lung_{RECRUITED}$, with a power of 90% and a two-sided significance level of 0.05. Since, no data is available for a similar population with both ARDS and obesity, we opted to increase our sample size by 10 additional subjects (maximum of 21 subjects).

Continuous variables are expressed as mean ± SD or median [interquartile range]. The distribution of continuous variables was assessed by the Shapiro-Wilk test of normality. The differences between the two phases ($Lung_{ARDSnet}$ and $Lung_{RECRUITED}$) were tested using Student's t-test or Wilcoxon signed rank for paired samples as appropriate for the data distribution. The p-values, means or medians of differences, and confidence intervals were reported as appropriate for the data type and distribution. Categorical

variables are expressed as count (n) and proportion (%).

A mixed linear model was used to compare overdistension and collapse during multiple PEEP levels (decremental PEEP trial) between patients with ARDS and with obesity or not. Same mixed linear model was used to compare the pressure-volume curves for the same groups and PEEP levels.

Data analysis was performed with R studio, version 8.0.2.

Appendix 2. Experimental study

Healthy swine with normal pleural pressure

Preparation and procedures

Six male Yorkshire swine (33±4 kg actual body weight) were delivered to the study site and, before performing any procedure related to the study, were premedicated intramuscularly with: acepromazine (0.1 mg/kg), ketamine cloridate (5mg/kg) and midazolam (0.5 mg/kg). They were cleaned, shaved and placed on a bed in the supine position. Two ear veins were cannulated and intravenous propofol (0.3 mg/kg) was administered for intubation. The end-tidal CO_2 ($ETCO_2$) monitoring confirmed the endotracheal tube correct position. The maintenance of anesthesia and paralysis were continuously provided by intravenous ketamine cloridate (2mg/kg/h), midazolam (0.4 mg/kg/h), thiopental (2.5 mg/kg/h) and pancuronium (0.2 mg/kg/h). Before assessing the right jugular vein, 150 mg of amiodarone, diluted in 100 ml of 5% dextrose (Baxter), was infused at a rate of 100 mL/h to prevent cardiac arrhythmias. A fluid bolus of ringer lactate (1000 ml) was administered over a period of 60 minutes followed by a maintenance infusion of 2 ml/kg/h for the entire study period.

Ventilatory monitoring

A nasogastric tube equipped with esophageal balloon (Avea GS SmarthCath, Carefusion) was inserted. The methods for correct placement and appropriate balloon inflation were previously addressed at Appendix 1 (clinical study) section of this supplemental material. Flow, airways pressure (P_{AW}) and P_{ES} were continuously recorded, including during end-inspiratory and end-expiratory pauses (zero flow). The tidal volume (Vt) was calculated as the integral of the expiratory flow-time waveform

from a mean respiratory cycle (for each pig and at each study phase). The mean respiratory cycle was obtained by resampling and interpolating a group of cycles (5-10 cycles) using Pneumobench Analysis 9.0, developed with LabVIEW (National Instruments). The derived ventilation parameters were calculated as follow:

- Plateau pressure (Plateau P) = airway pressure in the end of an inspiratory pause
- PEEP = airway pressure in the end of an expiratory pause
- Driving pressure (DP)= Plateau pressure - PEEP
- Compliance = Vt / DP
- End-expiratory P_{ES} = esophageal pressure in the end of an expiratory pause
- End-inspiratory P_{ES} = esophageal pressure in the end of an inspiratory pause
- End-inspiratory transpulmonary pressure ($P_L I$) = Plateau Pressure – Inspiratory P_{ES} (6)
- End-expiratory transpulmonary pressure ($P_L E$) = PEEP – Expiratory P_{ES} (6)

Cardiac and hemodynamic monitoring

The right femoral artery was cannulated, guided by ultrasound (Vivid i, GE Healthcare), a 5Fr catheter (Teleflex) was placed and connected to a pressure transducer (Edwards Lifesciences LLC), thus, invasive arterial pressure (AP) was measured continuously, and arterial blood gas samples were drawn per protocol phase.

A pulmonary artery catheter (CCOmbo V, Edwards Lifescience) was inserted in the left jugular vein to measure cardiac output (CO), central venous pressure (CVP), and pulmonary artery pressure (PAP) (Figure E3). The cannulation of the femoral and pulmonary arteries provided derived hemodynamic parameters calculated as follow:

- $MAP = AP_{SYSTOLE} + (2 \times AP_{DIASTOLE}) / 3$

- $PAP_{MEAN} = PAP_{SYSTOLE} + (2 \times PAP_{DIASTOLE}) / 3$
- Wedge pressure = measured after inflating the pulmonary artery balloon
- Systemic vascular resistance (SVR) = (MAP – CVP) / CO
- Pulmonary vascular resistance (PVR) = (PAP_{MEAN} – Wedge) / CO

  One pressure catheter (Millar SPR-524, AD Instruments) was placed in each cardiac ventricle and pressures were monitored continuously. In the right ventricle (RV), the catheter was introduced via the right jugular vein; and, in the left ventricle (LV), via the left carotid artery. The catheters position was checked by respective traces and transthoracic echocardiography (TTE). Before calculating the transmural pressure for each ventricle, the P_{ES} was converted from cmH_2O to mmHg (P_{ES} mmHg = P_{ES} cmH_2O x 0.735). The right and left ventricular systolic pressures (RVSP and LVSP, respectively) and the right and left ventricular diastolic pressures (RVDP and LVDP, respectively) were recorded. The transmural pressure (TM) was calculated as the pressure difference between the cardiac chamber (right and left ventricle) and P_{ES} during inspiratory (Table E3) and expiratory pauses, (Table 4) and during systole and diastole (18):

  TM RVSP = RVSP – Inspiratory P_{ES} and TM RVSP = RVSP – Expiratory P_{ES}

  TM RVDP = RVDP – Inspiratory P_{ES} and TM RVDP = RVDP – Expiratory P_{ES}

  TM LVSP= LVSP – Inspiratory P_{ES} and TM LVSP= LVSP – Expiratory P_{ES}

  TM LVDP= LVDP – Inspiratory P_{ES} and TM LVDP= LVDP – Expiratory P_{ES}

Hemodynamic management

The hemodynamic management was tailored to maintain MAP > 65 mmHg and urine output > 0.5 ml/kg/h. If necessary, further fluid replacement with tetrastarch (Voluven) 250 ml + 250 ml over a period of 30 minutes would be performed. Norepinephrine was added if volume replacement was deemed unsatisfactory and infusion would start at 0.05 mcg/kg/minute.

Vasoactive-inotropic score (VIS)

VIS was measured during this study protocol, this index provided comparable values of possible vasoactive agents administered per study phase. VIS was calculated as a weighted sum of all administered inotropes and vasoconstrictors. The formula used was: dopamine dose (mcg/kg/min) + dobutamine dose (mcg/kg/min) + 100 X epinephrine dose (mcg/kg/min) + 10 X milrinone dose (mcg/kg/min) + 10,000 X vasopressin dose (U/kg/min) + 100 X norepinephrine (mcg/kg/min) + 10 X phenylephrine dose (mcg/kg/min) (12, 13).

Electrical impedance tomography

The dedicated EIT belt was placed around the swine chest. The measurements and analysis utilized the same methodology previously described at Appendix 1 (clinical study) section of this supplemental material (Figure E1, panel B).

At the end of the procedures, a lung recruitment maneuver (LRM) was performed with pressure control ventilation (PCV) of 15 cmH_2O, PEEP of 15 cmH_2O, 10 breaths/min of respiratory rate (RR), inspiratory: expiratory (I:E) of 1:1 and F_IO_2 of 100%. PEEP was

increased in steps of 5 cmH_2O every 30 sec until a plateau pressure of 50 cmH_2O and PEEP was maintained for 2 min. At the end of the LRM, the ventilator was set in volume control ventilation (VCV) of 7 mL/kg, PEEP of 10 cmH_2O, 25 breaths/min of RR, I:E:1:2, and F_IO2 of 100% for 10 minutes. After the 10 minutes stabilization, an arterial blood gas analysis was performed (ABL 750 flex, Radiometer Medical) and a PaO_2/F_IO_2 < 400 mmHg was considered exclusion criterion.

Experimental study protocol

Swine were ventilated on VCV with a Vt of 6-7 ml/kg. The RR was set to keep the $ETCO_2$ between 35 and 40 mmHg and the PEEP was adjusted per study phase. The F_IO_2 was 100% throughout the study protocol.

The protocol compared two phases:

1) $Lung_{PEEP7}$: PEEP was set at 7 cmH_2O and after 30 minutes data was collected (ventilator settings, respiratory system mechanics, hemodynamic measurements, ABG and EIT).

2) $Lung_{PEEP19}$: firstly, we performed LRM in PCV with DP of 15 cmH_2O, RR of 20 bpm and PEEP was increased in a staircase fashion until a Plateau P of 50 cmH_2O (for 2 minutes). After the LRM, we switched again the ventilator mode to VCV and dropped the PEEP to 19cmH_2O. The swine were ventilated for 30 minutes before data collection (ventilator settings, respiratory system mechanics, hemodynamic measurements, ABG and EIT).

ARDS-induced swine with high pleural pressure

Preparation and procedures

Nine female *Landrace* swine (29 ± 4 kg actual body weight) were delivered to the study site and preparation and procedures were the same aforementioned for the healthy swine group.

ARDS with high pleural pressure model

Two previously described experimental models, one of ARDS (19, 20), and one of high pleural pressure (21), were combined in this study. First, abdominal loading was increased by adding external weights to the swine abdomen, resulting in an increased in P_{PL} of 5 cmH_2O. Next, surfactant was depleted through multiple lung lavages (utilizing 30ml/kg of saline solution at 37ºC) combined with injurious ventilation (low PEEP, high inspiratory pressure/tidal volumes for 3 hours) resulting in a stable experimental model of ARDS (constant PaO_2/F_IO_2 <100 mmHg for at least 10 minutes with a PEEP of 10 cmH_2O) (22).

Experimental study protocol

Swine were ventilated on VCV with a Vt of 6-7 ml/kg. The RR was set to keep the $ETCO_2$ between 35 and 40 mmHg and the PEEP was adjusted per study phase. The F_IO_2 was 100% throughout the study protocol.

The protocol compared two phases:

1) $Lung_{COLLAPSED}$: PEEP was set at 5 cmH_2O and after 30 minutes data was collected (ventilator settings, respiratory system mechanics, hemodynamic measurements, ABG, TTE and EIT).

2) $Lung_{RECRUITED}$: firstly, we performed LRM in PCV with DP of 15 cmH_2O, RR of 20 bpm and PEEP was increased in a staircase fashion until a Plateau P of 50 cmH_2O (for 2 minutes). After the LRM, we switched again the ventilator mode to VCV (5ml/kg of body weight) and dropped the PEEP 2 by 2 cmH_2O every 30 seconds until the baseline level of 5 cmH_2O. By the end of the decremental PEEP trial, the $Lung_{RECRUITED}$ was determined by the PEEP with the best C_{RS} plus 2 cmH_2O following the open lung approach, previously described (23, 24) . Finally, another LRM was performed, in the same settings as before, and, the ventilator was readjusted to VCV (5ml/kg of body weight) with the elected PEEP. The swine were ventilated for 30 minutes before data collection (ventilator settings, respiratory system mechanics, hemodynamic measurements, ABG and EIT).

In the end of each experiment, the swine was sacrificed according to Sao Paulo City Law (Nº 10.309; April 22, 1987) and Sao Paulo State Law (Nº 11.977; August 25, 2005). The subjects received a bolus (4 ml) of anesthetics before the bolus (10 ml) of potassium chloride 19.1% administered in the central line. At MGH, we were compliant with the institution internal protocol.

Statistical analysis of the swine study

The primary aim of the swine study was to assess the cardiovascular response to the transition from atelectasis ($Lung_{PEEP7}$ and $Lung_{COLLAPSED}$) to after lung recruitment ($Lung_{PEEP19}$ and $Lung_{RECRUITED}$) in the setting of healthy swine with normal pleural

pressure and ARDS-induced swine with high pleural pressure.

We calculated the sample size with the goal of measuring significant lung recruitment estimation after the $Lung_{RECRUITED}$ strategy was employed. Assuming an average decrease in driving pressure of 4.7 cmH_2O after lung recruitment maneuver, as observed in a previous swine study of high pleural pressure without ARDS (21), a minimum of 7 swine were calculated as needed to obtain a significant difference between $Lung_{COLLAPSED}$ and $Lung_{RECRUITED}$, with a power of 90% and a two-sided significance level of 0.05. Two pigs were added (total n=9 pigs) to account for possible missing data.

Continuous variables are expressed as mean ± standard deviation (SD) or median [interquartile range]. The distribution of continuous variables was assessed by Shapiro-Wilk test of normality. The differences between the two phases ($Lung_{COLLAPSED}$ / $Lung_{PEEP7}$ and $Lung_{RECRUITED}$ / $Lung_{PEEP19}$) were compared with Student's t-test for paired samples. The p-values, means of differences, and confidence intervals were reported, as appropriate.

Data analysis was performed with R studio, version 8.0.2.

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Figures Legends

Figure E1. Electrical Impedance Tomography

The electrical impedance tomography belt is composed by 32 electrodes placed around the chest during the experimental and clinical procedures. A harmless electrical current is applied and received around the chest and images of ventilation and perfusion can be generated. Both ventilation and perfusion images were divided in three regions of interest (ROIs).

Panel A

Clinical Study

Panel B

Swine Study

Figure E2. Studies Design

Panel A

Patients with ARDS and Obesity

Daily screening was performed for potential subjects with ARDS and obesity. Esophageal balloon, electrical impedance tomography belt and recording system were placed and connected within one hour. Finally, two ventilation strategies – "$Lung_{ARDSnet}$" and "$Lung_{RECRUITED}$"- were performed, always in the same order and lasting 30 minutes each.

Panel B

Healthy Swine with Normal Pleural Pressure

The first two hours were dedicated to preparation and procedures. Then, the two study phases were applied.

Panel C

ARDS-Induced Swine with High Pleural Pressure

The first two hours were dedicated to preparation and procedures. The model of ARDS with high pleural pressure was completed at the fifth hour. Finally, two ventilation strategies – "$Lung_{COLLAPSED}$" and "$Lung_{RECRUITED}$"- were performed, always in the same order and lasting 30 minutes each.

Abbreviations: PCV = pressure control ventilation, ΔP = driving pressure, PEEP = positive end-expiratory pressure, P_{PL} = pleural pressure.

Figure E3. Swine Study. Systolic Pulmonary Artery Pressure during Respiration Phase at Two PEEP Levels.

Panel A

Healthy Swine with Normal Pleural Pressure

Systolic pulmonary artery pressure during inspiratory and expiratory phases at $Lung_{PEEP7}$ and $Lung_{PEEP19}$. No significant interaction was detected between the $PAP_{SYSTOLIC}$ and the respiratory phase (mixed linear model).

Panel B

ARDS-Induced Swine with High Pleural Pressure

Systolic pulmonary artery pressure during inspiratory and expiratory phases at $Lung_{COLLAPSED}$ and $Lung_{RECRUITED}$. No significant interaction was detected between the $PAP_{SYSTOLIC}$ and the respiratory phase (mixed linear model).

Abbreviations: $PAP_{SYSTOLIC}$ = systolic pulmonary artery pressure

Figure E4. Swine Study. Effect of Different Transpulmonary Pressures on Right and Left Ventricles Transmural Pressures in a Healthy Swine with Normal Pleural Pressure and in an ARDS-Induced Swine with High Pleural Pressure during PEEP Transitions (before, during and after Lung Recruitment Maneuver)

Panel A

Healthy swine with normal pleural pressure

P_L, TM RV and TM LV at $Lung_{PEEP7}$, during lung recruitment maneuver and at $Lung_{PEEP19}$. The black arrow shows the TM LV drop with the increase in P_L from $Lung_{PEEP7}$ and $Lung_{PEEP19}$.

Panel B

ARDS-Induced Swine with High Pleural Pressure

P_L, TM RV and TM LV at $Lung_{COLLAPSED}$, during lung recruitment maneuver and at $Lung_{RECRUITED}$. The black arrow shows TM RV drop with the increase in P_L whereas TM LV did not change with the increase in P_L.

Abbreviations: P_L= transpulmonary pressure (respiratory cycles and during an expiratory pause), TM RV = right ventricle transmural pressure, TM LV = left ventricle transmural pressure.

Table E1. Swine Study. Mechanics, Oxygenation, Collapse & Overdistension and Regional Compliance

	Healthy Swine with Normal P_{PL} (n=6)				ARDS-Induced Swine with High P_{PL} (n=9)			
	Lung PEEP7	**Lung PEEP19**	**Difference [CI]**	***P* Value**	**Lung COLLAPSED**	**Lung RECRUITED**	**Difference [CI]**	***P* Value**
Vt, mL/kg	6.5 ± 0.4	6.5 ± 0.4	0	>0.99	7.1 ± 0.3	7.2 ± 0.5	0.1 [-0.1, 0.3]	0.35
RR, bpm	27 ± 4	27 ± 4	0	>0.99	38 ± 3	38 ± 2	0 [-1, 2]	0.58
Plateau P, cmH_2O	13 ± 1	25 ± 1	12 [12, 13]	<0.01	27 ± 6	31 ± 4	4 [2, 7]	<0.01
PEEP, cmH_2O	7	19	12 [12, 12]	>0.99	7 ± 3	18 ± 3	11 [9, 12]	<0.01
DP, cmH_2O	6 ± 1	6 ± 1	0 [-0.5, 1.3]	0.30	19 ± 5	13 ± 3	-6 [-9, -4]	<0.01
Pes_{INSP}, cmH_2O	10 ± 1	16 ± 3	6 [4, 8]	<0.01	15 ± 2	18 ± 3	3 [1, 4]	<0.01
Pes_{EXP}, cmH_2O	7 ± 1	14 ± 2	7 [5, 7]	<0.01	11 ± 2	15 ± 3	4 [3, 5]	<0.01
PL_{INSP}, cmH_2O	3 ± 2	9 ± 3	6 [4, 8]	<0.01	11 ± 4	13 ± 3	2 [-0.3, 4]	0.08
PL_{EXP}, cmH_2O	-0.4 ± 1.4	5.4 ± 1.9	5.8 [4.6, 7.2]	<0.01	-4 ± 2	3 ± 2	7 [5, 8]	<0.01
DPL, cmH_2O	4 ± 1	4 ± 2	0 [-1, 1]	0.79	15 ± 4	10 ± 3	-5 [-7, -3]	<0.01
C_{RS}, mL/cmH_2O	37 ± 6	35 ± 7	-2 [-6, 2]	0.27	11± 3	17 ± 6	6 [3, 9]	<0.01
C_L, mL/cmH_2O	59 ± 16	61 ± 22	2 [-15, 19]	0.81	15 ± 4	24 ± 13	9 [1, 16]	0.03
C_{CW}, mL/cmH_2O	106 ± 27	102 ± 48	-4 [-68, 60]	0.88	58 ± 23	91 ± 46	33 [7, 60]	0.02
Collapse, %	20 ± 2*	0 ± 0*	-20*	-	55 ± 8	15 ± 6	-40 [-47, -33]	<0.01
Overdist., %	5 ± 4*	56 ± 7*	51*	-	5 ± 3	21± 9	16 [9, 22]	<0.01
PaO_2/FIO_2	540 ± 38	561 ± 50	21 [-26, 69]	0.30	72 ± 32	197 ± 60	125 [81, 170]	<0.01
Shunt, %	4 ± 2	2 ± 2	-2 [-4, 1]	0.26	68 ± 6	43 ± 12	-25 [-34, -16]	<0.01
Dead Space, %	5.3 ± 8.2	0.3 ± 9.3	-5 .0 [-8.5, -1.5]	0.01	40 ± 15	22 ± 11	-18 [-24, -11]	0.01

Definition of abbreviations: Vt= tidal volume, RR= respiratory rate, Plateau P= plateau pressure, PEEP= positive end-expiratory pressure, DP= driving pressure, PES_{INSP}= inspiratory esophageal pressure, PES_{EXP}= expiratory esophageal pressure, PL_{INS}= inspiratory transpulmonary pressure, PL_{EXP}= expiratory transpulmonary pressure, DPL= driving transpulmonary pressure, C_{RS}=compliance of the respiratory system, C_L= compliance of the lungs, C_{CW}= compliance of the chest wall, Overdist.= overdistension. Data presented as mean ± SD. *Collapse and overdistension measured in 2 healthy swine with normal pleural pressure.

Table E2. Swine Study. Hemodynamics

	Healthy swine with Normal P_{PL} (n=6)				ARDS-Induced Swine with High P_{PL} (n=9)			
	Lung PEEP7	**Lung** PEEP19	**Difference [CI]**	***P* Value**	**Lung** COLLAPSED	**Lung** RECRUITED	**Difference [CI]**	***P* Value**
HR, bpm	99 ± 24	104 ± 32	5 [-10,21]	0.39	129 ± 34	129 ± 18	0 [-21, 19]	0.92
MAP, mmHg	91 ± 11	68 ± 12	-23 [-37, -10]	<0.01	112 ± 16	107 ± 14	-5 [-16, 5]	0.28
PAP_{MEAN}, mmHg	17 ± 2	24 ± 2	7 [4, 9]	<0.01	40 ± 10	31 ± 5	-8 [-13, -4]	<0.01
CVP, mmHg	10 ± 4	16 ± 5	6 [4, 9]	<0.01	9 ± 4	11 ± 4	2 [1, 4]	<0.01
TM CVP, mmHg	4 ± 3	6 ± 6	2 [-1, 5]	0.17	0.9 ± 3.0	0.2 ± 2.9	-0.7 [-1.9, 0.6]	0.24
Wedge, mmHg	10 ± 3	15 ± 2	5 [4, 7]	<0.01	11 ± 4	14 ± 4	3 [2, 4]	<0.01
TM Wedge, mmHg	4 ± 2	5 ± 2	1 [-1, 3]	0.16	2.7 ± 3.6	2.6 ± 2.9	-0.1 [-1.3, 1.1]	0.80
CO, L/min	4.5 ± 1.1	3.1 ± 0.9	-1.4 [-2.0, -0.8]	<0.01	5.5 ± 1.1	4.8 ± 1.2	-0.7 [-1.4, 0.01]	0.053
SVR, WU	19 ± 6	17 ± 4	-2 [-6, 2]	0.27	19 ± 4	21 ± 6	2 [-2, 5]	0.28
PVR, WU	1.8 ± 0.6	2.9 ± 0.6	1.1 [0.5, 1.7]	<0.01	5.5 ± 2.1	3.9 ± 1.8	-1.5 [-2.2, -0.9]	<0.01
SvO_2, %	78 ± 7	59 ± 14	-19 [-27, -12]	<0.01	52 ± 14	75 ± 5	23 [11, 35]	<0.01
DO_2, mL/min	581 ± 148	390 ± 142	-191 [-260, -123]	<0.01	607 ± 153	692 ± 176	85 [-1,171]	0.052
VO_2, mL/min	176 ± 62	185 ± 86	10 [-20,40]	0.44	188 ± 64	181 ± 55	-7 [-38, 23]	0.59
O_2 EF, %	30 ± 7	48 ± 12	18 [11, 24]	<0.01	31 ± 6	26 ± 4	-5 [-11, 1]	0.08
VIS	0	0	0	0	7 ± 15	8 ± 16	1 [-1, 3]	0.35

Definition of abbreviations: HR= heart rate, MAP= mean arterial pressure, PAP_{MEAN}= mean pulmonary artery pressure, CVP=central venous pressure TM CVP= transmural central venous pressure, TM Wedge= transmural wedge pressure, CO= cardiac output, SVR= systemic vascular resistance, PVR= pulmonary vascular resistance, SvO_2= mixed venous saturation of oxygen, DO_2=oxygen delivery, VO_2=oxygen consumption, O_2EF= oxygen extraction fraction, VIS= vasoactive-inotropic score

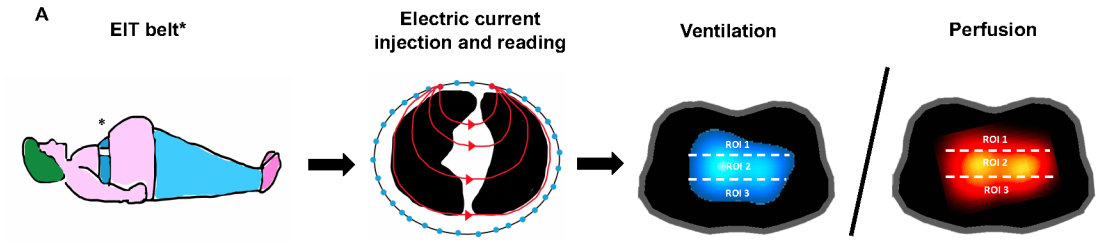
Data presented as mean ± SD.

Table E3. Swine Study. Ventricular Pressures and Respective Transmural Pressures during Different PEEP Levels during an Inspiratory Pause in Healthy Swine with Normal Pleural Pressure and ARDS-Induced Swine with High Pleural Pressure

Healthy Swine with Normal P_{PL}	**$Lung_{PEEP7}$**	**$Lung_{PEEP19}$**	**Difference [CI]**	***P* value**
Right Ventricle, mmHg (n=6):				
RVSP	28 ± 7	35 ± 6	7 [2,11]	0.01
TM RVSP	21 ± 7	23 ± 6	2 [-2,7]	0.25
RVDP	10 ± 4	17 ± 5	7 [3,10]	<0.01
TM RVDP	3 ± 4	5 ± 5	2 [-2,6]	0.26
Left Ventricle, mmHg (n=6):				
LVSP	103 ± 10	77 ± 13	-26 [-43, -10]	<0.01
TM LVSP	96 ± 10	65 ± 13	-31 [-48, -14]	<0.01
LVDP	11 ± 6	16 ± 6	5 [4,7]	<0.01
TM LVDP	4.0 ± 5.7	4.7 ±7.5	0.7[-1.7,3.2]	0.47
ARDS Swine with High P_{PL}	**$Lung_{COLLAPSED}$**	**$Lung_{RECRUITED}$**	**Difference [CI]**	***P* value**
Right Ventricle, mmHg (n=9):				
RVSP	53 ± 9	44 ± 3	-9 [-14, -4]	<0.01
TM RVSP	42 ± 8	31 ± 3	-11 [-16, -6]	<0.01
RVDP	18 ± 8	16 ± 1	-2 [-8,4]	0.46
TM RVDP	7 ± 8	3 ± 2	-4 [-10, 2]	0.16
Left Ventricle, mmHg (n=8):				
LVSP	136 ± 18	130 ± 12	-6 [-19, 7]	0.28
TM LVSP	125 ± 18	117 ± 13	-8 [-21, 5]	0.17
LVDP	25.6 ± 7.6	26.3 ± 8.8	0.7 [-1.6, 2.9]	0.49
TM LVDP	14 ± 8	13 ± 9	-1 [-4, 1]	0.28

Definition of abbreviations: RVSP= right ventricle systolic pressure at inspiration, TM RVSP= transmural right ventricle systolic pressure at inspiration, RVDP= right ventricle diastolic pressure at inspiration, TM RVDP= transmural right ventricle diastolic pressure at expiration, LVSP= left ventricle systolic pressure at inspiration, TM LVSP= transmural left ventricle systolic pressure at inspiration, LVDP= left ventricle diastolic pressure at inspiration, TM LVDP= transmural left ventricle diastolic pressure at inspiration. Data presented as mean ± SD. Confidence interval presented as mean of differences [CI 95%].

Figure E1


A
EIT belt*
Electric current injection and reading
Ventilation
Perfusion
*
ROI 1
ROI 2
ROI 3
ROI 1
ROI 2
ROI 3

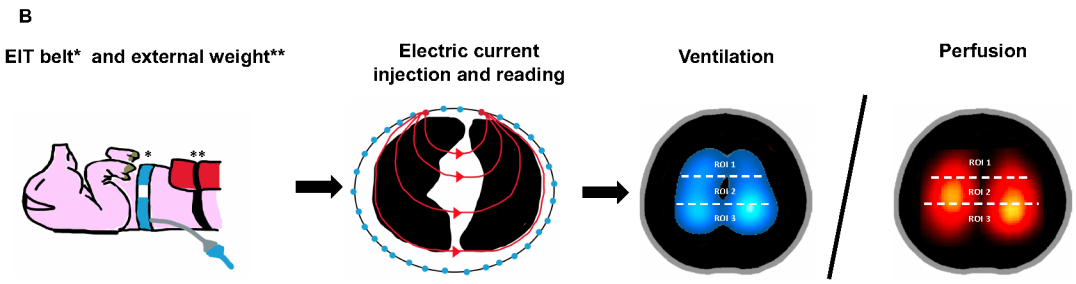
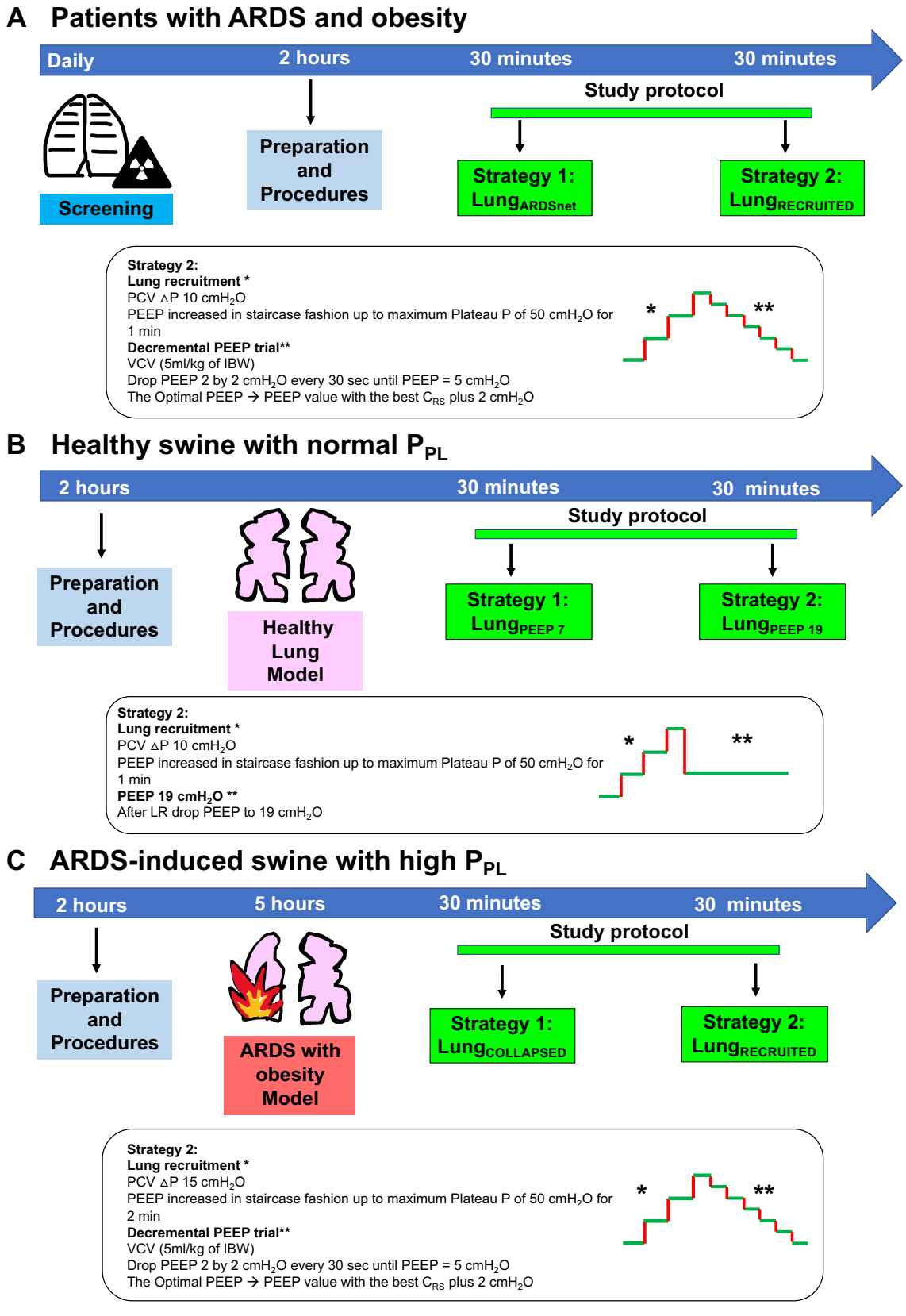

B
EIT belt* and external weight**
Electric current injection and reading
Ventilation
Perfusion
*
**
ROI 1
ROI 2
ROI 3
ROI 1
ROI 2
ROI 3

Figure E2


A Patients with ARDS and obesity
Daily
2 hours
30 minutes
30 minutes
Study protocol
Screening
Preparation and Procedures
Strategy 1: Lung ARDSnet
Strategy 2: Lung RECRUITED
Strategy 2:
Lung recruitment *
PCV ΔP 10 cmH2O
PEEP increased in staircase fashion up to maximum Plateau P of 50 cmH2O for 1 min
Decremental PEEP trial**
VCV (5ml/kg of IBW)
Drop PEEP 2 by 2 cmH2O every 30 sec until PEEP = 5 cmH2O
The Optimal PEEP → PEEP value with the best CRS plus 2 cmH2O
*
**
B Healthy swine with normal PPL
2 hours
30 minutes
30 minutes
Study protocol
Preparation and Procedures
Healthy Lung Model
Strategy 1: Lung PEEP 7
Strategy 2: Lung PEEP 19
Strategy 2:
Lung recruitment *
PCV ΔP 10 cmH2O
PEEP increased in staircase fashion up to maximum Plateau P of 50 cmH2O for 1 min
PEEP 19 cmH2O **
After LR drop PEEP to 19 cmH2O
*
**
C ARDS-induced swine with high PPL
2 hours
5 hours
30 minutes
30 minutes
Study protocol
Preparation and Procedures
ARDS with obesity Model
Strategy 1: Lung COLLAPSED
Strategy 2: Lung RECRUITED
Strategy 2:
Lung recruitment *
PCV ΔP 15 cmH2O
PEEP increased in staircase fashion up to maximum Plateau P of 50 cmH2O for 2 min
Decremental PEEP trial**
VCV (5ml/kg of IBW)
Drop PEEP 2 by 2 cmH2O every 30 sec until PEEP = 5 cmH2O
The Optimal PEEP → PEEP value with the best CRS plus 2 cmH2O
*
**

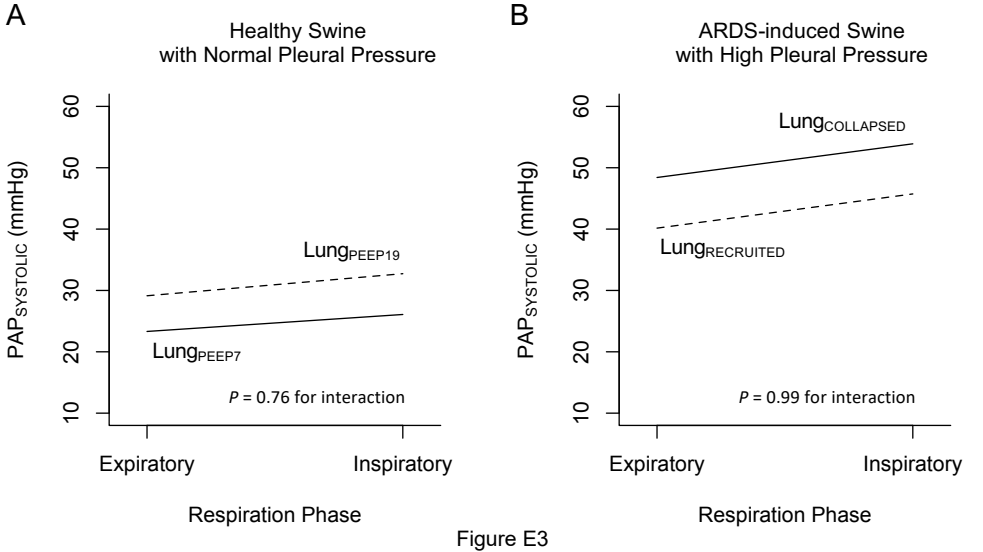
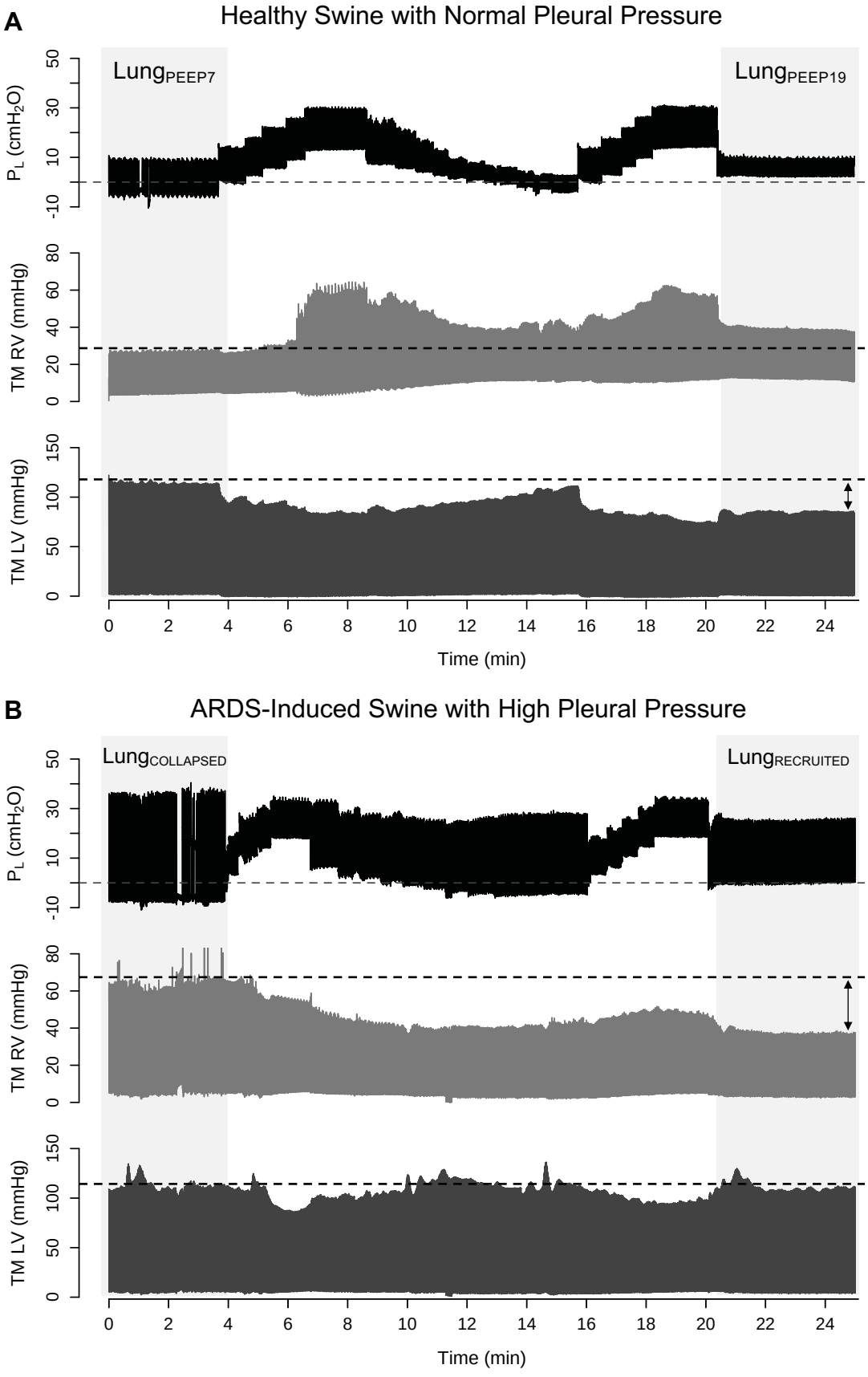

A
Healthy Swine
with Normal Pleural Pressure
PAP SYSTOLIC (mmHg)
10
20
30
40
50
60
Lung PEEP19
Lung PEEP7
P = 0.76 for interaction
Expiratory
Inspiratory
Respiration Phase
B
ARDS-induced Swine
with High Pleural Pressure
PAP SYSTOLIC (mmHg)
10
20
30
40
50
60
Lung COLLAPSED
Lung RECRUITED
P = 0.99 for interaction
Expiratory
Inspiratory
Respiration Phase

Figure E3

Figure E4


A
Healthy Swine with Normal Pleural Pressure
Lung PEEP7
Lung PEEP19
P_L (cmH2O)
TM RV (mmHg)
TM LV (mmHg)
Time (min)
B
ARDS-Induced Swine with High Pleural Pressure
Lung COLLAPSED
Lung RECRUITED
P_L (cmH2O)
TM RV (mmHg)
TM LV (mmHg)
Time (min)