

Negative Pressure Ventilation and Positive Pressure Ventilation Promote Comparable Levels of Ventilator-induced Diaphragmatic Dysfunction in Rats

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ABSTRACT

Background: Mechanical ventilation is a life-saving intervention for patients with respiratory failure. Unfortunately, a major complication associated with prolonged mechanical ventilation is ventilator-induced diaphragmatic atrophy and contractile dysfunction, termed ventilator-induced diaphragmatic dysfunction (VIDD). Emerging evidence suggests that positive pressure ventilation (PPV) promotes lung damage (ventilator-induced lung injury [VILI]), resulting in the release of signaling molecules that foster atrophic signaling in the diaphragm and the resultant VIDD. Although a recent report suggests that negative pressure ventilation (NPV) results in less VILI than PPV, it is unknown whether NPV can protect against VIDD. Therefore, the authors tested the hypothesis that compared with PPV, NPV will result in a lower level of VIDD.

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What We Already Know about This Topic

- Diaphragm dysfunction occurs with positive pressure ventilation

What This Article Tells Us That Is New

- Twelve hours of mechanical ventilation, both negative pressure and positive pressure, resulted in similar levels of ventilator-induced diaphragmatic dysfunction, and the levels of lung injury did not affect the magnitude of diaphragmatic dysfunction

Methods: Adult rats were randomly assigned to one of three experimental groups (n = 8 each): (1) acutely anesthetized control (CON), (2) 12h of PPV, and (3) 12h of NPV. Dependent measures included indices of VILI, diaphragmatic muscle fiber cross-sectional area, diaphragm contractile properties, and the activity of key proteases in the diaphragm.

Results: Our results reveal that no differences existed in the degree of VILI between PPV and NPV animals as evidenced by VILI histological scores (CON = 0.082 ± 0.001 ; PPV = 0.22 ± 0.04 ; NPV = 0.25 ± 0.02 ; mean \pm SEM). Both PPV and NPV resulted in VIDD. Importantly, no differences existed between PPV and NPV animals in diaphragmatic fiber cross-sectional area, contractile properties, and the activation of proteases.

Conclusion: These results demonstrate that NPV and PPV result in similar levels of VILI and that NPV and PPV promote comparable levels of VIDD in rats.

MECHANICAL ventilation (MV) is used clinically to maintain adequate pulmonary gas exchange in patients incapable of doing so on their own. Common indications for MV include respiratory failure, neuromuscular diseases, drug overdose, coma, and surgery. The use of MV in critical care medicine is common and it is estimated that approximately 33% of all adult patients admitted to an intensive care unit require MV.¹ Although MV can be a life-saving intervention, it may have severe side effects.² A major complication from prolonged MV is ventilator-induced diaphragm atrophy and contractile dysfunction, collectively termed ventilator-induced diaphragmatic dysfunction (VIDD). Importantly,

diaphragmatic weakness is predicted to be a major contributor to difficult “weaning” of patients from MV.³

The development of VIDDD can occur rapidly within the first 12 h of MV, and extended periods of MV result in a progressive increase in diaphragmatic weakness.^{4–6} This is associated with decreased protein synthesis⁷ and increased protein degradation.^{8–13} The cellular “trigger” within diaphragm muscle fibers to promote this rapid induction of VIDDD appears to be an increased production of reactive oxygen species resulting in oxidative stress and the activation of cellular proteases that degrade diaphragmatic proteins.^{9,14–16} At present, the precise mechanism(s) that promote this rapid ventilator-induced oxidant stress in the diaphragm remain largely unknown, although recent studies have implicated an inflammatory response in the development of VIDDD.^{17,18}

In this context, it has been shown that positive pressure MV can induce lung injury in patients with healthy lungs,¹⁹ including an induction of cytokine production in the lungs. Importantly, the onset of ventilator-induced lung injury (VILI) in healthy lungs has not only been reported with injurious ventilator settings,²⁰ but also by using low tidal volumes that are designed to minimize VILI.²¹ In lungs injured before MV, positive pressure MV results in over-distension of lung tissue and exacerbates the existing injury.²² Although the basis remains a topic of debate, recent data support the use of negative pressure ventilation (NPV) in injured lungs and suggest that this approach is less injurious compared with positive pressure ventilation (PPV).^{23,24} This raises the intriguing question of whether NPV, which has been reported to result in less lung injury than PPV,²⁴ can be used to ventilate patients without the development of VIDDD or at least resulting in a reduction of VIDDD, if VILI serves as a key factor promoting VIDDD. This unanswered question forms the basis for the current experiments.

Therefore, we compared the development of VIDDD in negative *versus* PPV in a clinically relevant animal model of MV. On the basis of reports that PPV promotes lung inflammation and production of proinflammatory cytokines, the so-called biotrauma hypothesis,² we hypothesized that NPV would result in less lung injury and lower levels of VIDDD compared with PPV.

Materials and Methods

Animals

Young, adult (approximately 4 months old), female Sprague–Dawley rats were maintained on a 12:12 h light–dark cycle and provided food and water *ad libitum* before experimental procedures. The Institutional Animal Care and Use Committee of the University of Florida, Gainesville, Florida, approved these experiments. Animals were randomly assigned to one of three experimental groups ($n = 8$ per group): (1) acutely anesthetized control (CON), (2) PPV, and (3) NPV. All animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg body weight). After reaching a surgical plane of

anesthesia, ventilated animals were tracheostomized and a catheter was inserted into the carotid artery to permit the continuous measurement of blood pressure and the collection of blood during the protocol for blood gas analysis. Further, the jugular vein was cannulated to permit continuous infusion of sodium pentobarbital (10 mg·kg⁻¹·h⁻¹). All surgical procedures were performed using aseptic techniques.

MV

Animals assigned to the PPV group were exposed to 12 h of MV *via* a pressure-controlled ventilator (Servo Ventilator 300; Siemens-Elma, Solna, Sweden) with the following settings: upper airway pressure limit, 20 cm H₂O; pressure control level above positive-end expiratory pressure (PEEP), 5–10 cm H₂O; respiratory rate, 80 breaths/min; PEEP, 1 cm H₂O.

Animals in the NPV group were also subjected to 12 h of artificial ventilation in an iron lung. Briefly, after insertion of the arterial and venous catheters and placement of the tracheal tube, rats in the NPV group were placed in an iron lung constructed from a fiberglass box. The animal's head protruded out of the box through a porthole at one end. A rubber latex sheet was sealed around the animal's neck to maintain an air tight environment within the iron lung. Cyclical negative pressure within the chamber was created by a vacuum pump (TOPO Small Animal Ventilator; Kent Scientific Corporation, Torrington, CT) and was cycled at a rate resulting in approximately 80 breaths/min.

In both ventilated groups, arterial blood samples (100 μ l per sample) were removed after 1, 3, 5, and 12 h and analyzed for arterial P_O₂, PCO₂, and pH using an electronic blood-gas analyzer (GEM Premier 3000; Instrumentation Laboratory, Lexington, MA). The end-tidal PCO₂ was continuously monitored in the NPV group using a microcapnograph (CI240; Columbus Instruments, Columbus, OH). Ventilator adjustments were performed in both the PPV and NPV animals if arterial PCO₂ exceeded 40 mmHg. Moreover, arterial P_O₂ in PPV was maintained above 60 mmHg throughout these experiments by increasing the F_IO₂ (22–26% oxygen). Arterial P_O₂ was maintained in the NPV animals by increasing alveolar ventilation when appropriate. In both the PPV and NPV animals, body temperature was maintained at 37° ± 1°C with a circulating water blanket (PPV) or a heating lamp (NPV). Both blood pressure and heart rate were continuously monitored. Ongoing care during these experiments included lubricating the eyes, expressing the bladder, removing airway mucus, rotating the animal, and passively moving the limbs (PPV animals). Animals also received an intramuscular injection of glycopyrrolate (0.04 mg/kg) before beginning MV, and every 2 h during MV, to reduce airway secretions.

At the completion of the experiment, the diaphragm was quickly removed and the costal diaphragm was divided into several segments. A strip of the medial costal diaphragm was immediately used for *in vitro* contractile measurements, a separate section was stored for histological measurements,

and the remaining portions of the costal diaphragm were rapidly frozen in liquid nitrogen and stored at -80°C for subsequent biochemical analyses.

Measurement of In Vitro Diaphragmatic Contractile Properties

We measured diaphragmatic contractile properties using previously described techniques.⁴ Briefly, upon sacrifice, a diaphragm muscle strip, including the tendinous attachments at the central tendon and rib cage, was dissected from the midcostal region. The strip was suspended vertically, with one end connected to an isotonic force transducer (model 310C; Aurora Scientific, Aurora, Ontario, Canada) within a jacketed tissue bath, and force output was recorded *via* a computerized data-acquisition system (Super Scope II; GW Instruments, Somerville, MA). The optimum contractile length (L_0) was determined by systematically adjusting the length of the muscle using a micrometer while evoking single twitches. Maximal isometric twitch force was determined by supramaximal stimulation with 120-V pulses at 160 Hz. The force-frequency curve was created by supramaximal stimulation with 120-V pulses ranging from 15 to 160 Hz. The duration of each train was 500 ms, which was sufficient to reach a force plateau.

Bronchoalveolar Lavage and Determination of Serum Cytokine Levels

Following diaphragm removal, the lungs with the attached trachea were excised. The right bronchus was ligated to allow lavage of the left lung only. A catheter was tied into the trachea, and bronchoalveolar lavage (BAL) was performed with the infusion of 1 ml of cold, sterile saline (0.9%) and then withdrawn. This step was repeated three times. The BAL fluid (BALF) was pooled and subsequently centrifuged at 400g for 10 min at 4°C . The resulting supernatant was frozen in liquid nitrogen and stored at -80°C for subsequent analysis of cytokines. To determine whether circulating blood cytokine levels were increased during the course of MV, 150 μl of arterial blood was collected at 1, 3, 5, and 12 h and placed on ice (4°C). After clotting, samples were centrifuged at 1,500g for 10 min at 4°C and serum was collected, snap frozen, and stored at -80°C . The concentrations of interleukin (IL)-1 β , IL-6, and keratinocyte-derived chemokine (KC) were determined in serum and BALF in duplicate, using a Luminex xMAP-based assay (Affymetrix, Santa Clara, CA).

Lung Histology

After sacrifice, the lungs were removed and the left main bronchus was closed using a clamp. The right lung was fixed with 2.5 ml of 4% buffered formalin, with a pressure of 20 cm H_2O and stored for 24 h at room temperature; the tissue was then stored at 4°C until sectioning. The lungs were sectioned in 3- μm serial slices from the center of each lobe and stained with hematoxylin and eosin. Lung damage was scored by an experienced blinded investigator, using the criteria proposed by Matute-Bello *et al.*²⁵

Myofiber Cross-sectional Area

Sections from frozen diaphragm samples were cut at 10 μm using a cryostat microtome (Shandon Inc., Pittsburgh, PA) and stained for dystrophin, myosin heavy chain I, and type IIa proteins for fiber cross-sectional area analysis, as described previously.¹² Cross-sectional area for each fiber type was determined using ImageJ software (Wayne Rasband at National Institute of Health, Bethesda MD).

Western Blot Analysis

Diaphragm tissue samples were homogenized 1:10 (wt/vol) in 5 mM Tris (pH 7.5) and 5 mM EDTA (pH 8.0), with a protease inhibitor cocktail (Sigma, St. Louis, MO) and centrifuged at 1,500g for 10 min at 4°C . The supernatant was collected and protein content was assessed by Bradford protein assay (Sigma). Proteins were separated by electrophoresis on 4–20% gradient polyacrylamide gels for approximately 1 h at 200 V and then transferred to nitrocellulose membranes. Membranes were incubated at 4°C with primary antibodies directed against the protein of interest. 4-Hydroxynonenal (Abcam, Cambridge, MA) was probed as a measurement indicative of oxidative stress. Proteolytic activity was assessed by analyzing cleaved (active) calpain-1 and cleaved caspase-3 (Cell Signaling Technology, Danvers, MA). Activation of the proteasome system of proteolysis was assessed by analyzing the expression of Ubiquitin E2-Ligase Atrogin and Muscle ring finger 1 protein (#AP2041, #MP3401; ECM Biosciences, Versailles, KY). Autophagic activity was assessed by calculating the ratio of microtubule-associated protein 1A/1B-light chain 3 (LC3) I *versus* LC3II (#2775; Cell Signaling Technology). Finally, α -tubulin (Santa Cruz Biotechnology, Santa Cruz, CA) was probed as a loading control to normalize equal protein loading and transfer. After incubation with primary antibodies, membranes were washed extensively in phosphate-buffered saline with 0.1% Tween 20 and incubated with secondary antibodies (GE Healthcare, Pittsburgh, PA). After washing, a chemiluminescent system was used to detect labeled proteins (GE Healthcare). Membranes were developed using autoradiography film and images of the film were captured and analyzed using the 440CF Kodak Imaging System (Kodak, New Haven, CT).

Statistical Analysis

Comparisons of parametric data between groups for each dependent variable were made by a one-way ANOVA and, when appropriate, a Tukey test (two-tailed) was performed *post hoc* (Prism 6; GraphPad Software Inc., La Jolla, CA). Analyses of nonparametric data were evaluated with the Kruskal–Wallis test followed by Dunn *post hoc* test. Cytokine data were transformed using the BoxCox transformation to achieve homoscedasticity when necessary. The relationship between variables was assessed with the Pearson correlation coefficient. Note that *P* values less than 0.05 were considered as statistically significant. Data are presented as means \pm SEM.

Results

Systemic and Biological Response to MV

PPV and NPV animals were ventilated for 12 h without complications and blood gas homeostasis was maintained throughout the experiments (table 1). The P_{aO_2} and the A-a gradient did not differ between the groups PPV and NPV. However, the P_{aCO_2} was significantly lower in the NPV group (with a concomitant increase in pH) when compared with the PPV group due to the increase in alveolar ventilation required to maintain arterial P_{O_2} above 60 mmHg. Finally, mean arterial blood pressure was significantly lower in the NPV group.

VILI

To determine whether lung damage differed between PPV and NPV, we compared the histopathological scores and the changes in the alveolar–arterial P_{O_2} gradient. The lung injury histopathological score was based on quantification of neutrophils in the alveolar space and interstitial space, hyaline membranes, proteinaceous debris filling the airspace, and alveolar septal thickening. Our results revealed that in control animals, 12 h of ventilation resulted in significant lung injury in both the PPV and NPV animals (fig. 1). Representative images of lung tissue from both PPV and NPV animals reveal a robust infiltration of macrophages and neutrophils in both experimental groups that were rarely observed in lung sections from nonventilated animals (fig. 2). However, contrary to work suggesting that NPV results in less lung injury than PPV,²⁴ the mean lung injury score between PPV and NPV groups did not differ. Further support for this conclusion is provided by the finding that no significant differences exist between the alveolar–arterial P_{O_2} gradients of these experimental groups (table 1).

Blood and BAL Cytokine Levels following MV

Our results reveal that no differences exist in circulating levels of IL-6, IL-1 β , and KC between NPV and PPV animals

Table 1. Physiological Response to Mechanical Ventilation

| Parameter | PPV | NPV |
|---|--------------------|--------------------|
| P_{aO_2} , mmHg | 57.80 \pm 2.13 | 72.40 \pm 10.16 |
| P_{aCO_2} , mmHg | 38.83 \pm 2.15 | 26.80 \pm 2.25* |
| Arterial pH | 7.40 \pm 0.01 | 7.47 \pm 0.03* |
| Peak airway pressure, cm H ₂ O | 8.25 \pm 0.41 | n/a |
| Mean arterial pressure | 78.75 \pm 3.09 | 68.29 \pm 3.11* |
| A-a gradient alveolar–arterial P_{O_2} difference | 60.86 \pm 3.36 | 66.23 \pm 7.50 |
| P_{aO_2}/F_{iO_2} | 267.96 \pm 10.31 | 344.64 \pm 48.32 |

All measurements were performed at the completion of 12 h of mechanical ventilation using NPV or PPV. Values are means \pm SEM.

* Significantly different between PPV and NPV.

n/a = not applicable; NPV = negative pressure ventilation; PPV = positive pressure ventilation.

and the nonventilated control animals (data not shown). This finding suggests that neither of our artificial ventilation protocols resulted in a magnitude of lung injury sufficient to promote a persistent release of cytokines into the circulation.

Our results reveal that compared with control animals, the BALF concentrations of both IL-6 and IL-1 β were significantly increased in both ventilation groups compared with controls (fig. 3). In contrast, only NPV resulted in a significant increase in KC levels in the BALF (fig. 3). Together, these findings provide additional support that both NPV and PPV promote similar levels of VILI. See table 2 for details of these data.

Impact of PPV and NPV on VIDD

NPV and PPV resulted in similar levels of fiber atrophy in diaphragm type I fibers and type IIa fibers in the PPV group (table 2 and fig. 4A). In contrast, neither NPV nor PPV resulted in atrophy of type IIb/x fibers in the diaphragm.

Compared with nonventilated control animals, diaphragmatic specific force was depressed in PPV and NPV at all frequencies above 30 Hz (fig. 4B). Importantly, no significant differences existed in diaphragmatic force production between PPV and NPV at any given stimulation frequency. Together, these results indicate that both NPV and PPV result in similar levels of ventilator-induced contractile dysfunction in the diaphragm.

Ventilator-induced Oxidative Stress and Protease Activation in the Diaphragm

Our results reveal that, compared with control, diaphragmatic levels of 4-hydroxynonenal were significantly increased in both PPV and NPV animals (fig. 5A). Importantly, no differences existed in diaphragmatic 4-hydroxynonenal levels between PPV and NPV animals.

Our results reveal that both PPV and NPV activate calpain and caspase-3 in the diaphragm and that no

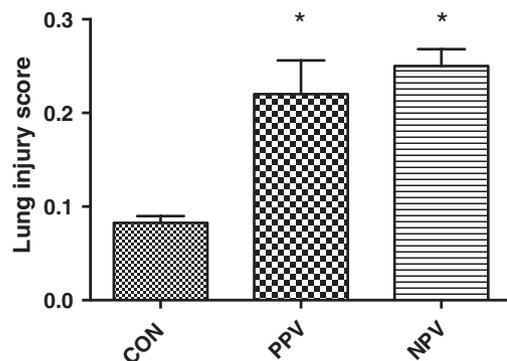


Fig. 1. Comparison of lung injury scores between positive pressure ventilation (PPV) and negative pressure ventilation (NPV) after 12 h of ventilation. PPV and NPV groups registered higher lung scores compared with control (CON) animals. No differences existed between PPV and NPV animals. * $P < 0.05$ versus control, $n = 8$ each.

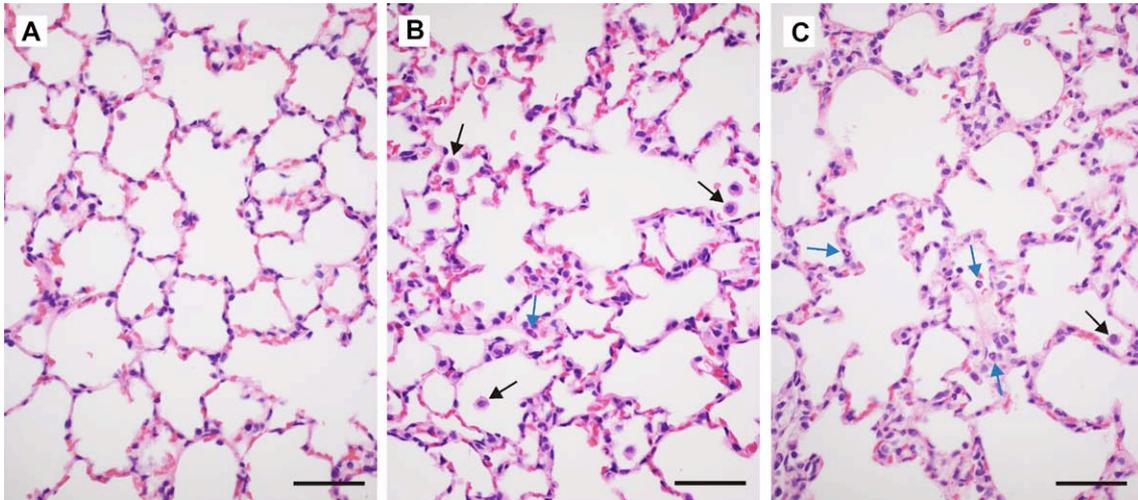


Fig. 2. Representative photographs of alveolar lung tissue from: (A) nonventilated (CON) animals, (B) positive pressure ventilation animals, (C) negative pressure ventilation animals. Note that both positive pressure ventilation (B) and negative pressure ventilation (C) resulted in increased infiltration of neutrophils (blue arrows) and alveolar macrophages (black arrows). In contrast, the infiltration of neutrophils was absent in the nonventilated (control) animals (A). Scale bars are 50 μm at $\times 400$ magnification.

differences exist in the levels of activation between PPV and NPV (fig. 5, B and C). Expression of the Ubiquitin E2-Ligase Atrogin-1 was significantly increased (fig. 6A) in both groups, although Muscle ring finger 1 protein levels did not differ between groups (fig. 6B). The ratio of LC3II/LC3I was significantly increased in both ventilation groups compared with controls, suggesting an increase in autophagy flux (fig. 6C). Together, these results indicate that PPV and NPV promote similar increases in oxidative

stress, protease, proteasome, and autophagic activation in the diaphragm.

Relationship between VILI and VIDD

During histological quantification of lung injury, we noted a wide range of lung injury scores across animals within both the NPV and PPV groups. We then questioned whether the magnitude of VILI significantly correlated with VIDD, independent of the mode of MV? (*i.e.*, diaphragm atrophy

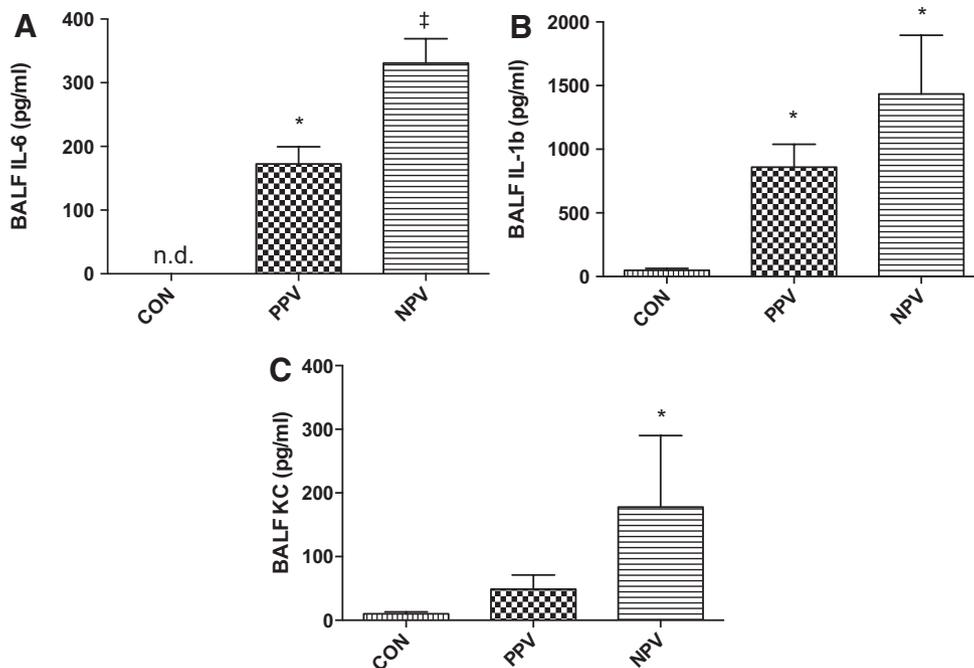


Fig. 3. Proinflammatory cytokines in bronchoalveolar lavage fluid (BALF) after positive pressure ventilation (PPV) or negative pressure ventilation (NPV). Shown are (A) interleukin (IL)-6, (B) IL-1 β , and (C) keratinocyte-derived chemokine (KC). * $P < 0.05$ versus control (CON), $\ddagger P < 0.05$ versus PPV, $n = 8$ each. n.d. = not detectable.

Table 2. Descriptive Statistics of Parameter of Ventilator-induced Lung Injury and Ventilator-induced Diaphragmatic Dysfunction

| | CON | PPV | NPV |
|--------------------------------------|---------------|---------------|----------------|
| Histo score | 0.082 ± 0.001 | 0.22 ± 0.04* | 0.25 ± 0.02* |
| BALF IL-6, pg/ml | n.d. | 172.4 ± 27* | 330.7 ± 38.4‡ |
| BALF KC, pg/ml | 10.1 ± 3.2 | 48.7 ± 22.6 | 177.9 ± 112.4* |
| BALF IL-1β, pg/ml | 49 ± 14.6 | 859 ± 180.8* | 1,434 ± 461.7* |
| CSA type I, μm ² | 995.1 ± 49.9 | 794.8 ± 45.8* | 834 ± 34.9* |
| CSA type IIa, μm ² | 1,024 ± 65.6 | 758.4 ± 40.6* | 884.6 ± 28.8 |
| CSA type IIb/x, μm ² | 2,484 ± 162.7 | 2,245 ± 198.2 | 2,355 ± 135.9 |
| Max tetanic force, N/cm ² | 25.2 ± 0.3 | 21.2 ± 0.3* | 21.3 ± 0.2* |

Data are presented as mean ± SEM.

**P* < 0.05 vs. control, ‡*P* < 0.05 vs. PPV and CON.

BALF = bronchoalveolar lavage fluid; CON = control; CSA = cross-sectional area; IL = interleukin; KC = keratinocyte-derived chemokine; n.d. = not detectable; NPV = negative pressure ventilation; PPV = positive pressure ventilation.

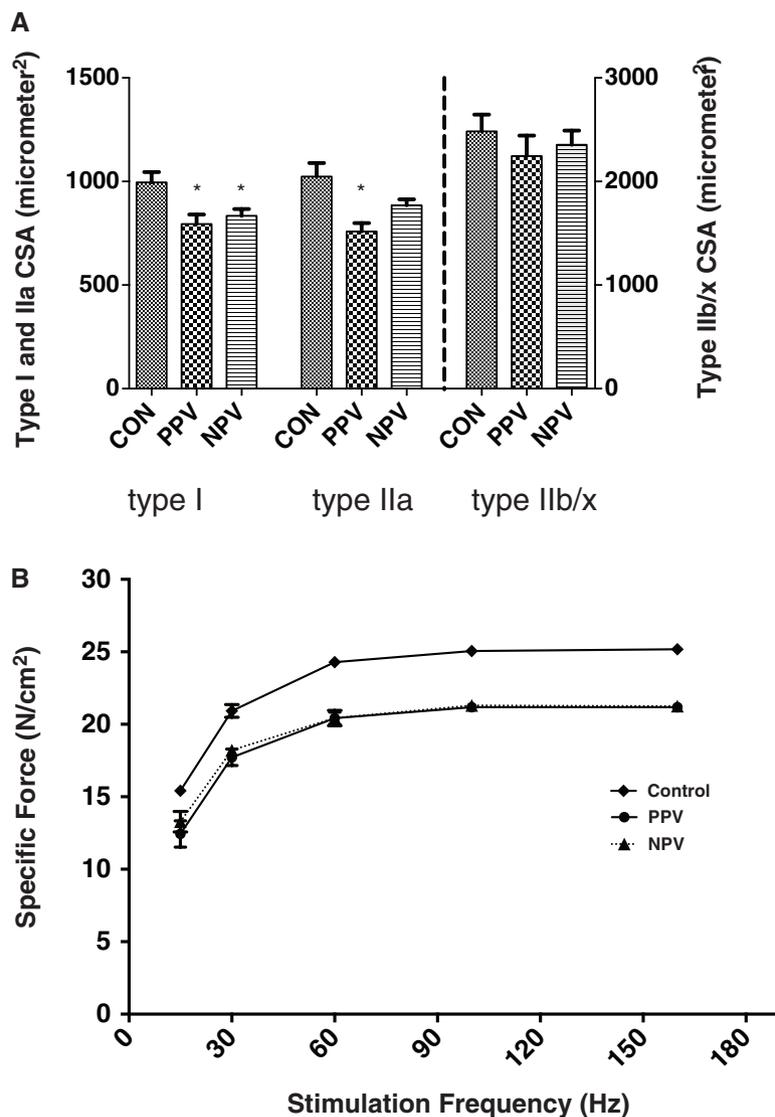


Fig. 4. Diaphragm atrophy (A) and contractile dysfunction (B) after negative pressure ventilation (NPV) or positive pressure ventilation (PPV). (A) Fiber cross-sectional area analysis (CSA) of diaphragm sections stained for dystrophin, myosin heavy chain I, and myosin heavy chain type IIa proteins. (B) Force-frequency curves for diaphragms from unventilated animals (CON) or from animals ventilated with either PPV or NPV. **P* < 0.05 versus control (CON), n = 8 each.

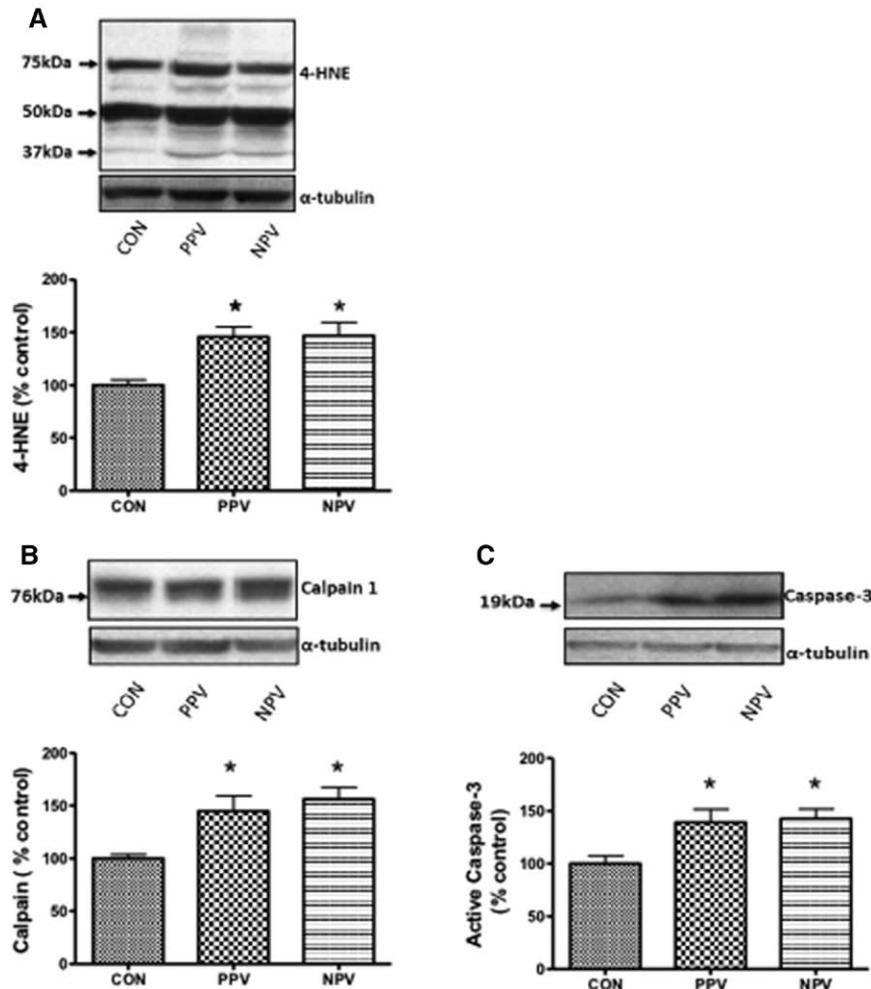


Fig. 5. Biochemical markers of oxidative stress and atrophy in the diaphragm. Shown are representative immunoblots and densitometric data for (A) 4-hydroxynonenal (4-HNE), (B) calpain 1, and (C) caspase-3. Values represent the mean percentage change \pm SD. CON = unventilated control animals; NPV = negative pressure ventilation; PPV = positive pressure ventilation. * $P < 0.05$ versus CON, $n = 8$ each.

and force generation). Our results indicate that lung injury scores are not significantly correlated with either diaphragmatic atrophy, or contractile dysfunction (table 3). Similarly, no significant correlations exist between the BALF cytokine levels and diaphragmatic fiber size and contractile properties (table 4).

Discussion

Overview of Major Findings

The current study reveals three new and important findings. First, prolonged ventilation using NPV or PPV results in similar levels of VIDD in a healthy animal model of MV. Second, both NPV and PPV promote similar levels of lung injury, although the degree of VILI varies widely across animals in both the NPV and PPV groups. Finally, the magnitude of VILI does not appear to influence the level of VIDD. A brief discussion of these results follows.

NPV and PPV Promote Comparable Levels of VIDD and Lung Injury

Our results do not support the hypothesis that compared with PPV, NPV results in protection against the development of VIDD. Indeed, NPV and PPV resulted in similar levels of both diaphragmatic atrophy and contractile dysfunction (fig. 4). Our hypothesis, that NPV would be protective against VIDD, evolved from a report indicating that NPV results in less lung injury than PPV,²⁴ and studies suggesting that an inflammatory process might be involved in the development of VIDD.^{17,18}

However, two major differences exist between the current study and the previous work of Grasso *et al.*²⁴ First, the Grasso *et al.* experiments used surfactant-depleted rabbits, whereas, the current study investigated healthy rats. Second, PEEP was not controlled during NPV in the current experiments, contrary to the NPV protocol used by Grasso *et al.*²⁴ Our decision to not regulate PEEP was based on the fact that PEEP was not regulated in patients who were ventilated

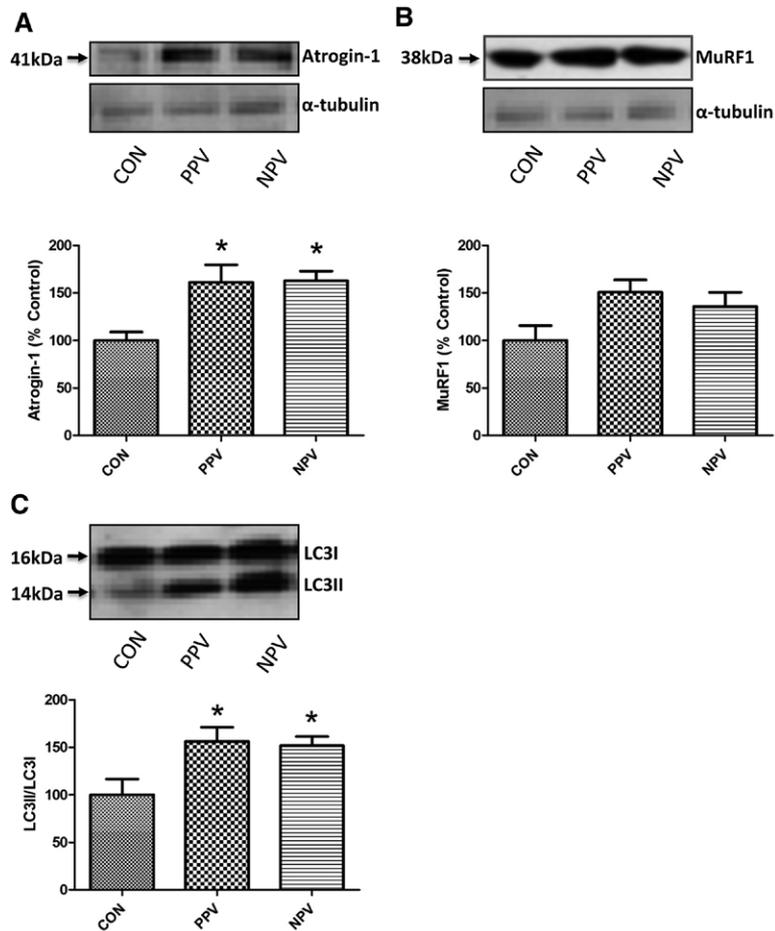


Fig. 6. Biochemical markers of proteasome activity and autophagy. (A) Atrogin, (B) muscle ring finger protein (MURF) 1, (C) LCII/LCI ratio as indicator of autophagic activation. Values represent the mean percentage change \pm SD. CON = unventilated control animals; LC = microtubule-associated protein 1A/1B-light chain 3; NPV = negative pressure ventilation; PPV = positive pressure ventilation. * $P < 0.05$ versus CON, $n = 8$ each.

in the “iron lung” ventilators during the worldwide polio epidemic, which peaked in the 1940s and 1950s. Indeed, many polio patients were ventilated in iron lung ventilators for many months without obvious signs of lung injury. Further, many applications of the modern cuirass ventilator do not control PEEP in patients.²⁶ Whether or not the failure to regulate PEEP in our NPV protocol played a major role in the magnitude of lung injury induced by NPV is unclear.

Levels of Lung Injury and VIDD Are Not Significantly Correlated

We examined the responses of the key proteolytic pathways (*i.e.*, calpains, caspase-3, proteasome system, and autophagy) in the diaphragm to both NPV and PPV. Both modes of MV resulted in similar levels of proteolytic activation. Further, biomarkers of oxidative stress (*i.e.*, oxidized proteins adducts, 4-hydroxynonenal) in the diaphragm were equally increased

Table 3. Correlations between Cytokine Concentrations Found in the Bronchial Lavage Fluid and Diaphragmatic Fiber Size and Diaphragm-specific Force Production

| | IL-6 | | IL-1 β | | KC | |
|---------------------------|----------------------|---------|----------------------|---------|----------------------|---------|
| | R ² Value | P Value | R ² Value | P Value | R ² Value | P Value |
| CSA of type I fibers | 0.137 | 0.21 | 0.036 | 0.49 | 0.040 | 0.49 |
| CSA of type IIa fibers | 0.006 | 0.79 | 0.000 | 0.99 | 0.008 | 0.76 |
| CSA of type IIb/x fibers | 0.005 | 0.81 | 0.042 | 0.46 | 0.038 | 0.50 |
| Specific force at 15 Hz | 0.061 | 0.91 | 0.000 | 0.97 | 0.028 | 0.55 |
| Max specific force 160 Hz | 0.039 | 0.51 | 0.016 | 0.63 | 0.018 | 0.62 |

CSA = cross-sectional area; IL = interleukin; KC = keratinocyte-derived chemokine.

Table 4. Correlations between Lung Injury Score and Diaphragmatic Fiber Size, Diaphragm-specific Force Production, and the Concentrations of Cytokines Found in the Bronchial Lavage Fluid

| | Lung Injury | |
|-----------------------------|----------------------|---------|
| | R ² Value | P Value |
| CSA of type I fibers | 0.156 | 0.14 |
| CSA of type IIa fibers | 0.008 | 0.75 |
| CSA of type IIb/x fibers | 0.126 | 0.19 |
| Specific force at 15 Hz | 0.000 | 0.94 |
| Max specific force (160 Hz) | 0.000 | 0.98 |
| IL-6 | 0.182 | 0.14 |
| IL-1 β | 0.063 | 0.35 |
| KC | 0.109 | 0.12 |

CSA = cross-sectional area; IL = interleukin; KC = keratinocyte-derived chemokine.

in the diaphragm after both NPV and PPV. We and others have reported that VIDD is directly linked to the existence of ventilator-induced oxidative stress in the diaphragm^{9,27} and that oxidative stress is required for MV-induced protease activation in the diaphragm.²⁸ Importantly, the current experiments demonstrate for the first time that these major components of the pathophysiology of VIDD are independent of the way the tidal volume is created (*i.e.*, NPV *vs.* PPV) during 12 h of ventilatory assistance.

Both NPV and PPV resulted in increases BAL levels of key cytokines (IL-6 and IL-1, KC in NPV), which are associated with VILI.² Further, increased circulating levels of IL-6 and IL-1 β are known to contribute to muscle atrophy.^{29,30} Nonetheless, our results reveal that circulating levels of these cytokines were not increased after 12 h of NPV or PPV.

Levels of lung injury after 12 h of NPV or PPV varied widely between animals, as assessed histologically and by quantification of cytokine levels in the lungs. No differences existed in the histologic injury score or BAL IL6/IL1 levels between animals in the NPV and PPV groups. However, compared with PPV animals, BAL levels of KC were significantly increased after 12 h of NPV. This result could be due to the lack of PEEP in the NPV group, thereby enhancing cyclic stress and lung injury.³¹ Nonetheless, the occurrence of VILI is not defined by the increase of proinflammatory mediators alone, but also by emerging histological lung injury and physiological dysfunction.²⁵ In the current study, arterial oxygenation was higher in the NPV group compared with the PPV group, indicating that NPV did not promote greater lung injury than the PPV treatment.

Therefore, to determine if the level of lung injury was related to the magnitude of VIDD, we examined the correlations between the lung injury score and diaphragmatic fiber size and contractile performance. Our results indicate that lung injury is not significantly correlated with the magnitude of diaphragmatic atrophy or contractile dysfunction (table 3), indicating that VIDD develops independent of

VILI. However, because both NPV and PPV animals experienced some level of lung injury, we cannot exclude the possibility that a threshold level of VILI (and possibly higher levels of atrophying cytokines) is a requirement for VIDD. Nonetheless, our results clearly show that increased levels of lung injury do not promote higher levels of ventilator-induced diaphragmatic atrophy and contractile dysfunction.

Clinical Implications

NPV was the most prevalent method of artificial ventilation until the 1960s.²⁶ However, after the development of positive pressure ventilators, the clinical usage of NPV has become uncommon and is not a part of the routine treatment for respiratory failure or surgery.²⁶ Nonetheless, it has been argued that NPV remains a useful method of artificial ventilation in select patients requiring respiratory support (*e.g.*, chemical lung injury, *etc.*).^{26,32} For example, potential advantages of NPV over PPV include the avoidance of endotracheal intubation and the associated complications, limited need for sedation, and the preservation of physiological functions, such as speech, cough, and swallowing.³³ Further, with the development of the modern cuirass ventilation instead of the older iron lung ventilation, this noninvasive type of NPV has regained some popularity in the pediatric field as a first line of treatment in acute respiratory failure and may prove useful in other clinical applications.^{26,33} Although NPV exhibits some advantages, there are also contraindications for the use of NPV, which include gastrointestinal bleeding, rib fractures, recent abdominal surgery, and sleep apnea syndrome.³³ Also, NPV can be associated with undesirable side effects, including depression, rib fracture, impaired sleep quality, and upper airway obstruction.³³ Hence, similar to PPV, the clinical application of NPV is not without problems, and the current results indicate that the use of NPV does not protect against VIDD.

VILI is acute lung injury that develops during MV. The cause and clinical relevance of VILI has been debated for years and many questions remain unanswered.³⁴ In the current study, we predicted that NPV would result in less VILI compared with PPV. This prediction was based on a report suggesting that compared with PPV, NPV results in less lung injury and better arterial oxygenation.²⁴ Nonetheless, our results did not confirm that previous study, because both PPV and NPV resulted in a similar degree of lung injury in previously healthy rats. Nonetheless, the current study contributes to the discussion about the clinical relevance of VILI in healthy individuals.³⁴

Experimental Limitations

Similar to all experimental designs, some limitations exist in our study. First, because our animals experienced lung injury during both NPV and PPV, this study cannot address the issue of whether VILI is required for the development of VIDD. Further, although our ventilated animals exhibited a range of lung injury scores, none of the ventilated animals

experienced critically high levels of lung injury. Therefore, our data cannot address the issue of whether a high level of VILI would exacerbate VIDDD compared with animals that exhibit relatively low levels of VILI. We chose “safe” ventilator settings (*i.e.*, low tidal volumes), which may not generate severe VILI to simulate clinical practice.³⁵ Importantly, even low tidal volumes can induce VILI and may have an impact on the lungs in preinjured patients.²¹

At the completion of the experiment, our NPV animals had a significantly lower P_{aCO_2} and mean arterial pressure compared with PPV animals. Nonetheless, these levels of P_{aCO_2} do not depress diaphragmatic contractility.³⁶ Further, since the mean arterial pressure in the NPV group remained above 60 mmHg, we do not believe that blood pressure influence our results.

The current experiments used an animal model of MV, and although many similarities exist between VIDDD in humans and animals, it is unknown if these results can be directly translated to humans. Further, we investigated the impact of only one duration (*i.e.*, 12h) of MV on VIDDD and whether longer durations of ventilatory support would have resulted in different outcomes between NPV and PPV remains unknown.

Finally, our results cannot address the question as to whether local production of proinflammatory cytokines in the lungs contributed to VIDDD because lung injury occurred in both the experimental models of artificial ventilation. Nonetheless, our results indicate that neither mode of MV resulted in increases in circulating cytokines and that cytokine levels in the BALF are not significantly correlated with VIDDD.

Conclusions

Our findings confirm that MV can cause diaphragmatic atrophy and contractile dysfunction. We demonstrate that NPV causes a similar extent of VIDDD and VILI as PPV does. Finally, we provide evidence that the level of VILI is not significantly correlated with the degree of VIDDD.

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