Continuous Negative Abdominal Pressure Reduces Ventilator-induced Lung Injury in a Porcine Model

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ABSTRACT

Background: In supine patients with acute respiratory distress syndrome, the lung typically partitions into regions of dorsal atelectasis and ventral aeration ("baby lung"). Positive airway pressure is often used to recruit atelectasis, but often overinflates ventral (already aerated) regions. A novel approach to selective recruitment of dorsal atelectasis is by "continuous negative abdominal pressure."

Methods: A randomized laboratory study was performed in anesthetized pigs. Lung injury was induced by surfactant lavage followed by 1 h of injurious mechanical ventilation. Randomization (five pigs in each group) was to positive end-expiratory pressure (PEEP) alone or PEEP with continuous negative abdominal pressure (-5 cm H_2O *via* a plexiglass chamber enclosing hindlimbs, pelvis, and abdomen), followed by 4 h of injurious ventilation (high tidal volume, 20 ml/kg; low expiratory transpulmonary pressure, -3 cm H_2O). The level of PEEP at the start was ≈ 7 (*vs.* ≈ 3) cm H_2O in the PEEP (*vs.* PEEP plus continuous negative abdominal pressure) groups. Esophageal pressure, hemodynamics, and electrical impedance tomography were recorded, and injury determined by lung wet/dry weight ratio and interleukin-6 expression.

Results: All animals survived, but cardiac output was decreased in the PEEP group. Addition of continuous negative abdominal pressure to PEEP resulted in greater oxygenation (Pa_{O_2}/fractional inspired oxygen 316 ± 134 *vs.* 80 ± 24 mmHg at 4 h, $P = 0.005$), compliance (14.2 ± 3.0 *vs.* 10.3 ± 2.2 ml/cm H_2O, $P = 0.049$), and homogeneity of ventilation, with less pulmonary edema ($\approx 10\%$ less) and interleukin-6 expression ($\approx 30\%$ less).

Conclusions: Continuous negative abdominal pressure added to PEEP reduces ventilator-induced lung injury in a pig model compared with PEEP alone, despite targeting identical expiratory transpulmonary pressure. (ANESTHESIOLOGY 2018; 129:163-72)

THE lungs of patients with adult respiratory distress syndrome are often compartmentalized into two regions, aerated *versus* atelectatic.[1] In the supine position, the aerated region is usually in nondependent lung and receives most of the tidal volume(V_T). The weight of the edematous lung and an increased pleural pressure gradient explain the propensity for atelectasis to develop in dependent regions[2–4]; this is accentuated by sedation and neuromuscular blockade, lowering diaphragm tone and permitting the abdominal contents to shift the diaphragm cephalad.[5,6]

The conventional approaches to recruitment of atelectasis are prone positioning and increasing airway pressure. Prone positioning in adult respiratory distress syndrome can successfully recruit dependent atelectasis and improve survival, but recent comprehensive data (459 intensive care units, 50 countries) make it clear that clinicians seldom use this (used in less than 20% of cases).[7]

Elevated airway pressure (*e.g.*, positive end-expiratory pressure [PEEP] or high frequency oscillatory ventilation] are commonly used to recruit atelectasis but have failed to improve outcome in clinical trials.[8–11] One reason may be that increased airway pressure tends to recruit atelectasis only after overinflating (and potentially injuring) already aerated lung.[12]

> **What We Already Know about This Topic**
>
> - Atelectasis commonly develops in dependent lung regions in patients with adult respiratory distress syndrome
> - Prone position and increased airway pressure may reverse atelectasis but often fail
> - In a pig model of adult respiratory distress syndrome, continuous negative abdominal pressure effectively recruited dorsal atelectasis
>
> **What This Article Tells Us That Is New**
>
> - In a pig adult respiratory distress syndrome model, addition of continuous negative abdominal pressure (-5 cm H_2O) to positive end-expiratory pressure (PEEP), compared with PEEP alone (where transpulmonary pressure was matched in each group), resulted in better oxygenation, compliance, and homogeneity of ventilation, as well as less lung injury
> - PEEP with continuous negative abdominal pressure might be a treatment option for adult respiratory distress syndrome by recruiting atelectasis and minimizing ventilator-induced lung injury, but its efficacy and long-term effects in patients are not yet known

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Reducing intraabdominal pressure could also recruit dependent atelectasis. For example, upright positioning can improve oxygenation, but it is usually not feasible in the critically ill.[13,14] Nonetheless, the effect is likely mediated through reduction of pleural pressure in the dependent (but not in nondependent) lung, as is observed after experimental removal of abdominal contents.[15] Because distending (transpulmonary) pressure is the difference between airway and pleural pressures, reducing the vertical "gradient" of pleural pressures observed in experimental removal of abdominal contents[15] would selectively increase distending pressure in dependent "atelectatic" lung but not in the nondependent lung.

Continuous negative abdominal pressure has been tested with the aim of decreasing abdominal pressure or to improve hemodynamics.[16–18] We[19] and others[17,18] have previously attempted this in pilot studies for dependent atelectasis, but the effectiveness was limited by incomplete application of abdominal pressure[17,18] or the use of a rodent model.[19]

We recently reported that continuous negative abdominal pressure can selectively recruit dependent atelectasis in a large-animal model and that the effect of the negative pressure (applied through the diaphragm) is different from that of simply increasing airway pressure.[20] Because of this distinctive effect, we investigate the hypothesis that addition of continuous negative abdominal pressure in injurious mechanical ventilation would reduce ventilator-associated lung injury (*i.e.*, oxygenation, compliance, pulmonary edema, and inflammatory cytokines in bronchoalveolar fluid) in a pig model.

Materials and Methods

Animal Preparation

The study was approved by the Ethical Committee for Experimental Studies, Hospital for Sick Children, Toronto, Canada (No. 34847). Yorkshire pigs (n = 10, 20.2 to 24.6 kg) were used. After induction of anesthesia (pentobarbital $10 \, mg \cdot kg^{-1} \cdot h^{-1}$) and muscle paralysis (rocuronium $0.02 \, mg \cdot kg^{-1} \cdot h^{-1}$), a tracheotomy was performed and an esophageal-gastric balloon catheter (NutriVent, Sidam, Italy) inserted, calibrated,[21] and filled with air (1.0 ml for esophageal balloon, *i.e.*, minimal nonstress volume; 1.5 ml for gastric balloon). Muscle paralysis was confirmed by the absence of negative deflection of esophageal pressure. Catheters were placed in the carotid artery to monitor arterial blood pressure, internal jugular vein, and pulmonary artery (for pulmonary artery pressure, cardiac output). We recorded baseline measurements with fractional inspired oxygen tension (F_{IO_2}) 1.0, V_T $10 \, ml \cdot kg^{-1}$, PEEP 5 cm H_2O, and rate 20 min^{-1} after lung recruitment (PEEP 10 cm H_2O, driving pressure 15 cm H_2O) for 2 min.

Experimental Lung Injury

Experimental lung injury was induced by repeated lung lavage with 30 ml · kg^{-1} saline solution (37°C),[22] and injury was considered stable after observing a Pa_{O_2}/F_{IO_2} ratio < 100 mmHg for 10 min at PEEP 5 cm H_2O. After confirming lung injury, animals were subjected to injurious mechanical ventilation for 1 h (Servo 300, Siemens-Elema AB, Sweden) with assisted pressure control (F_{IO_2} 1.0, rate 20 min^{-1}, pressure trigger –2 cm H_2O). A previously described combination of driving pressure and PEEP was used to provide stable lung injury[23]:

- driving pressure/PEEP = 39/1, 37/3, 35/5, 33/7, 31/9, 29/11, or 27/13 cm H_2O;
- driving pressure and PEEP were adjusted accordingly, every 15 min for 1 h, to maintain Pa_{O_2}/F_{IO_2} between 55 and 65 mmHg. We recorded measurements at F_{IO_2} 1.0, driving pressure 10 cm H_2O, PEEP 10 cm H_2O, and rate 25 min^{-1}.

Experimental Protocol

The experimental protocol is summarized in figure 1. After lung injury was established, the lungs were recruited (PEEP stepwise increased from 15 cm H_2O to 20 cm H_2O, to 25 cm H_2O with fixed driving pressure of 20 cm H_2O)[23] and assignment was random (but not blinded) to one of two groups (n = 5 each): PEEP (no continuous negative abdominal pressure) or PEEP plus continuous negative abdominal pressure.

Randomization was from a bag of coded letters. Mechanical ventilation was for 4 h, with V_T 20 ml · kg^{-1} (maintained by adjusting inspiratory pressure), respiratory rate 20 to 30 min^{-1} (targeting Pa_{CO_2} < 50 mmHg), inspiratory:expiratory ratio 1:2, and F_{IO_2} 1.0.

In order to facilitate progressive ventilator-induced lung injury, low expiratory transpulmonary pressure (P_L) of –3 cm H_2O was targeted in each group (immediately after lung recruitment) and was calculated with esophageal manometry, as: P_L = [PEEP – esophageal pressure (P_{es})], all at end-expiration.

Transpulmonary pressure was titrated in each group by adjustment of PEEP. All ventilator settings were adjusted every 15 min. In order to maintain target V_T (20 ml · kg^{-1}) and target expiratory transpulmonary pressure (–3 cm H_2O), plateau pressure and PEEP were adjusted accordingly. PEEP was increased by 2 cm H_2O if Pa_{O_2}/F_{IO_2} ratio was less than 55 mmHg (measured each hour).

Continuous Negative Abdominal Pressure

Continuous negative abdominal pressure was generated by a custom-made prototype device (fig. 2) that was connected to a negative pressure ventilator (Pegaso V; Dima Italia Srl, Italy). The lower half of the animal was placed inside the continuous negative abdominal pressure device, sealed at the level of xiphoid to prevent air leak, and a continuous negative abdominal pressure of –5 cm H_2O applied to the external abdomen wall. Gastric pressure (a surrogate of abdominal pressure) was decreased by ≈ –5 cm H_2O when continuous negative abdominal pressure of –5 cm H_2O was applied (table 1).
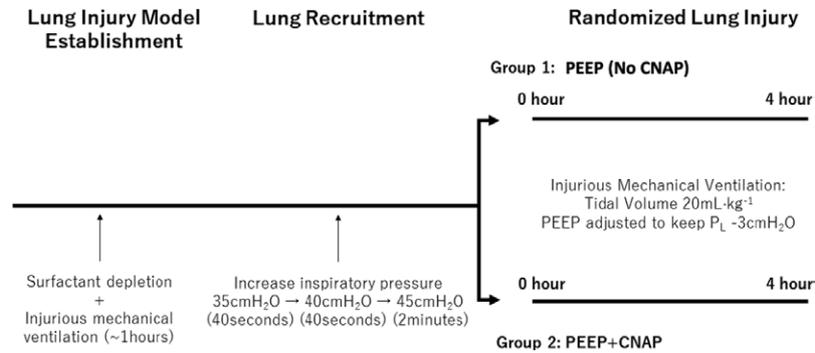
Fig. 1. Schematic of lung injury protocol: lung injury was established by surfactant lavage. Lung recruitment was performed, and the animals were randomized to either PEEP or PEEP plus CNAP (n = 5 per group); injurious mechanical ventilation was continued for 4 h. CNAP = continuous negative abdominal pressure; PEEP = positive end-expiratory pressure; P_L = transpulmonary pressure.

Fig. 2. Image of the CNAP prototype device during an experiment: the pig was ventilated with positive pressure through a tracheal tube by a Servo 300 mechanical ventilator (Siemens-Elema AB, Sweden). CNAP was generated by a custom-made prototype plexiglass device connected to a negative-pressure ventilator (Pegaso V; Dima Italia Srl, Italy). The lower of half of the pig was placed inside the CNAP device and sealed at the level of xiphoid with a soft band to prevent air leak. CNAP of −5 cm H_2O was applied to the external abdomen surface. CNAP = continuous negative abdominal pressure.

Electric Impedance Tomography

Electrical impedance tomography was recorded by the PulmoVista 500 (Dräger, Germany) with a 16-electrode silicon belt around the thorax at the sixth intercostal space (parasternal line). In all animals, electrical impedance tomography data were recorded for 2 min each hour and analyzed (Dräger EIT Analysis Tool 6.1).

The "center" of ventilation was calculated to evaluate the distribution of tidal ventilation[24] as follows. First, in electrical impedance tomography images taken in normal "fully recruited" lung (i.e., electrical impedance tomography images before injury), the most ventral and dorsal boundaries (i.e., pixels) available for ventilation were identified, and the range available for ventilation was defined as from 0% (most ventral) to 100% (most dorsal). Second, we defined the "center" of ventilation as an index to visualize the shifts in regional tidal ventilation in the ventrodorsal direction during progressive injury, compared to the reference ventilation zone observed in normal lung. The "center of ventilation" (homogeneity) reflects the distribution of tidal ventilation (i.e., inspiratory impedance change: ΔZ) along the ventraldorsal axis,[24] such that homogenous ventilation is represented as the bulk of the imaged ventilation at the axis midpoint (i.e., a 50% center of ventilation; Supplemental Digital Content 1, http://links.lww.com/ALN/B710). The "center" of ventilation (as a percent) was defined as: [ΔZ, dorsal half of lung] × 100 / [ΔZ, whole lung], which increases (approaching to 100%) when the ventilation is shifted into dependent lung regions.

Measurements and Definitions

Arterial blood gas measurements were performed with an ABL 835 (Radiometer, Denmark). Shunt was calculated by standard formulas, and plateau pressure (P_{plat}) was determined as the positive airway pressure (P_{aw}) at zero flow at end-inspiration. Definitions were as follows: inspiratory P_L = [P_{plat} − P_{es}], at end-inspiration; expiratory P_L = [PEEP − P_{es}], at end-expiration; compliance (respiratory system) = V_T/(P_{plat} − PEEP); compliance (chest wall) = V_T/(end-inspiratory

Table 1. Respiratory Data

	Time during Protocol							P Value		
	Baseline	Lung Injury	0 h	1 h	2 h	3 h	4 h	Group	Time	Interaction
P_{AO_2}/F_{IO_2}, mmHg										
PEEP	489±54	239±128	82±18	157±112	107±48	92±29	80±24	0.04	0.38	0.02
CNAP	489±40	248±114	222±168	210±202	296±155*¶	305±142*¶	316±134*§			
Pa_{CO_2}, mmHg										
PEEP	54±5	74±19	50±8	42±3	41±4	40±10	43±7	0.16	< 0.01	0.97
CNAP	60±16	78±21	45±7	37±6	38±6	36±8	36±7			
Tidal volume, ml/kg										
PEEP	10.0±0.1	7.7±0.5	20.0±0.4	20.0±0.4	20.0±0.1	20.0±0.4	20.3±0.2	0.28	0.07	0.59
CNAP	10.3±0.5	7.6±0.6	19.9±0.7	20.0±0.3	19.7±0.3	19.9±0.3	20.1±0.3			
Driving pressure, cm H_2O										
PEEP	13.0±1.3	15.8±0.5	42.0±6.1	42.5±5.1	44.1±6.4	45.1±6.8¶	45.8±7.3§	0.02	0.97	< 0.01
CNAP	13.8±2.5	15.9±0.2	34.3±7.7	34.5±7.0	32.1±5.9*	31.4±4.7#¶	31.3±5.0#¶			
Compliance of respiratory system, ml/cm H_2O										
PEEP	17.6±3.3	11.1±2.1	11.0±2.4	10.8±1.8	10.5±2.1	10.3±2.2	10.3±2.2	0.12	0.52	< 0.01
CNAP	16.6±3.4	10.2±1.7	13.1±3.4	13.0±3.4	13.7±3.4	14.0±2.9¶	14.2±3.0§			
Compliance of chest wall, ml/cm H_2O										
PEEP	54.0±8.1	50.2±14.1	58.9±11.4	64.9±12.2	65.0±12.5	66.8±13.3	65.8±9.0	0.85	0.23	0.40
CNAP	47.8±9.5	43.9±7.3	58.5±11.7	54.4±14.9	65.0±17.9	65.6±12.1	67.6±10.0			
Compliance of lung, ml/cm H_2O										
PEEP	26.7±6.4	14.3±2.4	13.8±3.7	13.2±3.0	12.8±3.3¶	12.4±3.4§	12.2±3.8§	0.13	0.99	< 0.01
CNAP	26.3±7.8	13.4±2.4	17.1±5.4	17.6±5.8	18.0±6.2	18.2±5.2	18.4±5.4			
Inspiratory transpulmonary pressure, cm H_2O										
PEEP	7.1±3.1	13.2±2.7	31.2±7.1	32.7±6.4	34.3±7.6¶	35.3±8.1§	37.6±8.1§	0.03	0.46	< 0.01
CNAP	8.4±2.5	13.9±1.1	24.4±6.9	23.5±6.8	22.4±6.5*	21.9±5.9*	22.2±5.7*			
Expiratory transpulmonary pressure, cm H_2O										
PEEP	−1.6±3.1	1.0±2.5	−2.9±1.3	−2.6±0.8	−2.6±0.9	−2.9±0.6	−2.6±0.4	0.88	0.72	0.67
CNAP	−0.6±1.0	1.7±1.1	−2.4±0.5	−2.7±0.5	−2.8±0.3	−2.9±0.7	−2.5±0.4			
Mean airway pressure, cm H_2O										
PEEP	10.1±0.6	16.1±0.2	20.6±3.1	21.0±2.7	21.6±3.0	22.8±3.4§	24.0±3.2§	< 0.01	0.04	< 0.01
CNAP	10.4±0.8	16.3±0.5	14.4±1.5#	15.1±2.1#	14.5±2.1#	14.2±1.7#	14.0±2.1#			
Shunt, %										
PEEP	14.5±7.3	22.6±8.2	34.5±9.7	28.0±12	30.1±6.2	29.8±6.0	32.8±3.4	0.01	0.45	0.47
CNAP	13.2±5.1	20.6±7.0	20.8±9.8	20.2±11	15.3±7.6	15.4±7.2	14.9±7.7			
Gastric pressure, cm H_2O										
PEEP	7.9±1.8	9.3±3.3	10.5±3.8	10.2±3.0	10.2±2.5	10.9±3.0	9.9±0.8	< 0.01	0.74	0.36
CNAP	7.0±1.3	8.4±1.5	4.6±1.7	4.7±1.2	4.9±1.1	4.5±0.7	4.7±1.1			
Locus of ventilation, %										
PEEP	46.8±2.6	49.4±3.6	46.6±6.2	43.4±8.0	41.8±6.9¶	40.0±8.1§	39.0±9.7§	0.06	0.04	< 0.01
CNAP	49.4±3.4	50.2±6.3	50.4±5.5	49±4.7	50.6±3.7	51.2±2.9*	51.2±3.7*			

*$P < 0.05$ compared with PEEP. #$P < 0.01$ compared with PEEP. ¶$P < 0.05$ compared with 0 h. §$P < 0.01$ compared with 0 h.

CNAP = continuous negative abdominal pressure; F_{IO_2} = fractional inspired oxygen tension; Pa_{CO_2} = arterial carbon dioxide tension; P_{AO_2} = partial pressure of alveolar oxygen; PEEP = positive end-expiratory pressure.

P_{es} – end-expiratory P_{es}); and compliance (lung) = V_T/(end-inspiratory P_L – end-expiratory P_L).

Cardiac output was obtained (Model-9520-A; Edwards Lifesciences, USA) by thermodilution (5-ml bolus iced, isotonic sodium chloride solution; average of three measurements taken independent of ventilatory cycle). Transmural vascular pressure was defined as the intravascular pressure minus mean P_{es} (reflecting extravascular thoracic pressure). "Time zero" represents the time of the first measurements taken immediately after continuous negative abdominal pressure was applied (and the corresponding time in animals without continuous negative abdominal

pressure), and all measurements were performed every hour.

End of Experiment

After 4 h of injurious mechanical ventilation, animals were killed with IV sodium pentobarbital (100 mg · kg^{-1}) and the lungs excised.

Wet to Dry Lung Weight

The right lower lobe of the lung was sectioned transversely and a tissue sample (2 × 2 × 2 cm) taken from the nondependent, middle, and dependent lung regions, weighed, placed in a warming

oven (37°C), and weighed daily until weight was stable. The combined weights were analyzed as one value per animal.

Molecular Markers of Inflammation

Bronchoalveolar fluid was collected from the left lung *via* the bronchoscopy (BF type 1T20D, Olympus, Japan), and cytokines (interleukin-6, -1β, -8, -10) quantitated in bronchoalveolar fluid and serum (before lung injury, 0 h, 2 h, and 4 h) with a Milliplex porcine Cytokine Immunoassay Kit (Millipore, USA) and processed with Luminex xMAP Technology (Luminex, USA). Elastase activity in bronchoalveolar fluid was determined by incubating 50 μl bronchoalveolar fluid with 150 μl of 1.25 mM methoxy succinyl-ala-pro-val-p-nitroanilide (specific synthetic elastase substrate) in a 96-well plate for 24 h at 37°C and expressed as increase in absorbance at 410 nm/mg protein.[25]

Each lung was biopsied (as above), the messenger RNA (mRNA) isolated (Purelink RNA mini kit; Invitrogen, Canada), and relative quantitative real-time polymerase chain reaction performed in duplicate on reverse transcribed complementary deoxyribonucleic acids (ABI Prism 7700 Sequence Detection System; Applied Biosystems, USA) and PowerSYBR Green (Invitrogen) reaction mix. Gene expression was calculated relative to 18S ribosomal ribonucleic acid control and normalized to a nonventilated control by use of the comparative cycle threshold (ΔΔCt) method. Nonventilated lung tissue was obtained from anesthetized, spontaneously breathing pigs euthanized for collection of normal tissues. A biopsy from the nondependent region was used as the calibrator for all ventilated lung samples in real-time polymerase chain reaction. The following primers were used: interleukin-6: forward—5′-TGG GTT CAA TCA GGA GAC CT-3′; reverse—5′-CAG CCT CGA CAT TTC CCT TA -3′, interleukin-1β: forward—5′-CCA GCC AGT CTT CAT TGT TCA G-3′; reverse—5′-TTT TGG GTG CAG CAC TTC AT-3′, early growth response gene-1: forward—5′-CAC CTG ACC GCA GAG TCT TT-3′; reverse—5′-TTT GGC TGG GGT AAC TCG TC-3′, cyclooxygenase-2: forward—5′-CCC TTC CTG CGG AAT GCA A-3′; reverse—5′-GGT TAG AAA AGG CTT CCC AGC-3′.

Lung myeloperoxidase activity was measured spectrophotometrically from lung biopsies homogenized in 0.5% hexodecyltrimethylammonium bromide and incubated with 0.2 mg/ml o-dianisidine and 0.001% H_2O_2.[26] One investigator (G.O.), who was blind to sampling region and group allocation, performed the analysis.

Statistical Analyses

Statistical analyses were performed with SigmaStat (12 Systat Software, USA). Sample size was not formally calculated, but it was based on experience. The results are expressed as mean ± SD. A two-way ANOVA for repeated measures was used to evaluate the effects of group and time on respiratory and hemodynamic variables, center of ventilation, and plasma cytokine levels. In *post hoc* analyses, Dunnett tests were used to compare repeated values with the first value (*i.e.*, at the start of the protocol, time 0). Tukey tests were used for between-group comparisons. A two-way ANOVA (lung region × group) was used to evaluate data from lung tissue. All tests were two-tailed, and differences were significant when $P < 0.05$.

Results

Respiratory Variables

All animals survived the protocol. The targets for V_T (20 ml · kg⁻¹) and expiratory transpulmonary pressure (–3 cm H_2O) were achieved in both groups (table 1). In order to maintain target expiratory transpulmonary pressure, PEEP was lower in the continuous negative abdominal pressure group (fig. 3). A Pa_{O_2}/F_{IO_2} of less than 55 mmHg never occurred. Oxygenation was better in the presence of continuous negative abdominal pressure. Respiratory system compliance improved during the protocol in the PEEP plus continuous negative abdominal pressure group (table 1). No between-group differences in chest wall compliance were observed, and lung compliance decreased significantly over time in the PEEP (no continuous negative abdominal pressure) group. Higher levels of driving pressure (plateau pressure – PEEP), plateau pressure, and inspiratory transpulmonary pressure were required to maintain the target tidal volume in the PEEP (no continuous negative abdominal pressure) group; in the PEEP plus continuous negative abdominal pressure group, inspiratory transpulmonary pressure was less than 25 cm H_2O throughout.

Homogeneity of Ventilation

In the PEEP (no continuous negative abdominal pressure) group, ventilation was predominantly distributed to the nondependent (ventral) regions, and this pattern of distribution became more pronounced over time (table 1; fig. 4). In contrast, in the PEEP plus continuous negative abdominal pressure group there was a homogenous distribution of ventilation at the start of the protocol, which was preserved over the course of the experiment (table 1; fig. 4).

Ventilator-induced Lung Injury

The overall injury was less in the PEEP plus continuous negative abdominal pressure group in terms of lung tissue wet/dry lung weight ratio (fig. 5A), interleukin-6 protein in bronchoalveolar fluid (fig. 5B), and interleukin-6 mRNA expression in lung tissue (fig. 5C). There were no differences in elastase, interleukin-1β, interleukin-10, or interleukin-8 protein in bronchoalveolar fluid (Supplemental Digital Content 2, http://links.lww.com/ALN/B711, and Supplemental Digital Content 3, http://links.lww.com/ALN/B712).

The regional patterns of injury differed between the groups. For example, the lung tissue interleukin-6 mRNA expression in nondependent lung was less in the PEEP plus continuous negative abdominal pressure *versus* PEEP (no continuous negative abdominal pressure) group (fig. 5C). There were no differences in myeloperoxidase or early growth
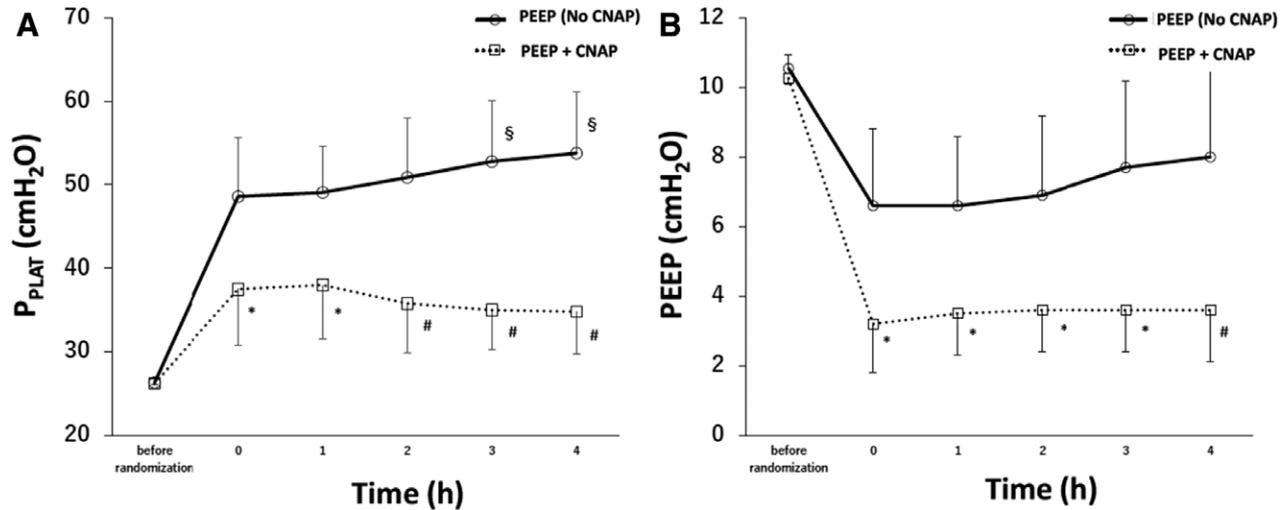
Fig. 3. P_{PLAT} and PEEP during lung injury: (*A*) In order to achieve target V_T (20 ml · kg^{-1}), the P_{PLAT} was increased in the PEEP group but not in the CNAP plus PEEP group. (*B*) In order to maintain target expiratory P_L, PEEP was lower in the CNAP plus PEEP group. These changes reflected more severe lung injury in the PEEP *vs.* CNAP plus PEEP groups. The data are expressed as mean ± SD. *$P < 0.05$ *versus* PEEP; #$P < 0.01$ *versus* PEEP; ¶$P < 0.05$ *versus* values at 0 h; §$P < 0.01$ *versus* values at 0 h. CNAP = continuous negative abdominal pressure; PEEP = positive end-expiratory pressure; V_T = tidal volume; P_L = transpulmonary pressure; P_{PLAT} = plateau pressure.
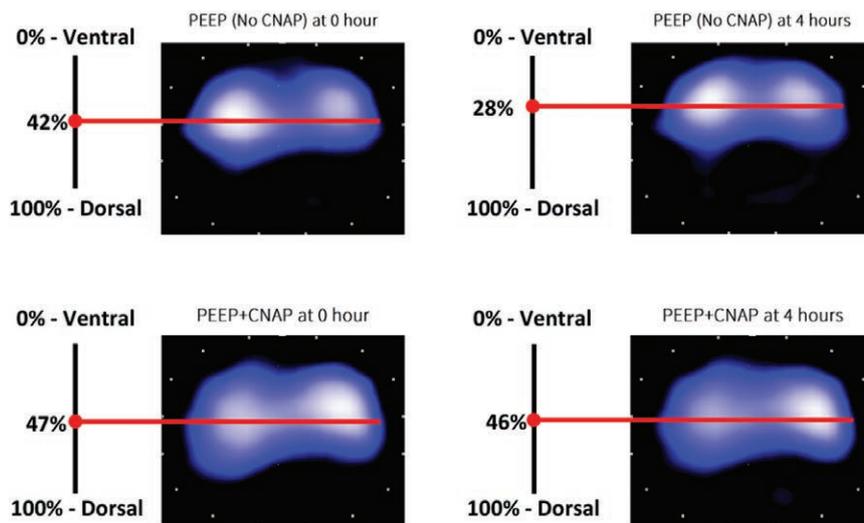

Fig. 4. Center of ventilation: the zone available for ventilation from 0% (most ventral) to 100% (most dorsal) was identified in normal "fully recruited" lung. During progressive lung injury, ventilation with PEEP (only) was predominantly distributed to the nondependent (ventral) regions, and this pattern of distribution became more pronounced over time. However, application of CNAP (plus PEEP) was associated with a homogenous distribution of ventilation over the course of the experiment. CNAP = continuous negative abdominal pressure; PEEP = positive end-expiratory pressure.

response gene-1 mRNA expression in lung tissue (Supplemental Digital Content 4, http://links.lww.com/ALN/B713, and Supplemental Digital Content 5, http://links.lww.com/ALN/B714). No difference was observed in interleukin-1β, -6, -8, and -10 in the serum between groups (Supplemental Digital Content 6, http://links.lww.com/ALN/B715).

Hemodynamics

Arterial pressure decreased over time in both groups (complete hemodynamic data: table 2), and cardiac output decreased (overall ≈30%) only in the PEEP (no continuous negative abdominal pressure) group (table 2), which was associated with higher mean positive airway pressure and higher plateau pressure at the end of the study in the PEEP (no continuous negative abdominal pressure) group.

Discussion

The current study demonstrates that a low level of continuous negative abdominal pressure (–5 cm H_2O) protects against ventilator-induced lung injury in a pig model, despite the same V_T and expiratory transpulmonary pressure. This technique
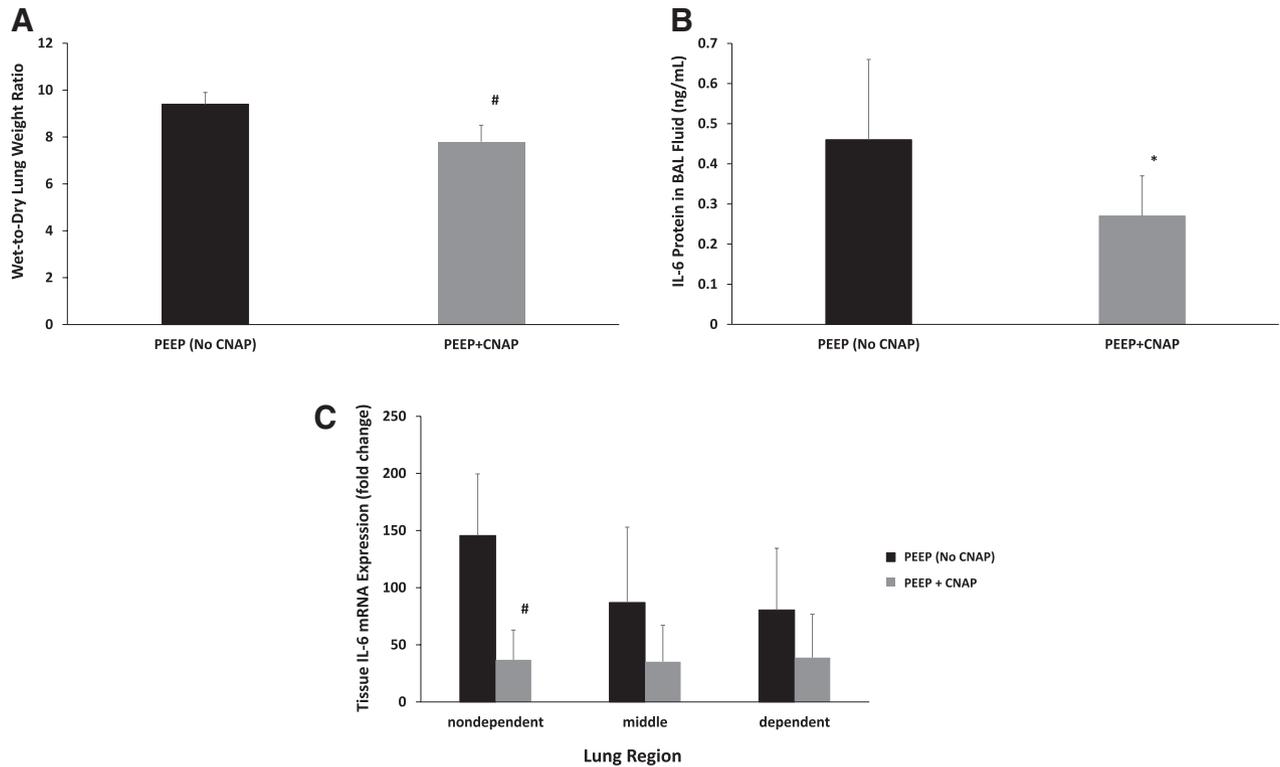
Fig. 5. Indices of lung tissue injury. All measurements were performed at the end of the randomized study of progressive lung injury. The use of CNAP plus PEEP (*vs.* PEEP alone) resulted in lower lung tissue wet/dry lung weight ratio (*A*), less IL-6 protein in bronchoalveolar fluid (*B*), and decreased IL-6 mRNA expression in nondependent lung (*C*). The data are expressed as mean ± SD. *$P < 0.05$ *versus* PEEP; #$P < 0.01$ *versus* positive end-expiratory pressure. BAL = bronchoalveolar lavage; CNAP = continuous negative abdominal pressure; IL-6 = interleukin-6; mRNA = messenger RNA; PEEP = positive end-expiratory pressure.

Table 2. Hemodynamic Data

	Baseline	Lung Injury	0 h	1 h	2 h	3 h	4 h	Group	Time	Interaction
			Time during Protocol					**P Value**		
Cardiac output, l/min										
PEEP	5.7±0.8	4.8±0.5	4.5±0.7	4.3±0.8	4.0±0.6	3.7±0.7	3.1±0.7§	0.64	0.01	0.02
CNAP	5.7±1.0	4.4±0.9	4.0±1.4	3.6±1.0	3.3±0.7	3.5±1.5	3.8±1.5			
Mean arterial pressure, mmHg										
PEEP	83.1±11	84.5±18	83.1±11	73.6±12	65.4±8.3	65.2±8.7	56.5±12	0.43	0.00	0.16
CNAP	75.2±12	93.2±14	76.0±8.9	62.5±7.9	61.4±8.3	63.8±8.9	61.9±9.7			
Mean central venous pressure, mmHg										
PEEP	8.4±2.1	9.2±0.8	10.6±2.9	9.3±2.1	10.2±1.4	10.9±0.9	11.5±1.8	0.00	0.01	0.28
CNAP	9.4±1.7	9.4±1.1	6.7±1.6	6.5±1.9	6.7±2.0	6.8±1.9	7.1±1.7			
Mean pulmonary artery pressure, mmHg										
PEEP	27.5±2.4	34.6±5.4	41.5±3.9	37.1±3.8	40.0±2.9	41.2±3.0	41.9±4.1	0.00	0.08	0.72
CNAP	30.0±5.2	37.0±5.0	36.9±8.4	30.6±5.3	32.4±4.0	33.5±2.9	32.3±4.6			
Pulmonary artery occlusion pressure, mmHg										
PEEP	13.1±1.3	13.1±1.0	14.0±1.8	13.5±1.4	13.5±1.4	14.4±1.1	15.7±2.3	0.08	0.14	0.64
CNAP	14.4±2.2	14.0±0.8	11.9±2.3	11.8±2.3	11.8±2.5	11.8±2.3	12.3±2.3			

*$P < 0.05$ compared with PEEP; #$P < 0.01$ compared with PEEP; ¶$P < 0.05$ compared with 0h; §$P < 0.01$ compared with 0h.
CNAP = continuous negative abdominal pressure; PEEP = positive end-expiratory pressure.

could constitute a clinically testable approach in adult respiratory distress syndrome. Continuous negative abdominal pressure was associated with an attenuated progression of lung injury, in terms of lung mechanics and gas exchange, and less injury at the end of the experiment, in terms of pulmonary edema and levels of interleukin-6. Protection was not due to a higher level of PEEP, as the PEEP was lower in the PEEP plus continuous negative abdominal pressure group.

The mechanism of protection against lung injury appears to be selective recruitment of dorsal atelectatic lung and a corresponding increase in the volume of ventilated ("baby") lung.[20] With dorsal atelectasis and positive pressure ventilation, ventilator-induced lung injury predominates in nondependent lung regions where most of tidal ventilation is received.[27,28] An increase in the volume of ventilated ("baby") lung through recruitment is thought to result in a broader distribution of each V_T, with correspondingly less local injurious stretch.[29,30] This homogeneity of ventilation (and thus better oxygenation) was observed in our study, where the distribution of V_T was more "centered" with continuous negative abdominal pressure. Moreover, the lower expression of the proinflammatory cytokine (*e.g.*, interleukin-6 mRNA) in the ventral lung with continuous negative abdominal pressure supports the notion that greater recruitment increases the volume of ventilated ("baby") lung and renders it less susceptible to volutrauma.

The current data suggest that continuous negative abdominal pressure recruits lung *via* a different mechanism compared with PEEP (or other means of increasing positive airway pressure). PEEP increases transpulmonary pressure at all regions by increasing positive airway pressure and thus recruits the lung nonselectively (*i.e.*, overinflates in the already aerated regions). Because of the vertical gradient of pleural pressure (nondependent transpulmonary pressure higher, dependent transpulmonary pressure lower), lung that is already aerated is further expanded before expansion occurs in atelectatic lung. Where this gradient is sufficiently great, the effects of increasing PEEP may be confined (solely) to overexpansion of already aerated (nondependent) lung, potentially contributing to ventilator-induced lung injury.[31] Indeed, worse oxygenation and larger shunt fraction was observed in the PEEP group, accompanied by a shift of ventilation toward ventral regions as PEEP was increased.

In contrast, continuous negative abdominal pressure selectively recruits dependent lung where atelectasis is usually predominant. This may occur by decreasing dependent pleural pressures (*i.e.*, increasing transpulmonary pressure) in dependent—but not in nondependent—lung. Continuous negative abdominal pressure lowers intraabdominal pressure and might thereby reduce dependent pleural pressure,[20] which would be consistent with a previous report that removal of abdominal contents in animals lowered pleural pressures to the greatest extent in dependent (*vs.* nondependent) lung.[15] The hemodynamic impact of mechanical ventilation is potentially important[32]; indeed, this may have contributed to the recent negative study of high frequency oscillatory ventilation, a technique that involves sustained elevation of airway pressure.[10]

In the current study, arterial pressure decreased in both groups, and cardiac output decreased in the PEEP group at 4h. Positive airway pressure[33] and continuous negative abdominal pressure can each reduce cardiac preload (*i.e.*, end-diastolic volume[19]), but this is attenuated during continuous negative abdominal pressure by decreasing positive airway pressure. The mean positive airway pressure at the study end was 24 cm H_2O in PEEP *versus* 14 cm H_2O in continuous negative abdominal pressure; this was probably because the lung injury was greater in PEEP alone and targeting the same transpulmonary pressure and V_T resulted in lower values of PEEP and plateau pressure in the continuous negative abdominal pressure group. It is possible that the lower cardiac output in the PEEP group was due to the higher mean positive airway pressure and lower ventricular preload. Thus, continuous negative abdominal pressure, by sparing higher mean positive airway pressure, may have attenuated the impact on preload and thereby preserved cardiac output.

A key question is whether these effects could be replicated by using the prone position. There are key parallels between use of continuous negative abdominal pressure and ventilation in the prone position. Prone positioning increases oxygenation and reduces lung injury—and it improves outcome;[34] it also changes the gradient of pleural pressures.[35]

In contrast to continuous negative abdominal pressure, prone position involves several changes that impact distribution of ventilation.[36] Importantly, prone position involves a major change in body position and may raise concerns about complications including dislodgment of tubes or pressure injuries. These "real-world" concerns mean may explain why, notwithstanding clinical trials showing improved mortality,[34] prone positioning is employed in only 16% of patients with severe adult respiratory distress syndrome.[7] While the relative impact of prone positioninig *versus* continuous negative abdominal pressure (or the two techniques combined) has yet to be determined, the infrequent use of prone positioning in clinical practice suggests a potential role for continuous negative abdominal pressure.

The current data are consistent with the possibility that negative pressure to generate constant (*i.e.*, nonphasic) downward displacement of the diaphragm reduces lung injury during positive pressure ventilation. These data are in apparent contrast to multiple studies indicating that during positive pressure ventilation, spontaneous effort (*i.e.*, dynamic downward displacement of the diaphragm, negative pleural pressure) can, in severe lung injury, worsen injury and worsen outcome.[23,37–40] The key difference between the two circumstances is that repetitive spontaneous effort causes repetitive overstretch and tidal recruitment—and injury—of atelectatic lung[23,38,39]; by contrast, continuous negative abdominal pressure results in ongoing recruitment, thereby minimizing the impact of inspired V_T. Indeed, continual downward displacement of the diaphragm (achieved by continuous phrenic nerve stimulation) reduces atelectasis in uninjured lungs during anesthesia by preventing abdominal pressure transmission.[6] The impact of continuous negative abdominal pressure during preserved spontaneous effort is currently unknown.

In summary, we demonstrated that continuous negative abdominal pressure ameliorates ventilator-induced lung injury in a short-term *in vivo* large-animal model.

However, important questions remain. It is unknown whether these effects are reproducible in lung injury resulting from more clinically relevant ventilatory settings (*i.e.*, low V_T, higher PEEP), different etiologies (*e.g.*, direct *vs.* indirect injury[7]), or where atelectasis is not primarily distributed in dependent lung. Since we utilized a recruitable lung injury model, the impact of continuous negative abdominal pressure is unclear in cases of adult respiratory distress syndrome with low potential for recruitment (although in such cases, it is likely that no recruitment strategy will work). In this regard, it is important to recognize the limitations of electric impedance tomography, in terms of resolution and imaging, and it is possible that because not all dependent alveoli will be atelectatic, some may be overdistended. We analyzed lung injury regionally, and the size of samples was very small in relation to the size of the porcine lung (the opposite is the case, for example, in many studies of mice or rats). While we did not formally (or statistically) test for reproducibility, the samples were spatially distinct, and the interpretation of the samples was blinded. Finally, the clinical effects on hemodynamics, and the practical usability in patients, are unknown. In conclusion, continuous negative abdominal pressure protects against ventilator-associated lung injury, likely by selective recruitment of atelectatic lung.

Research Support

Competing Interests

Drs. Yoshida, Engelberts, and Kavanagh have applied for a patent on a continuous negative abdominal pressure device. The remaining authors declare no competing interests.

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