ANESTHESIOLOGY

Prone Position Minimizes the Exacerbation of Effort-dependent Lung Injury: Exploring the Mechanism in Pigs and Evaluating Injury in Rabbits

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Prone positioning during mechanical ventilation for patients with acute lung injury has been shown to increase oxygenation and possibly improve outcome
- It is now widely used for patients with COVID-19 failing routine ventilation protocols
- Its use during spontaneous ventilation has increased as result of the pandemic, yet detailed data on its ventilatory effects have not been well established

What This Article Tells Us That Is New

- The authors utilized porcine and rabbit models of lung injury to evaluate pulmonary mechanics, distribution of ventilation, and biochemical and histologic effects on lung injury with varying positive end-expiratory pressure levels
- Independent of positive end-expiratory pressure levels, prone positioning reduced maldistribution of lung stress and reduced effort-dependent evidence of lung injury

ABSTRACT

Background: Vigorous spontaneous effort can potentially worsen lung injury. This study hypothesized that the prone position would diminish a maldistribution of lung stress and inflation after diaphragmatic contraction and reduce spontaneous effort, resulting in less lung injury.

Methods: A severe acute respiratory distress syndrome model was established by depleting surfactant and injurious mechanical ventilation in 6 male pigs ("mechanism" protocol) and 12 male rabbits ("lung injury" protocol). In the mechanism protocol, regional inspiratory negative pleural pressure swing (intrabronchial balloon manometry) and the corresponding lung inflation (electrical impedance tomography) were measured with a combination of position (supine or prone) and positive end-expiratory pressure (high or low) matching the intensity of spontaneous effort. In the lung injury protocol, the intensities of spontaneous effort (esophageal manometry) and regional lung injury were compared in the supine position *versus* prone position.

Results: The mechanism protocol (pigs) found that in the prone position, there was no ventral-to-dorsal gradient in negative pleural pressure swing after diaphragmatic contraction, irrespective of the positive end-expiratory pressure level (–10.3 ± 3.3 cm H_2O *vs.* –11.7 ± 2.4 cm H_2O at low positive end-expiratory pressure, $P = 0.115$; –10.4 ± 3.4 cm H_2O *vs.* –10.8 ± 2.3 cm H_2O at high positive end-expiratory pressure, $P = 0.715$), achieving homogeneous inflation. In the supine position, however, spontaneous effort during low positive end-expiratory pressure had the largest ventral-to-dorsal gradient in negative pleural pressure swing (–9.8 ± 2.9 cm H_2O *vs.* –18.1 ± 4.0 cm H_2O, $P < 0.001$), causing dorsal overdistension. Higher positive end-expiratory pressure in the supine position reduced a ventral-to-dorsal gradient in negative pleural pressure swing, but it remained (–9.9 ± 2.8 cm H_2O *vs.* –13.3 ± 2.3 cm H_2O, $P < 0.001$). The lung injury protocol (rabbits) found that in the prone position, spontaneous effort was milder and lung injury was less without regional difference (lung myeloperoxidase activity in ventral *vs.* dorsal lung, 74.0 ± 30.9 μm · min^{-1} · mg^{-1} protein *vs.* 61.0 ± 23.0 μm · min^{-1} · mg^{-1} protein, $P = 0.951$). In the supine position, stronger spontaneous effort increased dorsal lung injury (lung myeloperoxidase activity in ventral *vs.* dorsal lung, 67.5 ± 38.1 μm · min^{-1} · mg^{-1} protein *vs.* 167.7 ± 65.5 μm · min^{-1} · mg^{-1} protein, $P = 0.003$).

Conclusions: Prone position, independent of positive end-expiratory pressure levels, diminishes a maldistribution of lung stress and inflation imposed by spontaneous effort and mitigates spontaneous effort, resulting in less effort-dependent lung injury.

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Spontaneous breathing using respiratory muscles is physiologically normal and therefore has been traditionally facilitated during mechanical ventilation.[1] Negative deflection ("swing") in pleural pressure resulting from diaphragmatic contraction is evenly transmitted across the whole lung surface, creating a uniform increase in transpulmonary pressure at any given airway pressure (P_{aw}): this is called "fluid-like" behavior.[2] Thus, spontaneous breathing achieves homogeneous inflation at lower levels of P_{aw} during mechanical ventilation, improving ventilation/perfusion and gas exchange, and preserving diaphragm function.[1,3] Although such benefits of spontaneous breathing have been reported during mechanical ventilation, it may also potentially injure the lungs and diaphragm when spontaneous effort is vigorous and/or when lung injury is severe.[3–6]

In the severely injured lung, negative deflection in pleural pressure resulting from diaphragmatic contraction is partially used on local lung deformation (*i.e.*, dense, atelectatic area resisting dynamic shape changes) and thus is not evenly transmitted to the entire lung; this is called "solid-like" behavior.[2,7] Such a maldistribution of lung stress imposed by spontaneous breathing is known to cause injurious inflation patterns (*e.g.*, pendelluft, local volutrauma[2,7]). In addition, several factors increase the strength and injury potential of spontaneous breathing effort in severe acute respiratory distress syndrome (ARDS), including acidemia, hypercapnia, and hypoxemia,[8] as well as reduced lung volume due to dorsal atelectasis.[9,10]

Turning to the prone position gravitationally translocates atelectasis (dense solid-like lung tissue resisting dynamic shape changes) from the dorsal to ventral lung, as is obvious from previous studies.[11] Because the dorsal lung (facing muscular parts of diaphragm) is now open and less solid-like atelectatic in the prone position, it might diffuse the inspiratory stress after diaphragmatic contraction from being local and injurious to generalized and less injurious (*e.g.*, less pendelluft, less local volutrauma). In several studies, the prone position is also shown to have the similar effect of recruiting lung and increasing lung volume as higher positive end-expiratory pressure (PEEP).[11,12] Lung recruitment may minimize the injurious effect of spontaneous effort (*e.g.*, large tidal volume, high transpulmonary pressure) by increasing lung volume, shortening diaphragm length, and thereby generating less force from the diaphragm.[6,9,10,13–15]

The prone position has been traditionally used under passive conditions (*e.g.*, more than 90% of patients in the prone position received muscle paralysis for more than 5 days),[16] and the interaction of the prone position with spontaneous breathing has not been evaluated well in severe ARDS. Based on this reasoning, we hypothesized that if spontaneous effort is permitted while in the prone position, it would diminish a maldistribution of lung stress and inflation imposed by spontaneous effort and decrease spontaneous effort, resulting in less lung injury.

We tested this hypothesis in established models of severe ARDS. First, in the "mechanism" protocol using pigs, to evaluate regional lung stress and the corresponding inflation pattern caused by spontaneous effort, we measured the impact of PEEP (high and low) and position (supine and prone) on regional lung inflation (electrical impedance tomography in pigs) and regional inspiratory negative pleural pressure swings (intrabronchial balloon manometry[2,17] in pigs). Second, in the "lung injury" protocol using rabbits, we measured the impact of position on the strength of spontaneous effort (negative deflection in esophageal pressure [P_{eso}] in rabbits), and on regional injury associated with spontaneous effort (total protein in bronchoalveolar lavage, lung myeloperoxidase activity in rabbits).

Materials and Methods

Two series of animal experiments (pigs, rabbits) were conducted from 2017 through 2018 (before the COVID-19 pandemic), both approved by the Animal Care Committee of the Hospital for Sick Children in Toronto (Toronto, Ontario, Canada; approval No. 45697). The animals were cared for in accordance with the hospital's standards for the care and use of laboratory animals.

Series 1 Mechanism Protocol: Anesthetized Pig Experiments

The schematic of study protocol is described in figure 1A. Six male Yorkshire pigs (n = 6; 30.9 to 39.3 kg) were anesthetized with 7 mg · kg^{-1} · h^{-1} ketamine and 2 mg · kg^{-1} · h^{-1} propofol and tracheostomized. Negative toe pinch was confirmed throughout the protocol. An esophageal balloon catheter (NutriVent, Sidam, Italy) was inserted to measure P_{eso}, filled with 1.0 ml as a minimal nonstress volume, and calibrated.[18] Neuromuscular blockade rocuronium bromide boluses of 0.5 mg · kg^{-1} were used to prevent spontaneous breathing effort when necessary.

Lung Injury. Experimental lung injury was induced in the supine position by repeated saline lung lavage (30 ml · kg^{-1}, 37°C),[19] and surfactant depletion was considered stable when the Pao_2/fractional inspired oxygen tension (Fio_2) ratio was less than 100 mmHg for 10 min, at a PEEP of 5 cm H_2O. Injurious mechanical ventilation was commenced and continued for 60 min using assisted pressure control: Fio_2, 1.0; rate, 25 breaths/min; and pressure trigger, –2 cm H_2O (Servo 300, Siemens-Elema AB, Sweden). Ventilator-induced lung injury was induced with the following driving pressure/PEEP combinations adjusted every 15 min to maintain Pao_2 of greater than 55 to 65 mmHg: 41/1, 39/3, 37/5, 35/7, 33/9, 31/11, or 29/13 cm H_2O.[13]

Experimental Protocol. The animals were then randomly assigned to four acquisition periods (each period comprised high or low PEEP and supine or prone position):

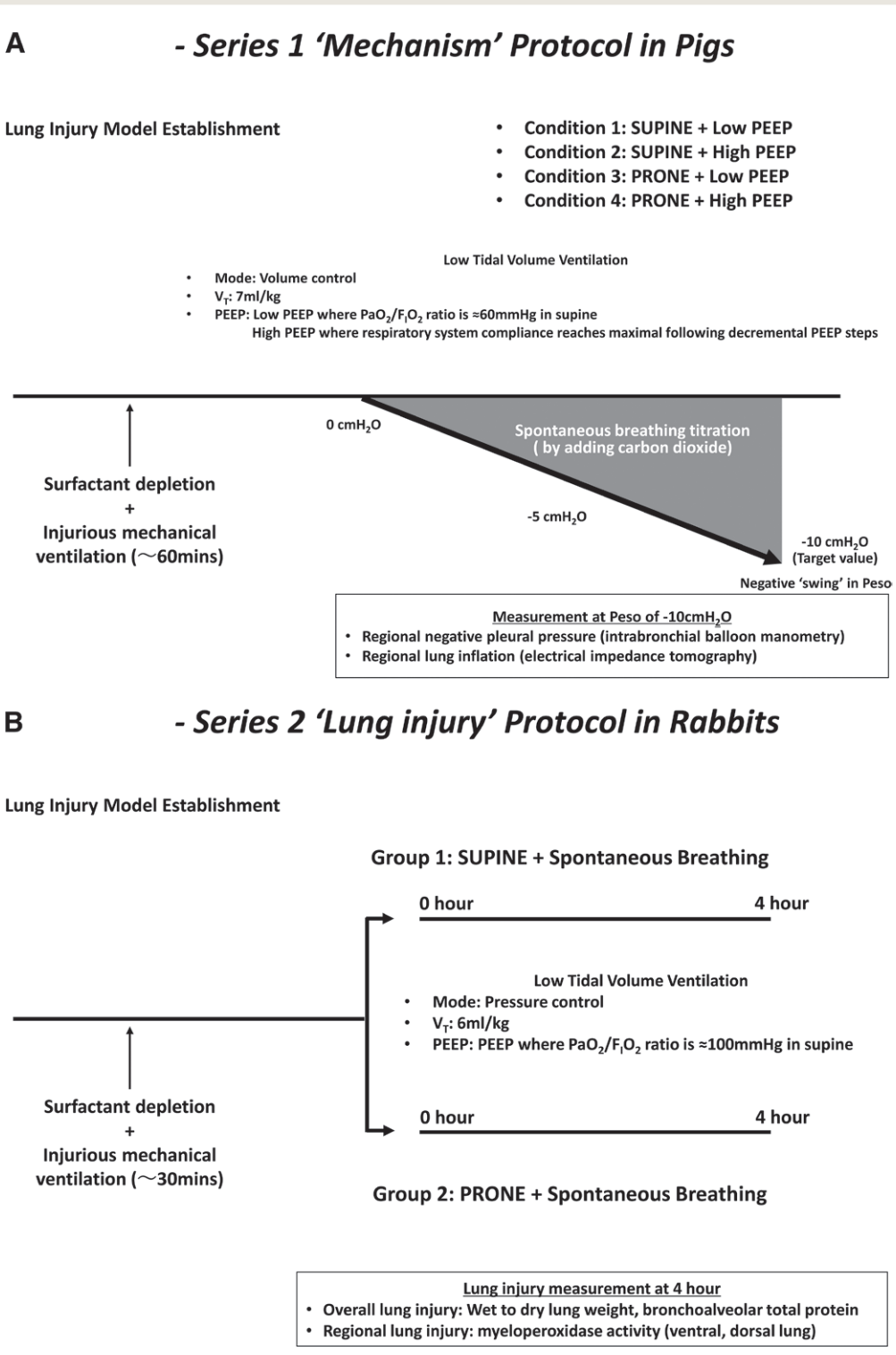

Fig. 1. Schematic of protocol. (*A*) Series 1 mechanism protocol in pigs. Lung-injured pigs (n = 6) were randomly assigned to each of four acquisition periods (conditions 1 to 4), and spontaneous effort was subsequently facilitated by adding carbon dioxide until a negative swing in esophageal pressure (P_{eso}) of –10 cm H_2O was reached. Then, the regional lung stress and lung inflation pattern were analyzed by intrabronchial balloon manometry and electrical impedance tomography, respectively. (*B*) Series 2 lung injury protocol in rabbits. Lung-injured rabbits (n = 12) were randomized to either group 1 (supine and spontaneous breathing) or group 2 (prone and spontaneous breathing). Lung injury was evaluated after 4-h preservation of spontaneous effort under mechanical ventilation. FIO_2, fractional inspired oxygen tension; PEEP, positive end-expiratory pressure; V_T, tidal volume.

- Low PEEP, supine
- High PEEP, supine
- Low PEEP, prone
- High PEEP, prone

Randomization was from a bag of coded letters. Static respiratory system compliance was measured with decremental PEEP steps (modified from Yoshida *et al.*[20]), starting at a PEEP of 20 cm H_2O and reducing by 2 cm H_2O every 30 s until an oxygen saturation measured by pulse oximetry of approximately 90% was reached. Ventilation was set at F_{IO_2} 1.0, inspiratory pressure was set at 15 cm H_2O, and the respiratory rate was 40 breaths/min. At a PEEP of 20 cm H_2O, the Pa_{O_2}/F_{IO_2} ratio was approximately 400 mmHg in all animals. High and low PEEP were defined as follows:

- High PEEP is the PEEP at which respiratory system compliance is maximal after decremental PEEP steps
- Low PEEP is the PEEP at which oxygen saturation measured by pulse oximetry is approximately 90% (Pa_{O_2} is approximately 60 mmHg)

The lungs were fully recruited in the supine position to homogenize lung volume history before randomization to each acquisition period and ventilated for approximately 15 min for stabilization. In each acquisition period, low tidal volume (V_T) ventilation employed assisted volume-controlled ventilation: V_T, 7 ml · kg^{-1}; rate, 30 breaths/min; inspiratory to expiratory ratio, 1:2 (no inspiratory pause); pressure trigger, –2 cm H_2O; and F_{IO_2}, 1.0.

At the start of each acquisition period, the absence of respiratory effort was confirmed by a lack of negative deflection in P_{eso}. Spontaneous breathing effort was subsequently facilitated by adding carbon dioxide (up to 0.10) until a negative swing in P_{eso} of –10 cm H_2O was reached. It usually took approximately 30 min to reach the target value of P_{eso}. The animals were sacrificed with IV sodium pentobarbital.

Electric Impedance Tomography. In all animals (n = 6), electrical impedance tomography data were recorded (PulmoVista 500, Dräger, Germany) continuously during the spontaneous breathing titration period (from paralysis to P_{eso} of –10 cm H_2O). Local lung inflation was analyzed after division of the image into four equal zones, from zone 1 (most ventral) to zone 4 (most dorsal), where each zone comprised 25% of the ventrodorsal distance and encompassed the complete area of the lung encircled by the band. We considered zone 1 (the most ventral one) and zone 4 (the most dorsal one) as representative of ventral lung and dorsal lung to be analyzed, respectively. The magnitude of local lung inflation imposed by spontaneous effort was estimated by the size of passive V_T during muscle paralysis to achieve the same degree of local lung inflation.[2] This estimation in each sequence was performed when ΔP_{eso} was –10 cm H_2O (*i.e.*, the same intensity of spontaneous effort). After measuring the magnitude of local lung inflation (represented by ΔZ in electrical impedance tomography) when ΔP_{eso} was –10 cm H_2O at a fixed, global V_T of 7 ml/kg during assisted volume-controlled ventilation, we paralyzed the animal and started to increase V_T setting during volume-controlled ventilation, until the same magnitude of local lung inflation (represented by ΔZ in electrical impedance tomography) developed in the dorsal lung.

Pleural Pressure Measurement. The local negative swing in pleural pressure was determined in nondependent and dependent regions (one pig did not survive; n = 5) by balloon catheter occlusion of subsegmental bronchi *via* a fibreoptic bronchoscope, as follows: nondependent region, left B; dependent region: left lower lobe beyond D4. The occluded subsegments were connected to a differential pressure transducer through the intrabronchial balloon catheter without airflow influx, thereby allowing continuous measurement of changes in occluded subsegment pressure. The pressure swings in the occluded subsegments were used as surrogates for negative pleural pressure swings, as described previously.[2,17] The occluded lung regions were filled with air until the alveolar pressure inside each target subsegmental region reached 20 (or 30) cm H_2O in nondependent (and dependent) lung regions, respectively, assuming that this opening pressure was sufficient to recruit the occluded lung regions. Simultaneous pressure recording of negative pleural pressure swings and ΔP_{eso} were performed, while preserving spontaneous effort. All measurements were performed when ΔP_{eso} was –10 cm H_2O.

Series 2 Lung Injury Protocol: Anesthetized Rabbit Experiments

A schematic of study protocol is shown in figure 1B. Twelve New Zealand white rabbits (adult, male, 2.9 to 3.9 kg) were anesthetized with intravenous propofol (10 to 100 mg · kg^{-1} · h^{-1}) and ketamine (1 to 5 mg · kg^{-1} · h^{-1}) and tracheostomized. Negative toe pinch was confirmed throughout the protocol. An esophageal balloon (SmartCath, Bicore, USA) was inserted to measure P_{eso} and filled with air (0.3 ml as minimal nonstress volume), and its position was verified.[18]

Lung Injury. Experimental lung injury was induced in the supine position by repeated lung lavage,[19] and surfactant depletion was considered stable when the Pa_{O_2}/F_{IO_2} ratio was less than 150 mmHg for 10 min at a PEEP of 3 cm H_2O. Injurious mechanical ventilation using assisted pressure control consisted of V_T of approximately 15 ml · kg^{-1} (by adjusting inspiratory pressure), and a PEEP of 2 cm H_2O. PEEP was adjusted (increased or decreased) by 2 cm H_2O to maintain a Pa_{O_2}/F_{IO_2} of 55 to 65 mmHg after 15 min and continued for 30 min.

Experimental Protocol. The lungs were fully recruited in the supine position, and PEEP was set at where the Pa_{O_2}/F_{IO_2} ratio was approximately 100 mmHg in the supine position. Then the animals were randomly assigned to one of two groups (n = 6 for each group):

- Supine plus spontaneous breathing
- Prone plus spontaneous breathing

Randomization was from a bag of coded letters. The animals were then ventilated for 4 h using low V_T ventilation, using pressure-controlled ventilation: V_T, 6 ml · kg^{-1} (by adjusting inspiratory pressure); respiratory rate, 60 to 120 breaths/min (targeted to $Paco_2$ of less than 50 mmHg); inspiratory time, 0.2 s; minimum flow trigger; and Fio_2 adjusted to target Pao_2 of 100 mmHg. All of the animals (n = 12) survived the protocol. After 4 h of mechanical ventilation, the animals were sacrificed with IV sodium pentobarbital, and the lungs were excised.

Wet to Dry Lung Weight. The right upper and middle lobes of the lung were weighed, placed in a warming oven (37°C), and weighed daily until the weight was stable.

Lung Inflammation. Bronchoalveolar fluid was collected from the left whole lung by injecting 10 ml of normal saline three times; then the total protein in the bronchoalveolar fluid was quantified. Lung myeloperoxidase activity was measured[21] from lung biopsies; a lung tissue sample (8 × 8 × 8 mm) was taken from the nondependent and dependent right middle lobes. One investigator (G.O.), who was blind to sampling regions and group allocation, performed the analysis.

Lung Histology. The right lower lobe was fixed with intratracheal insufflation of 10% formalin of 15 ml for at least 24 h. The right lower lobe was sectioned transversely (5-mm slices) and embedded in paraffin. In addition, 3-μm slices were stained with hematoxylin and eosin. Representative histologic images in each group are presented.

Definitions. The definitions of pulmonary pressures are as follows:

- Negative swing in P_{eso}: ΔP_{eso} was determined from the amount of decrease (spontaneous breathing) in P_{eso} from the start of inspiration.
- Negative swing in pleural pressure: Δ pleural pressure was determined from the amount of decrease (spontaneous breathing) in pleural pressure from the start of inspiration.
- Maximal (inspiratory) transpulmonary pressure: Peak transpulmonary pressure equaled the maximal value of [$P_{aw} - P_{eso}$] cm H_2O, usually corresponding to the time of the most negative value of P_{eso} (maximum inspiration).
- Plateau (inspiratory) transpulmonary pressure: Plateau transpulmonary pressure equaled [plateau P_{aw} – end-inspiratory P_{eso}] cm H_2O.
- Plateau pressure: P_{aw} measured during a short inspiratory hold (*i.e.*, zero flow phase).
- Driving pressure equaled [plateau P_{aw} – PEEP] cm H_2O.
- Peak Δ transpulmonary pressure: Peak Δ transpulmonary pressure equaled [P_{aw} – PEEP – (ΔP_{eso})] cm H_2O, corresponding to the time of maximal value of peak Δ transpulmonary pressure.
- Compliance of the respiratory system equaled [V_T/(driving pressure)] mL · cm H_2O^{-1}.

Statistical Analysis

Statistical analyses were performed using SPSS13.0 for Windows (SPSS, USA). The study was exploratory, and the sample size was not formally calculated, but it was based on experience. Normal distribution of data was checked with histography. The results are expressed as mean ± SD. One-way ANOVA was used to compare myeloperoxidase activities among regions. Two-way ANOVA with repeated measures evaluated the effects of time and group on respiratory variables. Two-way ANOVA was applied to evaluate the effects of lung regions (ventral *vs.* dorsal) and condition differences on lung stress and lung inflation imposed by spontaneous effort. In the *post hoc* analysis, a Dunnett's test was used to compare repeated values with the value at the start of the protocol (*i.e.*, 0 h), and Tukey's pairwise multiple comparison test was used to determine condition differences. Unpaired *t* tests were used to compare the wet to dry ratio and bronchoalveolar fluid protein. All tests were two-tailed, and differences were considered significant when $P < 0.05$.

Results

Mechanism Protocol in the Anesthetized Pig

Respiratory Variables. V_T was low and similar (volume-controlled ventilation: 6.7 ± 0.6 to 6.9 ± 0.5 ml/kg) in all four conditions ("condition" $P = 0.772$ by two-way repeated ANOVA) at baseline (paralyzed) and throughout titration of spontaneous effort ("time" $P = 0.081$ by two-way repeated ANOVA; Supplemental Digital Content table S1, http://links.lww.com/ALN/C801). The development of spontaneous breathing did not alter global V_T (as anticipated, given the volume-controlled ventilation). The swing (deflection) in esophageal pressure (ΔP_{eso}) increased until it reached –10 cm H_2O during spontaneous effort titration as per protocol in all groups (Supplemental Digital Content table S1, http://links.lww.com/ALN/C801).

Local Pleural Pressure during Spontaneous Effort. The regional distribution of pleural pressure (fig. 2) was measured and evaluated under the same amount of spontaneous effort in all conditions (*i.e.*, ΔP_{eso} = –10 cm H_2O). The magnitude of negative inspiratory pleural pressure in the dorsal (dependent) lung was almost twofold greater than negative inspiratory pleural pressure in the ventral (nondependent) lung at low PEEP in the supine position (Δ pleural pressure in ventral *vs.* dorsal lung: –9.8 ± 2.9 cm H_2O *vs.* –18.1 ± 4.0 cm H_2O; $P < 0.001$; fig. 2A). High PEEP in the supine position significantly reduced a ventral to dorsal gradient in inspiratory Δ pleural pressure (dorsal Δ pleural pressure in low PEEP *vs.* high PEEP: –18.1 ± 4.0 cm H_2O *vs.* –13.3 ± 2.3 cm H_2O; $P < 0.001$; fig. 2A *vs.* fig. 2B). In the prone position, however, there was no ventral to dorsal gradient in local Δ pleural pressure after diaphragmatic contraction, irrespective

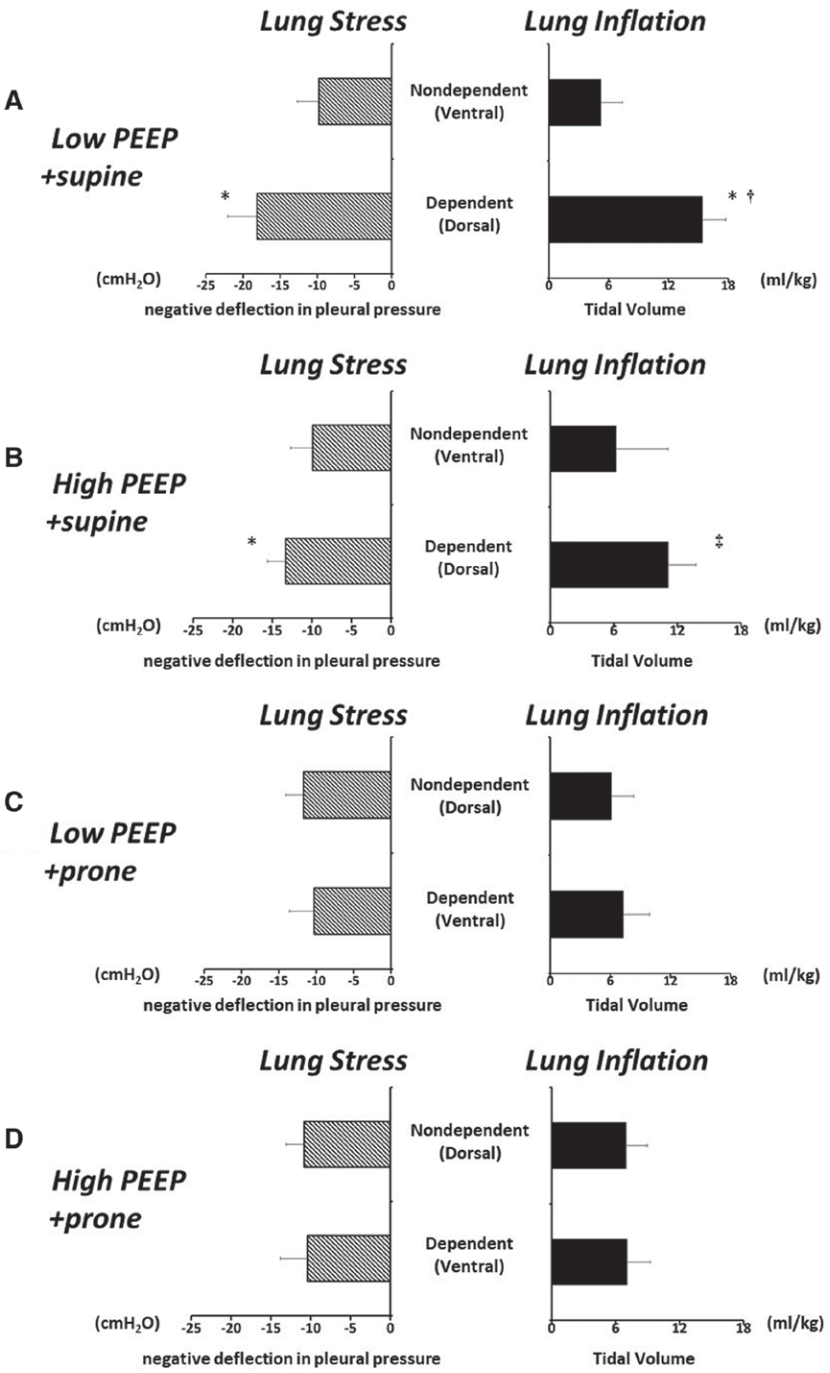

Fig. 2. Local pleural pressure and inflation imposed by spontaneous effort: series 1 mechanism protocol in pigs. The data are expressed as mean ± SD (*error bars*). The magnitudes of inspiratory negative pleural pressure (*left*) and lung inflation (*right*) were estimated at negative swing in esophageal pressure (P_{eso}) of approximately –10 cm H_2O and tidal volume (V_T) of approximately 7 ml/kg during volume-controlled ventilation in all conditions (*A* to *D*). The *x axes* in the *left panels* represent negative swings in pleural pressure measured by balloon catheter occlusion technique. In contrast, the *x axes* in the *right panels* represent the V_T levels required during controlled breaths (muscle paralysis) to obtain the same magnitude of local inflation (ΔZ in electrical impedance tomography) imposed by spontaneous effort (P_{eso} approximately –10 cm H_2O). (*A*) In low positive end-expiratory pressure (PEEP) and supine, a passive V_T of almost 16 ml/kg was required to obtain the same magnitude of local inflation imposed by spontaneous effort in the dependent lung, the same lung region where higher lung stress was concentrated. (*B*) High PEEP in supine decreased the ventral to dorsal gradient of negative pleural pressure swing, leading to decrease the degree of local dependent lung inflation imposed by spontaneous effort. In contrast, in the prone position, the distribution of lung stress and inflation during spontaneous effort was the same as during muscle paralysis, either at low PEEP (*C*) or high PEEP (*D*). *$P < 0.01$ compared with values in nondependent lung regions within groups; †$P < 0.01$ compared with values in dependent lung regions among all other conditions; ‡$P < 0.05$ compared with values in dependent lung regions among all other conditions.

of the PEEP level (Δ pleural pressure in ventral [dependent] *vs.* dorsal [nondependent] lung: −10.3 ± 3.3 cm H_2O *vs.* −11.7 ± 2.4 cm H_2O at low PEEP, $P = 0.115$; −10.4 ± 3.4 cm H_2O *vs.* −10.8 ± 2.3 cm H_2O at high PEEP, $P = 0.715$; fig. 2, C and D).

Local Lung Inflation during Spontaneous Effort versus *Muscle Paralysis.* When comparing the regional distribution of lung inflation, the strength of spontaneous effort was matched among animals (*i.e.*, ΔP_{eso} equaled −10 cm H_2O) under a fixed global V_T (volume-controlled mode: approximately 7 ml/kg, Supplemental Digital Content table S1, http://links.lww.com/ALN/C801). Local distribution of lung inflation imposed by spontaneous effort in electrical impedance tomography reflected the ventral to dorsal gradient in negative Δ pleural pressure during diaphragmatic contraction.

In the supine position during low PEEP, spontaneous effort increased local lung inflation in the dependent (dorsal) lung, in the same region where more negative Δ pleural pressure was localized (fig. 2A). At low PEEP and supine, a significantly larger passive V_T (15.4 ± 2.3 ml/kg, $P = 0.009$ *vs.* high PEEP and supine, $P < 0.001$ *vs.* high PEEP and prone, $P < 0.001$ *vs.* low PEEP and prone) was required to achieve inspiratory inflation of the dependent lung (fig. 2A) comparable to that achieved during spontaneous effort, despite limiting global V_T to approximately 7 ml/kg. The magnitude of local dependent lung inflation imposed by spontaneous effort was significantly less at high PEEP and supine ($P = 0.009$ *vs.* low PEEP and supine), and thus the distribution of lung inflation was similar among lung regions (passive V_T required in ventral [nondependent] *vs.* dorsal [dependent] lung: 6.2 ± 4.9 ml/kg *vs.* 11.2 ± 2.6 ml/kg; $P = 0.062$; fig. 2B). In the prone position, the distribution of lung inflation was not altered by spontaneous effort at low PEEP (passive V_T required in ventral [dependent] *vs.* dorsal [nondependent] lung: 7.3 ± 2.6 ml/kg *vs.* 6.1 ± 2.2 ml/kg; $P = 0.512$; fig. 2C) and at high PEEP (passive V_T required in ventral [dependent] *vs.* dorsal [nondependent] lung: 7.1 ± 2.2 ml/kg *vs.* 7.0 ± 2.0 ml/kg; $P = 0.943$; fig. 2D).

Lung Injury Protocol in the Anesthetized Rabbit

Respiratory Variables. The dose of propofol and ketamine was similar in the supine position *versus* the prone position (propofol: 19 ± 5 mg · kg^{-1} · h^{-1} *vs.* 22 ± 5 mg · kg^{-1} · h^{-1}, $P = 0.394$; ketamine: 3.1 ± 1.9 mg · kg^{-1} · h^{-1} *vs.* 2.1 ± 0.7 mg · kg^{-1} · h^{-1}, $P = 0.273$). The values of V_T (approximately 6 ml/kg) were similar in both groups ("group" $P = 0.853$ by two-way repeated ANOVA) throughout the protocol ("time" $P = 0.837$ by two-way repeated ANOVA). Oxygenation (Pao_2/Fio_2) was greater during spontaneous effort in the prone *versus* supine position (group $P = 0.008$ by two-way repeated ANOVA; table 1). In the supine position, oxygenation increased transiently for approximately the first hour after commencement of spontaneous breathing and decreased thereafter (table 1). Respiratory system compliance decreased over time in the supine position with spontaneous effort but did not decrease in the prone position with spontaneous effort. Respiratory system compliance was higher after 2 h in the prone position *vs.* supine position (respiratory system compliance at 4 h: 2.1 ± 0.9 ml/cm H_2O *vs.* 1.1 ± 0.2 ml/cm H_2O; $P = 0.034$; table 1).

Spontaneous Effort in Supine versus *Prone Position.* The intensity of spontaneous effort in terms of frequency (estimated by respiratory rate) and magnitude (estimated by negative ΔP_{eso}) was significantly less in the prone *versus* supine groups (table 1; fig. 3A) despite the use of the same doses of sedatives, the maintenance of constant Pao_2 (approximately 100 mg by adjusting Fio_2), and the same value of $Paco_2$ (table 1). The deflections in ΔP_{eso} became significantly more negative in the supine position but remained constant in the prone position (ΔP_{eso} at 4 h: −3.9 ± 1.3 cm H_2O *vs.* −1.6 ± 1.1 cm H_2O; $P = 0.008$; fig. 3A). Spontaneous respiratory rate (and thus minute ventilation) was significantly higher in the supine *vs.* prone groups (table 1). At all times during spontaneous breathing after time zero, the peak Δ transpulmonary pressure (at maximum inspiration) was greater in the supine group *versus* the prone group (fig. 3B).

Lung Injury in Supine versus *Prone Position.* Overall lung injury was less in the prone *versus* supine groups in terms of wet/dry lung weight ratio (fig. 4A) and protein concentration in bronchoalveolar fluid (fig. 4B). The regional patterns of injury also differed between the groups. In the supine group, the lung tissue myeloperoxidase expression was higher in the dependent (dorsal) lung, in the same regions where spontaneous effort increased lung stress and inflation (67.5 ± 38.1 μm/min/mg protein *vs.* 167.7 ± 65.5 μm/min/mg protein in the nondependent *vs.* dependent lung; $P = 0.003$; fig. 5A), but there were no regional differences in myeloperoxidase expression in the prone group (61.0 ± 23.0 μm/min/mg protein *vs.* 74.0 ± 30.9 μm/min/mg protein in the nondependent *vs.* dependent lung; $P = 0.951$; fig. 5B). The distribution of "histologic" injury in each group is presented with illustrative sections (Fig. 5).

Discussion

The prone position in severe ARDS has been traditionally used under passive conditions (*i.e.*, under muscle paralysis or deep sedation).[16] The current data suggest that the prone position could be an option to minimize lung injury from spontaneous effort in severe ARDS. This is because the prone position, independent of PEEP levels, diminishes the maldistribution of lung stress and thus the asymmetric, injurious lung inflation associated with spontaneous effort, and also because the prone position mitigates the magnitude of spontaneous efforts.

Ventilator-induced Lung Injury *versus* Effort-dependent Lung Injury

Using histology, computed tomography, and positron emission tomography imaging of [^{18}F]fluoro-2-deoxy-D-glucose,

Table 1. Respiratory Parameters in the Anesthetized Rabbit (n = 6/Each Group)

Parameter	Group	Lung Injury	Measurement				
			0 h after Start of Lung Injury Protocol	1 h after Start of Lung Injury Protocol	2 h after Start of Lung Injury Protocol	3 h after Start of Lung Injury Protocol	4 h after Start of Lung Injury Protocol
Pa_{O_2}/F_{IO_2}, mmHg	Supine	100 ± 15	204 ± 84	298 ± 138	219 ± 71	148 ± 75	106 ± 32
	Prone	113 ± 19	343 ± 159	422 ± 159	432 ± 161*	418 ± 171*	420 ± 179*
Pa_{CO_2}, mmHg	Supine	40 ± 9	65 ± 17	51 ± 6	44 ± 10†	39 ± 9†	39 ± 5†
	Prone	40 ± 10	63 ± 11	56 ± 10†	41 ± 7†	40 ± 8†	38 ± 11†
Peak airway pressure, cm H_2O	Supine	22.7 ± 3.7	25.6 ± 4.8	28.7 ± 6.5†	29.1 ± 3.7†	29.4 ± 4.0†	29.1 ± 4.0†
	Prone	21.3 ± 4.3	23.9 ± 9.1	24.0 ± 8.6	22.2 ± 9.0	20.6 ± 8.1*†	20.6 ± 8.6*†
Plateau airway pressure, cm H_2O	Supine	20.5 ± 2.9	21.7 ± 3.2	26.5 ± 5.5†	26.6 ± 3.4†	26.7 ± 3.9†	26.3 ± 3.6†
	Prone	19.3 ± 3.8	21.1 ± 8.5	20.5 ± 7.8	20.0 ± 7.9	18.3 ± 7.2*†	18.3 ± 7.6*†
Mean airway pressure, cm H_2O	Supine	13.0 ± 2.6	13.6 ± 3.1	14.6 ± 3.6	14.8 ± 2.5	14.3 ± 3.2	14.3 ± 2.5
	Prone	12.0 ± 4.6	12.8 ± 5.5	12.7 ± 5.3	12.2 ± 5.4	11.7 ± 4.8†	11.9 ± 4.9†
Positive end-expiratory pressure, cm H_2O	Supine	8.4 ± 2.6	8.4 ± 3.0	8.3 ± 3.0	8.1 ± 3.1	8.1 ± 3.3	8.0 ± 2.9
	Prone	7.5 ± 4.2	7.5 ± 3.7	7.6 ± 3.8	7.6 ± 3.8	7.1 ± 3.4	7.3 ± 3.6
Tidal volume, ml/kg	Supine	5.5 ± 1.2	6.0 ± 0.4	6.2 ± 0.7	6.1 ± 0.6	6.1 ± 0.4	6.2 ± 0.5
	Prone	5.4 ± 1.7	5.9 ± 0.5	6.2 ± 0.2	6.1 ± 0.5	6.1 ± 0.6	6.1 ± 0.9
Respiratory rate, breaths/min	Supine	40 ± 0	107 ± 23	113 ± 19	113 ± 11	112 ± 10	112 ± 12
	Prone	40 ± 0	84 ± 4	81 ± 5*	82 ± 7*	78 ± 7*	78 ± 8*
Minute volume, l/min	Supine	0.8 ± 0.2	2.2 ± 0.4	2.4 ± 0.6	2.4 ± 0.5	2.4 ± 0.4	2.4 ± 0.5
	Prone	0.8 ± 0.3	1.8 ± 0.2*	1.8 ± 0.2*	1.7 ± 0.1*	1.7 ± 0.1*	1.7 ± 0.3*
Respiratory system compliance, ml/cm H_2O	Supine	1.6 ± 0.3	1.5 ± 0.1	1.1 ± 0.4†	1.0 ± 0.2†	1.0 ± 0.2†	1.1 ± 0.2†
	Prone	1.6 ± 0.5	1.7 ± 0.8	1.8 ± 0.8	1.8 ± 0.8*	2.0 ± 0.7*	2.1 ± 0.9*
Peak transpulmonary pressure, cm H_2O	Supine	18.8 ± 3.6	22.9 ± 2.9	28.0 ± 5.7†	29.1 ± 2.6†	28.2 ± 2.4†	28.5 ± 2.4†
	Prone	16.8 ± 3.3	19.6 ± 8.8	19.0 ± 8.6	18.3 ± 8.4*	16.8 ± 8.5*†	16.4 ± 7.9*†
Plateau transpulmonary pressure, cm H_2O	Supine	16.4 ± 3.0	18.4 ± 1.9	22.2 ± 4.7†	22.4 ± 2.9†	21.5 ± 2.6†	22.3 ± 2.0†
	Prone	14.4 ± 2.9	14.4 ± 8.0	13.6 ± 7.9*	13.8 ± 7.3*	12.6 ± 7.7*	12.2 ± 7.2*

*$P < 0.05$ compared with supine. †$P < 0.05$ compared with 0 (at the start of the protocol) within groups.
F_{IO_2}, fractional inspired oxygen tension.

previous studies revealed that ventilator-induced lung injury occurred in nondependent (ventral) lung regions in animal models of ARDS (rats, rabbits, pigs)[6,22,23] and patients with ARDS.[24,25] During a controlled breath, ventilation is likely to shift to nondependent (ventral) lung regions because of spatial heterogeneity of lung aeration, *i.e.*, more atelectasis in the more dependent (dorsal) lung, and therefore a small percentage of the nondependent lung is more susceptible to higher inspiratory stress and strain in the supine position. On the other hand, the prone position decreases such spatial heterogeneity of lung aeration, leading to more even distribution of tidal strain and [^{18}F]fluoro-2-deoxy-D-glucose uptake.[26] Therefore, the prone position is known to reduce ventilator-induced lung injury[12] and improve mortality in severe ARDS[16]

The current study confirmed that spontaneous effort altered the locus of lung injury: the bulk of effort-dependent lung injury occurred in the dependent (dorsal) lung,[6,27,28] the same region where spontaneous inspiratory effort increased greater inspiratory lung stress and caused overinflation (figs. 2 and 5). Of note, the combination of low levels of PEEP and the supine position appears to pose the greatest risk of effort-dependent lung injury (fig. 2).

The maldistribution of lung stress during spontaneous effort was most manifested in the supine position with low PEEP. While the strength of spontaneous effort (measured as ΔP_{eso} equals −10 cm H_2O) was maintained to be the same among all conditions in the pig experiments, lower PEEP in the supine position was associated with the highest local lung stress in the dependent lung and thus the greatest magnitude of local lung inflation in the dependent lung. Therefore, overall lung injury from spontaneous effort was greater in the supine position (fig. 4), and the lung tissue myeloperoxidase expression and lung histologic injury were higher in dependent (dorsal) lung (fig. 5).

Mechanisms of Protection: Impact of Position

Prone position (*vs.* supine position) was effective to minimize effort-dependent lung injury, as evident from better gas exchange, better respiratory system compliance, lower wet/dry lung weight ratio, lower bronchoalveolar fluid protein concentration, and less lung tissue myeloperoxidase activity. The overall burden of lung injury was less, and there was no difference between degrees of injury in dorsal *versus* ventral lung. Several mechanisms were revealed from this study.

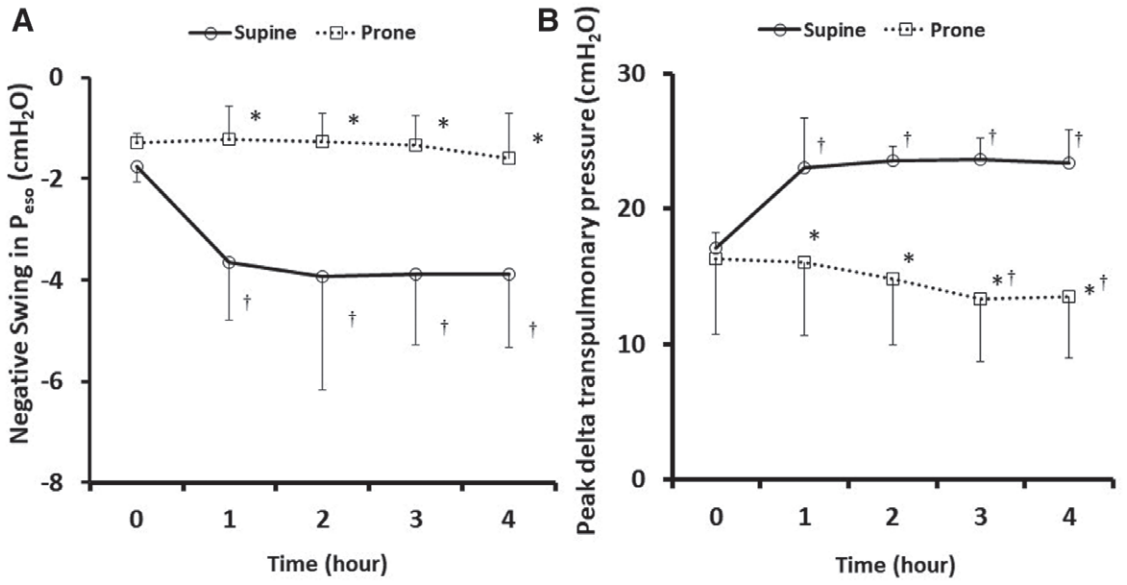

Fig. 3. Intensity of spontaneous effort and dynamic lung stress in supine *versus* prone- series 2 lung injury protocol in rabbits. The data are expressed as mean ± SD (*error bars*). The intensity of inspiratory effort (series 2 rabbit) was evaluated as the magnitude of the negative swing in esophageal pressure (ΔP_{eso}). (*A*) ΔP_{eso} was less in the prone position *versus* the supine position throughout the protocol. (*B*) As a result, peak Δ transpulmonary pressure, a surrogate of dynamic lung stress, was less in the prone position *versus* the supine position throughout the protocol. Peak Δ transpulmonary pressure decreased over time in the prone position. $P < 0.05$ compared with supine; †$P < 0.05$ compared with 0 h (at the start of the protocol) within groups.

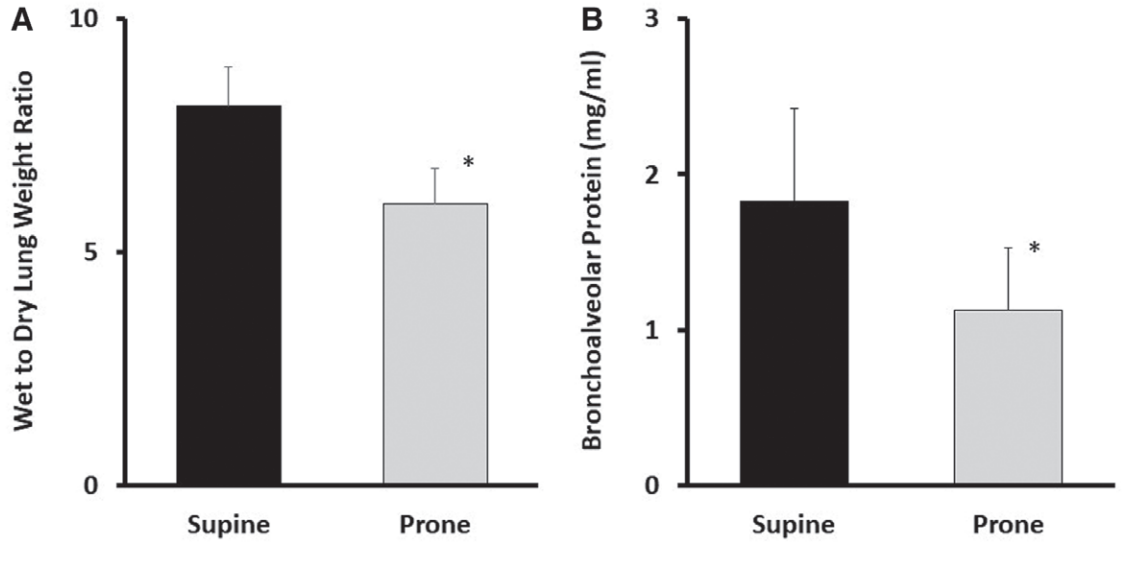

Fig. 4. Overall lung injury in the supine position *versus* prone position: series 2 lung injury protocol in rabbits. The data are expressed as mean ± SD (*error bars*). (*A*) Wet/dry lung weight ratio was less in the prone position. (*B*) Protein in bronchoalveolar fluid was less in the prone position. *$P < 0.01$ compared with supine.

First, the prone position had no ventral to dorsal gradient in local Δ pleural pressure after diaphragmatic contraction, and therefore the magnitude of local lung inflation during spontaneous breathing is the same as under V_T at approximately ≈7 ml/kg during muscle paralysis (fig. 2, C and D). This might be explained partially by the gravitational

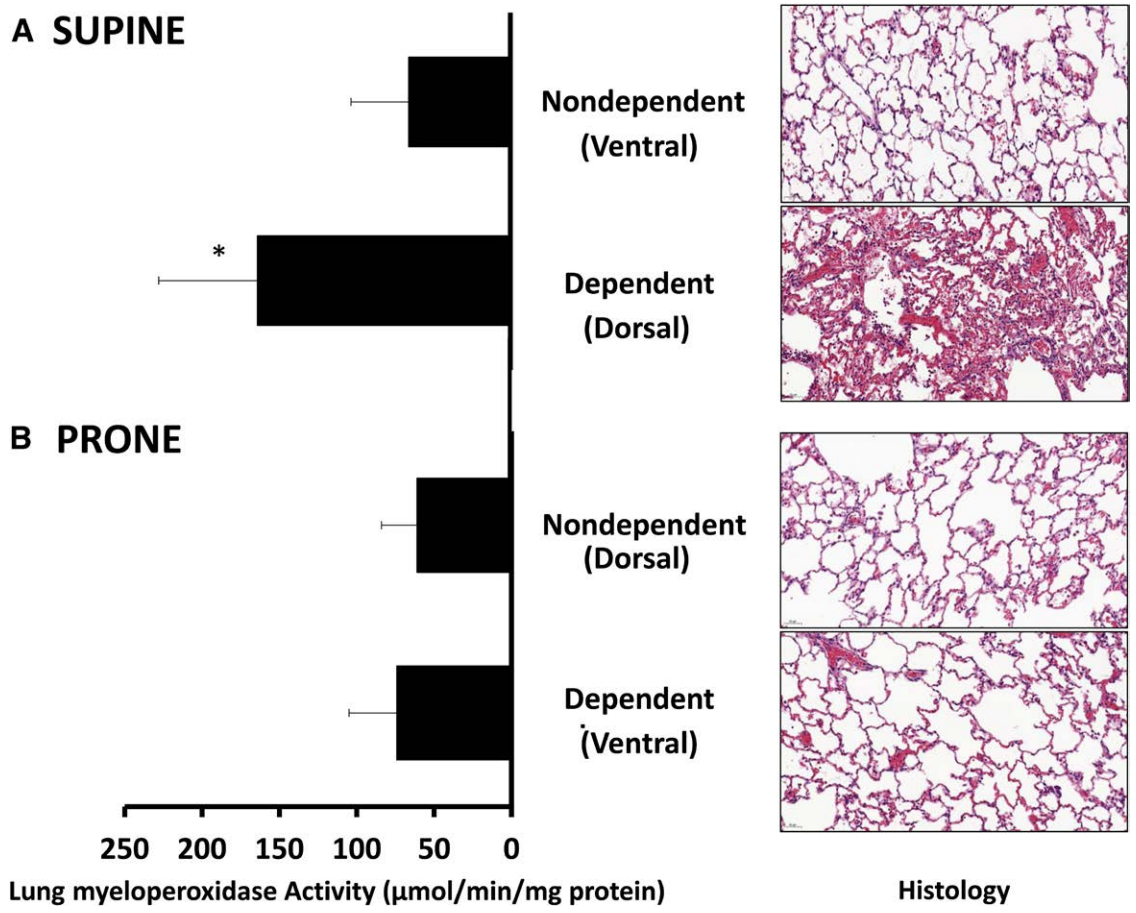

Fig. 5. Regional lung injury in the supine position *versus* prone position: series 2 lung injury protocol in rabbits. The data are expressed as mean ± SD (*error bars*). The regional patterns of injury differed between the groups. (*A*) The lung tissue myeloperoxidase expression was higher in the dependent (dorsal) lung, in the same region where spontaneous effort increased lung inflation, *versus* the nondependent (ventral) lung in the supine position. Representative images (original magnification, ×20; hematoxylin and eosin) are shown. (*Right upper*) Nondependent lung in the supine position. (*Right lower*) Dependent lung in the supine position. In accordance with the regional patterns of lung tissue myeloperoxidase expression, in the supine position, spontaneous effort increased dependent lung injury, *i.e.*, more hyaline membrane formation, severe alveolar hemorrhaging, more neutrophil infiltration into the alveoli and interstitium. (*B*) There were no regional differences in myeloperoxidase expression in the prone position. Representative images (original magnification, ×20; hematoxylin and eosin) are shown. (*Right upper*) Nondependent lung in the prone position. (*Right lower*) Dependent lung in the prone position. In the prone position, the magnitude of lung injury was not different between nondependent lung and dependent lung. *$P < 0.01$ compared with all other regions.

translocation of atelectatic solid-like lung tissue (which impedes pressure transmission) from the dorsal to ventral lung. This explanation is likely because "baby lung" is considered a functional entity (but not an anatomical entity) that can change its location with position and level of PEEP.[11,29] Importantly, previous studies show that dorsal muscular regions of the diaphragm move more than ventral regions of the diaphragm during spontaneous breathing, regardless of the body position.[30,31] Thus, the prone position decreases atelectatic solid-like lung tissue in the dorsal lung facing the well-moved, dorsal muscular regions of the diaphragm, which may facilitate the uniform transmission of Δ pleural pressure to the entire lung surface from where it was generated, after diaphragmatic contraction.

Second, the intensity of spontaneous effort (indicated by ΔP_{eso}, respiratory rate in fig. 3A and table 1) was lower in the prone position (*vs.* supine position), despite matching levels of sedation. Because the prone position mitigates injurious spontaneous effort, it resulted in lower peak Δ transpulmonary pressure (*i.e.*, dynamic lung stress). Of note, the benefit of the prone position in reducing the intensity of spontaneous effort was documented not only in rabbits (fig. 3A) but also in humans (infants,[32,33] patients with ARDS,[34] hypoxic patients with COVID-19[35]). Several plausible explanations are offered. First, the prone position increases end-expiratory lung volume in some patients (probably depending on lung recruitablity, the shape of the chest wall, the presence of abdominal

hypertension, and the presence of support).[11] In this study, the prone position was effective to recruit lung and increase lung volume in rabbits, suggested by higher respiratory system compliance in the prone position (table 1). Higher lung volume shortens diaphragm length, resulting in less force generation from the diaphragm.[9,10] Second, the prone position *per se* is known to shorten diaphragm length even with the same end-expiratory lung volume as in the supine position, probably due to altered chest wall configuration and diaphragm geometry.[36,37] Of course, the force generated by diaphragmatic contraction decreases as its length shortens.[38]

Therefore, the current study adds a promising technique to facilitate safe spontaneous breathing during mechanical ventilation in severe ARDS. It may synergize the benefits of spontaneous breathing (less muscle atrophy, more physiologic) with the benefits of the prone position *per se* (less ventilator-induced lung injury, more opening of well perfused regions). The current data support a larger clinical study as a next step to confirm the benefits of the prone position to render spontaneous effort less injurious in patients with ARDS whose spontaneous effort is vigorous.

Spontaneous Breathing and Prone Position Related to COVID-19

In the era of the COVID-19 pandemic, the indication of the prone position has been expanding: the prone position is now applied to nonintubated, hypoxic patients with COVID-19 (before intubation, not as severe as moderate-to-severe ARDS), hoping that being awake in the prone position might improve gas exchange, decrease the strength of spontaneous effort, minimizing the risk of effort-dependent lung injury, and thereby avoiding tracheal intubation.[35,39,40] A few case reports observed that the prone position was associated with better gas exchange and lower respiratory rate,[41,42] and a recent large randomized clinical trial has confirmed that being awake in the prone position significantly improved oxygenation, decreased the respiratory rate, and decreased the incidence of treatment failure and the need of intubation.[35] Therefore, the beneficial effects of the prone position to mitigate effort-dependent lung injury has been found not only in mechanically ventilated patients with ARDS[34] but also in nonintubated hypoxemic patients with COVID-19.[35] The current physiologic study may reveal potential protective mechanisms of the prone position from spontaneous breathing.

Limitations

There are several limitations to the current work. First, we utilized two different species (pigs and rabbits). Larger animals are closer to human physiology, so they are suitable for exploring the mechanism. The smaller animals are known to have a shorter (and steeper) trajectory of lung injury, so rabbits are more suitable for evaluating injury in a shorter time period; the overall consistent results in rabbits (location of injury) and pigs (location of lung stress and inflation) are reassuring. Second, different ventilatory modes were used (volume-controlled in pigs, pressure-controlled in rabbits). No differences in the patterns and magnitudes of dependent lung inflation imposed by spontaneous effort were observed between the volume-controlled mode and the pressure-controlled mode.[7] Thus, the difference in ventilatory mode does not affect interpretation of the data. Third, the current study lacked paralyzed groups in the lung injury protocol. We chose supine and spontaneous breathing as a control group to compare with prone and spontaneous breathing. We cannot separate completely the benefits of lowering spontaneous effort from those of prone position *per se*. Fourth, our study included a single sex (males), the rationale being minimizing data variability. The potentially confounding effects of this sex bias on the meaning of single-sex experimental data should be considered.[43]

Conclusions

The current animal study found that the prone position, independent of PEEP levels, diminished a maldistribution of lung stress and thus asymmetric, injurious lung inflation associated with spontaneous effort and mitigated spontaneous effort, resulting in less effort-dependent lung injury.

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Competing Interests

The authors declare no competing interests.

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References

1. Putensen C, Muders T, Varelmann D, Wrigge H: The impact of spontaneous breathing during mechanical ventilation. Curr Opin Crit Care 2006; 12:13–8
2. Yoshida T, Torsani V, Gomes S, De Santis RR, Beraldo MA, Costa EL, Tucci MR, Zin WA, Kavanagh BP, Amato MB: Spontaneous effort causes occult pendelluft during mechanical ventilation. Am J Respir Crit Care Med 2013; 188:1420–7
3. Goligher EC, Dres M, Fan E, Rubenfeld GD, Scales DC, Herridge MS, Vorona S, Sklar MC, Rittayamai N, Lanys A, Murray A, Brace D, Urrea C, Reid WD, Tomlinson G, Slutsky AS, Kavanagh BP, Brochard LJ, Ferguson ND: Mechanical ventilation-induced diaphragm atrophy strongly impacts clinical outcomes. Am J Respir Crit Care Med 2018; 197:204–13
4. Yoshida T, Uchiyama A, Matsuura N, Mashimo T, Fujino Y: The comparison of spontaneous breathing and muscle paralysis in two different severities of experimental lung injury. Crit Care Med 2013; 41:536–45
5. Güldner A, Pelosi P, Gama de Abreu M: Spontaneous breathing in mild and moderate *versus* severe acute respiratory distress syndrome. Curr Opin Crit Care 2014; 20:69–76
6. Morais CCA, Koyama Y, Yoshida T, Plens GM, Gomes S, Lima CAS, Ramos OPS, Pereira SM, Kawaguchi N, Yamamoto H, Uchiyama A, Borges JB, Vidal Melo MF, Tucci MR, Amato MBP, Kavanagh BP, Costa ELV, Fujino Y: High positive end-expiratory pressure renders spontaneous effort noninjurious. Am J Respir Crit Care Med 2018; 197:1285–96
7. Yoshida T, Nakahashi S, Nakamura MAM, Koyama Y, Roldan R, Torsani V, De Santis RR, Gomes S, Uchiyama A, Amato MBP, Kavanagh BP, Fujino Y: Volume-controlled ventilation does not prevent injurious inflation during spontaneous effort. Am J Respir Crit Care Med 2017; 196:590–601
8. Spinelli E, Mauri T, Beitler JR, Pesenti A, Brodie D: Respiratory drive in the acute respiratory distress syndrome: Pathophysiology, monitoring, and therapeutic interventions. Intensive Care Med 2020; 46:606–18
9. Pengelly LD, Alderson AM, Milic-Emili J: Mechanics of the diaphragm. J Appl Physiol 1971; 30:797–805
10. Laghi F, Harrison MJ, Tobin MJ: Comparison of magnetic and electrical phrenic nerve stimulation in assessment of diaphragmatic contractility. J Appl Physiol (1985) 1996; 80:1731–42
11. Gattinoni L, Taccone P, Carlesso E, Marini JJ: Prone position in acute respiratory distress syndrome: Rationale, indications, and limits. Am J Respir Crit Care Med 2013; 188:1286–93
12. Broccard A, Shapiro RS, Schmitz LL, Adams AB, Nahum A, Marini JJ: Prone positioning attenuates and redistributes ventilator-induced lung injury in dogs. Crit Care Med 2000; 28:295–303
13. Yoshida T, Roldan R, Beraldo MA, Torsani V, Gomes S, De Santis RR, Costa EL, Tucci MR, Lima RG, Kavanagh BP, Amato MB: Spontaneous effort during mechanical ventilation: Maximal injury with less positive end-expiratory pressure. Crit Care Med 2016; 44:e678–88
14. Teggia Droghi M, De Santis Santiago RR, Pinciroli R, Marrazzo F, Bittner EA, Amato MBP, Kacmarek RM, Berra L: High positive end-expiratory pressure allows extubation of an obese patient. Am J Respir Crit Care Med 2018; 198:524–5
15. Grieco DL, Maggiore SM, Roca O, Spinelli E, Patel BK, Thille AW, Barbas CSV, de Acilu MG, Cutuli SL, Bongiovanni F, Amato M, Frat JP, Mauri T, Kress JP, Mancebo J, Antonelli M: Non-invasive ventilatory support and high-flow nasal oxygen as first-line treatment of acute hypoxemic respiratory failure and ARDS. Intensive Care Med 2021; 47:851–66
16. Guérin C, Reignier J, Richard JC, Beuret P, Gacouin A, Boulain T, Mercier E, Badet M, Mercat A, Baudin O, Clavel M, Chatellier D, Jaber S, Rosselli S, Mancebo J, Sirodot M, Hilbert G, Bengler C, Richecoeur J, Gainnier M, Bayle F, Bourdin G, Leray V, Girard R, Baboi L, Ayzac L; PROSEVA Study Group: Prone positioning in severe acute respiratory distress syndrome. N Engl J Med 2013; 368:2159–68
17. Martin CJ, Young AC, Ishikawa K: Regional lung mechanics in pulmonary disease. J Clin Invest 1965; 44:906–13
18. Baydur A, Behrakis PK, Zin WA, Jaeger M, Milic-Emili J: A simple method for assessing the validity of the esophageal balloon technique. Am Rev Respir Dis 1982; 126:788–91
19. Lachmann B, Robertson B, Vogel J: *In vivo* lung lavage as an experimental model of the respiratory distress syndrome. Acta Anaesthesiol Scand 1980; 24:231–6
20. Yoshida T, Engelberts D, Otulakowski G, Katira B, Post M, Ferguson ND, Brochard L, Amato MBP, Kavanagh BP: Continuous negative abdominal pressure recruits lungs at lower distending pressures. Am J Respir Crit Care Med 2018; 197:534–7
21. Rodriguez-Palacios A, Kodani T, Kaydo L, Pietropaoli D, Corridoni D, Howell S, Katz J, Xin W, Pizarro TT, Cominelli F: Stereomicroscopic 3D-pattern profiling of murine and human intestinal inflammation reveals unique structural phenotypes. Nat Commun 2015; 6:7577
22. Tsuchida S, Engelberts D, Peltekova V, Hopkins N, Frndova H, Babyn P, McKerlie C, Post M, McLoughlin P, Kavanagh BP: Atelectasis causes alveolar injury in

nonatelectatic lung regions. Am J Respir Crit Care Med 2006; 174:279–89
23. Borges JB, Costa EL, Suarez-Sipmann F, Widström C, Larsson A, Amato M, Hedenstierna G: Early inflammation mainly affects normally and poorly aerated lung in experimental ventilator-induced lung injury. Crit Care Med 2014; 42:e279–87
24. Bellani G, Messa C, Guerra L, Spagnolli E, Foti G, Patroniti N, Fumagalli R, Musch G, Fazio F, Pesenti A: Lungs of patients with acute respiratory distress syndrome show diffuse inflammation in normally aerated regions: A [^{18}F]-fluoro-2-deoxy-D-glucose PET/CT study. Crit Care Med 2009; 37:2216–22
25. Bellani G, Guerra L, Musch G, Zanella A, Patroniti N, Mauri T, Messa C, Pesenti A: Lung regional metabolic activity and gas volume changes induced by tidal ventilation in patients with acute lung injury. Am J Respir Crit Care Med 2011; 183:1193–9
26. Motta-Ribeiro GC, Hashimoto S, Winkler T, Baron RM, Grogg K, Paula LFSC, Santos A, Zeng C, Hibbert K, Harris RS, Bajwa E, Vidal Melo MF: Deterioration of regional lung strain and inflammation during early lung injury. Am J Respir Crit Care Med 2018; 198:891–902
27. Kiss T, Bluth T, Braune A, Huhle R, Denz A, Herzog M, Herold J, Vivona L, Millone M, Bergamaschi A, Andreeff M, Scharffenberg M, Wittenstein J, Vidal Melo MF, Koch T, Rocco PRM, Pelosi P, Kotzerke J, Gama de Abreu M: Effects of positive end-expiratory pressure and spontaneous breathing activity on regional lung inflammation in experimental acute respiratory distress syndrome. Crit Care Med 2019; 47:e358–65
28. Hurtado DE, Erranz B, Lillo F, Sarabia-Vallejos M, Iturrieta P, Morales F, Blaha K, Medina T, Diaz F, Cruces P: Progression of regional lung strain and heterogeneity in lung injury: Assessing the evolution under spontaneous breathing and mechanical ventilation. Ann Intensive Care 2020; 10:107
29. Gattinoni L, Pesenti A: The concept of "baby lung." Intensive Care Med 2005; 31:776–84
30. Krayer S, Rehder K, Vettermann J, Didier EP, Ritman EL: Position and motion of the human diaphragm during anesthesia-paralysis. Anesthesiology 1989; 70:891–8
31. Tomita K, Sakai Y, Monma M, Ohse H, Imura S: Analysis of diaphragmatic motion with prone positioning using dynamic MRI. J Phys Ther Sci 2004; 16: 85–89
32. Dimitriou G, Greenough A, Pink L, McGhee A, Hickey A, Rafferty GF: Effect of posture on oxygenation and respiratory muscle strength in convalescent infants. Arch Dis Child Fetal Neonatal Ed 2002; 86:F147–50
33. Baudin F, Emeriaud G, Essouri S, Beck J, Portefaix A, Javouhey E, Guerin C: Physiological effect of prone position in children with severe bronchiolitis: A randomized cross-over study (BRONCHIO-DV). J Pediatr 2019; 205:112–9.e4
34. Yoshida T, Tanaka A, Roldan R, Quispe R, Taenaka H, Uchiyama A, Fujino Y: Prone position reduces spontaneous inspiratory effort in patients with acute respiratory distress syndrome: A bicenter study. Am J Respir Crit Care Med 2021; 203:1437–40
35. Ehrmann S, Li J, Ibarra-Estrada M, Perez Y, Pavlov I, McNicholas B, Roca O, Mirza S, Vines D, Garcia-Salcido R, Aguirre-Avalos G, Trump MW, Nay MA, Dellamonica J, Nseir S, Mogri I, Cosgrave D, Jayaraman D, Masclans JR, Laffey JG, Tavernier E; Awake Prone Positioning Meta-Trial Group: Awake prone positioning for COVID-19 acute hypoxaemic respiratory failure: A randomised, controlled, multinational, open-label meta-trial. Lancet Respir Med 2021; 9:1387–95
36. Rehan VK, Nakashima JM, Gutman A, Rubin LP, McCool FD: Effects of the supine and prone position on diaphragm thickness in healthy term infants. Arch Dis Child 2000; 83:234–8
37. Sprung J, Deschamps C, Margulies SS, Hubmayr RD, Rodarte JR: Effect of body position on regional diaphragm function in dogs. J Appl Physiol (1985) 1990; 69:2296–302
38. Evans CL, Hill AV: The relation of length to tension development and heat production on contraction in muscle. J Physiol 1914; 49:10–6
39. Marini JJ, Gattinoni L: Management of COVID-19 respiratory distress. JAMA 2020; 323:2329–30
40. Coppo A, Bellani G, Winterton D, Di Pierro M, Soria A, Faverio P, Cairo M, Mori S, Messinesi G, Contro E, Bonfanti P, Benini A, Valsecchi MG, Antolini L, Foti G: Feasibility and physiological effects of prone positioning in non-intubated patients with acute respiratory failure due to COVID-19 (PRON-COVID): A prospective cohort study. Lancet Respir Med 2020; 8:765–74
41. Sartini C, Tresoldi M, Scarpellini P, Tettamanti A, Carcò F, Landoni G, Zangrillo A: Respiratory parameters in patients with COVID-19 after using noninvasive ventilation in the prone position outside the intensive care unit. JAMA 2020; 323:2338–40
42. Damarla M, Zaeh S, Niedermeyer S, Merck S, Niranjan-Azadi A, Broderick B, Punjabi N: Prone positioning of nonintubated patients with COVID-19. Am J Respir Crit Care Med 2020; 202:604–6
43. Vutskits L, Clark JD, Kharasch ED: Reporting laboratory and animal research in Anesthesiology: The importance of sex as a biologic variable. Anesthesiology 2019; 131:949–52