Negative-Pressure Ventilation
Better Oxygenation and Less Lung Injury

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Rationale: Conventional positive-pressure ventilation delivers pressure to the airways; in contrast, negative pressure is delivered globally to the chest and abdomen.

Objectives: To test the hypothesis that ventilation with negative pressure results in better oxygenation and less lung injury than with positive pressure.

Methods: Anesthetized, surfactant-depleted rabbits were ventilated for 2.5 hours in pairs (positive or negative). Tidal volume was 12 ml · kg−1, normocapnia was maintained by adjusting respiratory rate, and FIO2 was 1.0.

Measurements and Main Results: Lung injury was assessed with histologic scoring, perfusion using thermodilution (global perfusion), and injected intravascular microspheres (regional perfusion); and dynamic computed tomography was used to determine inflation patterns. Negative pressure was associated with a higher PAO2, a lower PA–PETCO2 gradient (despite identical minute ventilation), and injected intravascular microspheres (regional perfusion); and dynamic computed tomography was used to determine inflation patterns. Negative pressure was associated with a higher PAO2, a lower PA–PETCO2 gradient (despite identical minute ventilation), and less lung injury. Lung perfusion (global and regional) was similar with positive and negative pressure. Positive end-expiratory pressure applied to the airway was more efficiently transmitted to the pleural space than comparable levels of negative end-expiratory pressure applied to the chest wall; however, the oxygenation associated with any level of end-expiratory lung volume was greater when achieved by negative versus positive pressure. Dynamic computed tomography suggested that lung distension achieved with negative pressure is characterized by greater proportions of normally aerated lung (with less atelectasis) during inspiration and at end-expiration.

Conclusions: Negative-pressure ventilation results in superior oxygenation that is unrelated to lung perfusion and may be explained by more effective inflation of lung volume during both inspiration and expiration.

Keywords: lung injury; mechanical ventilation; oxygenation

Inflation of the lung occurs when the difference between pressure inside versus outside the lung overcomes tissue and airway factors that impede distension. Such transpulmonary pressure represents the difference between the pressure in the alveolus (i.e., inside the lung) versus that in the pleural cavity (i.e., outside the lung). With positive-pressure ventilation (PPV), the transpulmonary pressure is increased by making the alveolar pressure more positive; in contrast, with negative-pressure ventilation (NPV), the transpulmonary pressure is increased by making the pleural pressure more negative.

In the simplest possible scenario, the lung may be considered as a uniform, homogeneous, distensible sphere; in such a situation, a given increase in the distending transpulmonary pressure would result in exactly the same degree of lung distension whether caused by the alveolar pressure being made more positive or by the pleural pressure being made equally—but inversely—more negative. Although the distribution of inflation may be similar with positive versus negative pressure in normal lungs, the distribution may be more complex in injured lungs (1–4).

In the context of acute lung injury, the distribution of open—or atelectatic—lung units is far from homogeneous (5, 6), as is the distribution of pleural pressure (7). Such heterogeneity in regional ventilation has long been recognized, and has been the rationale for many imaginative therapies, including prone positioning (8), external chest wall compression, perfluorocarbon administration, and abdominally directed ventilator assist (9).

Although PPV delivering high tidal volumes is known to cause ventilator-associated lung injury, the effects of comparable tidal volumes generated with negative pressure are unknown. It is possible that negative pressure, because it is distributed across a broad surface of the chest wall and abdomen, may result in more homogeneous distension, which would improve oxygenation and be less injurious.

We hypothesized that NPV would result in better oxygenation and less lung injury in the surfactant-depleted rabbit; we found this hypothesis to be true. Compared with PPV, negative pressure resulted in greater FRC where transpulmonary pressure was similar, and resulted in greater oxygenation where
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FRC was similar. NPV may distend lungs in a fundamentally different manner to positive pressure, resulting in more homogeneous ventilation, less injury, and superior oxygenation.

METHODS

After institutional ethics approval (conforming to the guidelines of the Canadian Committee for Animal Care), female New Zealand white rabbits (2.5–3.5 kg) were used in all experiments. Anesthesia was induced, and the model of surfactant depletion using saline lavage was used as previously reported (10). Stable physiologic conditions were obtained before group allocation, and animals excluded where baseline inclusion (i.e., hemoglobin, acid-base status, oxygenation, compliance, hemodynamic status) criteria were not met (10). Five experimental series were performed.

Series 1: Lung Injury and Oxygenation

During baseline conditions, a Harvard ventilator (Harvard Apparatus, South Natick, MA) was used. When baseline criteria were satisfied, the animals were allocated, in pairs, to either a positive- or a negative-ventilation strategy (Figure E1 of the online supplement). The “positive” ventilation strategy consisted of the following: VT of 12 ml · kg⁻¹ · min⁻¹, FiO₂ of 1.0, positive end-expiratory pressure (PEEP) titrated to keep PaCO₂ between 65 and 130 mm Hg, and respiratory rate titrated to keep PaCO₂ between 35 and 45 mm Hg; a pressure-controlled mode was used (Newport E100i; Newport Medical Instruments Inc., Newport Beach, CA). The negative-ventilation animal in that pair was ventilated as follows: VT of 12 ml · kg⁻¹ and FiO₂ of 1.0, and negative end-expiratory pressure was adjusted to match exactly the PEEP in the corresponding “positive” animal; the respiratory rate was titrated to keep PaCO₂ between 35 and 45 mmHg, and the ventilation was performed using a commercially available device (Hayek 1000; Breasy Medical Equipment Ltd., London, UK) in a custom-made whole-body chamber where the head and neck protruded through an airtight neck seal. Tidal volume was measured in all cases using continuous spirometry (Cosmo 8100; Nova Metric Medical Systems Inc., Wallingford, CT). After ventilation for 2.5 hours, the animals were exsanguinated under anesthesia, the lung–heart block removed via sternotomy, and the left lung inflation fixed (10% neutral buffered formalin) for histologic scoring, as previously described (11), by a pathologist blinded to group allocation.

Series 2: Global Pulmonary Perfusion

Eight additional animals were anesthetized and had a pulmonary artery catheter inserted via the femoral vein. The heart–lung block was exposed by thoracotomy, and the pericardium opened without disturbing the pleura or diaphragmatic ligaments. The catheter was then advanced until the tip was in the pulmonary artery, and the chest closed. At the end of the experiment, we visually confirmed the catheter location. After allocation to either positive or negative -pressure ventilation (settings as described above) and stabilization, the right ventricular cardiac output was measured by a 4 Fr thermodilution catheter (Arrow; 7 cm; Reading PA), as previously described (12). Each rabbit was ventilated with positive and negative ventilation. As an additional control, four animals were ventilated with the above negative-pressure device that was directed to the chest only.

Series 3: Regional Pulmonary Perfusion

The animal preparation and ventilation were performed as in series 1. Colored microspheres (NuFlow microspheres; Interactive Medical Technologies, Irvine, CA) were suspended in a saline/Tween 80 solution (0.05%) by sonication in an ultrasonic bath and were injected via the external jugular vein. Three different colored microspheres were injected, one color each at 30, 60, and 90 minutes after the start of experimental ventilation. Five minutes after the last injection of microspheres, the chest was opened, the inferior vena cava transected, the pulmonary artery gently flushed with saline, and the heart–lung block removed. Lung tissue samples collected from the dependent and the nondependent lung regions were homogenized and the number of spheres of each color per gram of tissue calculated (13).

Series 4: Transpulmonary Pressures, Lung Volumes, and Oxygenation

Esophageal pressure, a surrogate for pleural pressure, was measured using a water-filled conventional feeding tube (8.0 Fr) connected to a pressure transducer, placed in the lower one-third of the esophagus, and calibrated as previously described (14). The end-expiratory lung volume (EELV), a surrogate for functional residual capacity was measured by occluding the endotracheal tube at end-expiration, discontinuing ventilation, releasing the occlusion, and measuring the volume of exhaled gas with a spirometer (Bear Neonatal Volume Monitor NVM1; Bear Medical Systems, Riverside, CA) until flow ceased. End-expiratory pressures of 4, 6, 8, and 10 cm H₂O were established for both positive pressure and negative pressure, and the resulting values for transpulmonary pressure, PaO₂, and EELV were recorded. Eight additional measurements were made of end-expiratory pressure (outside the above range) to capture a broader range of transpulmonary pressure and EELV.

Series 5: Computed Tomography Scanning

After surfactant depletion, each rabbit was ventilated using both PPV and NPV alternately (half the group commenced with PPV and half with NPV). When the ventilation strategy was changed, the lungs were recruited to clear the lung history. The rabbit was ventilated with a targeted transpulmonary pressure of 5 cm H₂O at end-expiration and 25 cm H₂O at peak inspiration. Arterial blood gas was taken and ventilation was maintained for 15 minutes before scanning to ensure...
stable conditions. When the animal was stable, the respiratory rate was changed (Ti = 1.5 s, Tc = 6 s; for positive and negative) to allow comparisons of the two forms of ventilation at end-expiration.

Dynamic computed tomography (CT) was performed on an eight-detector multislice CT scanner (GE Lightspeed; GE Medical Systems, Milwaukee, WI). Imaging consisted of simultaneous acquisition of four slices (one volume) every 0.2 seconds. A radiopaque marker was inserted at peak inspiration, to mark the start of an entire respiratory cycle.

An automated software program was developed that allowed segmentation of the lungs from the soft tissues of the thorax using a wavelet-based algorithm for edge detection, and aeration versus atelectasis was defined as follows: aerated lung (~900 to ~500 Hounsfield units [HU]) and atelectatic lung (~300 to +200 HU) (15).

Data Acquisition and Statistical Analysis

Data were acquired and processed using the computerized software ANADAT/LABDAT (version 4, McGill University, Montreal, PQ, Canada), and are presented as mean ± SD or proportions. Groups were compared using unpaired t tests or analysis of variance, and statistical significance was inferred where \( P < 0.05 \).

RESULTS

Series 1: Gas Exchange and Lung Injury

Oxygenation was similar in both groups (n = 9, each group) during baseline ventilation (Figure 1), but was significantly greater with NPV versus PPV throughout the experimental period. Respiratory rate and \( P_{aCO_2} \) were similar in both groups, and did not significantly change in either group over the course of the experiment (Table 1); however, the differences between the arterial and end-tidal \( PCO_2 \) were similar at baseline, and were greater in the positive-pressure group throughout the experimental period (Figure 2).

The composite histologic lung injury score was significantly lower in NPV versus PPV (Figure 3). The details of the individual elements of the histologic injury—alveolar versus airway—are provided in Table 2.

The wet-to-dry lung weight ratios (positive vs. negative: 10.3 ± 1.6 vs. 9.7 ± 0.7), as well as the protein concentration in the bronchoalveolar lavage fluid (positive vs. negative: 5.8 ± 2.1 vs. 4.2 ± 1.8), were similar in both groups, consistent with protective effects of positive airway pressure against alveolar edema (16).

Series 2: Global Pulmonary Perfusion

Eight animals were ventilated sequentially with PPV and NPV using a whole-body device. Global lung perfusion was not different between PPV versus NPV (Figure 4). Because a whole-body device was used, the lung perfusion was measured in an additional group (n = 4) in which negative pressure was applied using a device applied only to the chest (Curiss; Breasy Medical Equipment, Ltd., London, U.K.) and positive pressure applied in the usual way; with the negative-pressure chest device, the global lung perfusion was significantly greater than with the PPV (Figure 4).

Series 3: Regional Pulmonary Perfusion

Two groups (n = 3 in each) of animals were studied to examine the regional distribution of perfusion in negative versus positive pressure, by examining the distribution of injected microspheres, where perfusion is proportional to the number of spheres per gram of sampled lung tissue (13). The relative (i.e., ratio of) perfusion between the upper (nondependent) and lower (dependent) lung regions was similar in the positive- versus negative-pressure groups (Figure 5).

Series 4: Transpulmonary Pressures, Lung Volumes, and Oxygenation

PPV (n = 6) or NPV (n = 5) was used to determine the relationship among applied expiratory pressure, transpulmonary pressure, FRC, and oxygenation over a range of applied positive and negative end-expiratory pressures. At each of these levels,
conditions were stabilized for 30 minutes, and the corresponding transpulmonary pressures and PaO₂, measured, followed by measurement of the EELV. The transpulmonary pressure generated by negative end-expiratory pressure was consistently less than that generated for a comparable level of PEEP (Figure 6A). For given levels of transpulmonary pressure, the overall EELV achieved was consistently greater when distension was with negative versus positive pressure (Figure 6B). Finally, for a given level of EELV, the resultant PaO₂ (FIO₂ with negative versus positive pressure (Figure 6B). For given levels of transpulmonary pressure, the overall EELV achieved was consistently greater when distension was with negative versus positive pressure (Figure 6B). For given levels of transpulmonary pressure, the overall EELV achieved was consistently greater when distension was with negative versus positive pressure (Figure 6B). For given levels of transpulmonary pressure, the overall EELV achieved was consistently greater when distension was with negative versus positive pressure (Figure 6B).

**DISCUSSION**

The current study demonstrates that a given level of EELV attained with negative pressure is more effective (i.e., associated with better oxygenation) than a comparable lung volume achieved with positive pressure. This effect appears to be due to the differential distribution of lung volume in which negative pressure is associated with greater volumes of normally aerated lung. These effects were not restricted to end-expiration, because dynamic CT indicated that a similar pattern develops during inspiration with negative pressure. The superior gas exchange was unrelated to global or regional pulmonary perfusion (at least in terms of dependent vs. nondependent perfusion), and the lesser gradient between arterial versus end-tidal PCO₂ suggested that the recruitment involved optimally “perfused” lung tissue rather than overdistended pulmonary dead space. In addition, NPV may be associated with less ventilator-induced lung injury. These observations could have important implications for how ventilation is delivered, and suggest that assessment of lung recruitment might ideally include a measure of pulmonary dead space.

**Applied Pressure and End-Expiratory Volume**

The characteristics of the EELV were fundamentally different in PPV compared with NPV. This is not readily explained on the basis of a unitary “transpulmonary pressure,” because such a measurement (in our case, esophageal pressure) is not a weighted average of all the regional transpulmonary pressures throughout the lungs. Indeed, regional differences may be on the basis of liquid airway plugs that have previously been postulated to cause regional atelectasis (17).

Nonetheless, using the esophageal pressure to calculate the transpulmonary pressure (18), we report that the transpulmonary pressure was greater with a given level of positive pressure (i.e., applied to the airways, internal), compared with application of the same magnitude of negative pressure (i.e., applied to the chest wall, external). This is readily understood, as has been previously demonstrated (19), because the negative pressure must take effect through the chest wall before impacting on

![Figure 4](image1.png)

**Figure 4.** Global pulmonary perfusion was similar with positive- versus negative-pressure ventilation in which negative pressure was applied using a whole-body device (P = not significant [NS]); however, when negative pressure was applied using a chest device only, the pulmonary perfusion was significantly greater than with positive-pressure ventilation (P < 0.05).

![Figure 5](image2.png)

**Figure 5.** Regional lung perfusion, measured using injected colored microspheres, was expressed as the ratio of blood flow in the dependent versus nondependent lung tissue. There was no significant difference in the regional flow ratio between positive- and negative-pressure ventilation. NS = not significant.
the pleural space, and the greater the chest wall elastance, the greater the gradient between the external (applied) pressure and the intrapleural (effective) pressure.

However, the relationship between the effective pressure (measured at the midesophagus) and the resultant lung volume was more complex. When the levels of transpulmonary pressure were ranked in intervals, it was apparent that, for any given level of transpulmonary pressure, the developed EELV was less for positive pressure than it was for negative pressure. Of course, it is not possible that negative pressure confers a greater compliance than positive pressure in any given regional unit, but, over a whole lung and chest wall, there are significant differences (20). Comparison of negative pressure, because it applies pressure to the overall lung surface, with positive pressure supports previous reports that a singular measurement of pleural pressure (i.e., esophageal manometry) does not represent all of the regional pleural pressure gradients (21).

One of two conditions is necessary to demonstrate that this is the case: either ventilation can be applied through different routes (i.e., regionally, or positive vs. negative as in the current study) or pleural pressure can be measured on a regional basis and not assumed from a measurement at a single site.

In summary, although this is complex and its basis uncertain, we offer the following explanation. The different effects of positive pressure versus negative pressure on lung distension might result from a more even distribution of aeration associated with negative pressure. A comparable pressure gradient between the trachea and the outside of the chest wall would result in greater recruitment if the distribution of aeration has made the lung more compliant; in this context, the transpulmonary pressure would be lower and the transthoracic pressure higher, and the sum of both would remain constant.

Distribution of Tidal Volume and End-Expiratory Volume

The distribution of lung volumes was also considered during respiration, using dynamic CT. The spatial differences were striking, with dependent atelectasis persisting over most of the respiratory cycle in PPV. Such patterns have previously been reported in patients undergoing PPV in the setting of acute lung injury (6). In contrast, with NPV, we observed a different topographic distribution, in which normally aerated lung volumes were greater and atelectasis was reduced. The findings in the current study are similar to the altered distribution of lung volume as has been demonstrated for ventilation in the prone versus supine position (22, 23).

Pulmonary Perfusion

Two important issues concerning pulmonary perfusion are reported. First, there were no differences in pulmonary perfusion, either on a global or a regional basis, between PPV and NPV; global or regional perfusion does not therefore explain the oxygenation differences, suggesting that such differences must be explained by differences in lung aeration.

NPV can have very different hemodynamic effects compared with positive pressure. In general, positive pressure decreases ventricular afterload and preload. Negative pressure applied only to the chest (19), in contrast to that applied to the whole body (4), could conceivably augment right ventricular preload.

**Figure 6.** The relationships among applied end-expiratory pressure, transpulmonary pressure, and end-expiratory lung volume (n = 6, positive; n = 5, negative). (A) The transpulmonary pressures corresponding to end-expiratory pressure (EEP) applied as either positive (via airway) or negative (via chest and abdominal wall) end-expiratory pressure, are shown. Overall, for given levels of EEP, the transpulmonary pressure was less when applied with negative-compared with positive-pressure ventilation (\(^*P < 0.05\), ANOVA), reflecting the elastance of the chest wall that must be overcome by the negative pressure. The number of the observations represented in (A) is 42 (4 observations reported per animal, 11 animals and 2 missing values). (B) The end-expiratory lung volume (EELV) resulting from the corresponding transpulmonary pressures is shown, and demonstrates that, for a given level of transpulmonary pressure, the overall EELV was greater when derived from negative versus positive pressure (\(^*P < 0.05\), ANOVA). The total number of observations represented in (B) is 49 (7 additional observations in the 11 animals). (C) The PaO\(_2\), levels associated with each level of EELV are shown. The overall PaCO\(_2\), values associated with given levels of EELV were greater where the EELV was achieved with negative-versus positive-pressure ventilation (\(^*P < 0.05\), ANOVA), suggesting that, with negative-pressure ventilation, EELV—or FRC—more effectively oxygenates the perfusing blood. The total number of observations represented in (C) is 50 (8 additional observations in the 11 animals).
and thereby increase lung perfusion and improve oxygenation; this could be particularly important where the circulation is critically dependent on right ventricular filling (e.g., hypovolemia or restrictive physiology). This phenomenon was confirmed in the current study by demonstrating no change in cardiac output when negative pressure was applied using the whole-body device, but there was a significant increase when negative pressure was applied to the chest only.

The second issue concerns ventilation of perfused lung versus dead space. The gradient between arterial and end-tidal CO2 reflects alveolar dead space, in which reduced dead space is characterized by a lower arterial to end-tidal PCO2 gradient, provided that the minute ventilation is unchanged (24). In the current study, this gradient was increased with positive pressure, without any differences in minute ventilation; this suggests that the recruited lung consisted of "perfused" rather than "dead space" lung (25).

Impact on Pulmonary Edema
Positive airway pressure is believed to attenuate the development of alveolar edema (26). The exact mechanism is unclear, but among other possibilities, the increased pressure on the alveolar side of the gas–blood barrier may attenuate the tendency for net fluid flux into the alveoli; indeed, others have shown that negative pressure can worsen established permeability edema (16). In the current study, negative pressure reduced the severity of lung injury, but did not alter the amount of lung water in terms of wet-to-dry lung weight ratio or histologic quantification of alveolar edema. It is possible that the injury-associated edema in the positive-pressure animals was offset by the reduced tendency for edema with positive pressure; if this were true, then the combination of simultaneous PPV and NPV could together lessen overall lung injury and attenuate the formation of alveolar edema.

Limitations of Study
An important limitation of this study is the use of a conventional measure of pleural pressure—that is, esophageal pressure. Although assessed using the best possible technique, this singular measure cannot take account of the regional differences in local pleural (and therefore transpulmonary) pressures, and can therefore not resolve the local pressure differences between the two types of ventilation.

The current study provides indirect evidence for the recruitment of better-perfused airspace with NPV compared with PPV. However, the regional VA/Q ratios were not measured and the evidence, although suggestive (i.e., CO2 clearance), is indirect. Finally, it is possible that the high FIO2 used exacerbated local injury in ventilated, overdistended regions, and further altered the distribution of injury. Indeed, we cannot be certain whether there is an important interaction between high FIO2 and high tidal volume, whereby high FIO2 caused a greater exacerbation of focal stretch-induced injury in regions that were overdistended and less synergistic injury in physically less stressed regions; the current design cannot exclude this possibility.

Clinical Implications
The clinical implications of any approach that improves oxygenation or diminishes lung injury are potentially very great. Extrapolation of the current experimental model to the clinical context is difficult because the chest wall compliance is far greater in a rabbit than an adult human (although the difference is less for neonates). Others have reported, however, that where the chest wall is less deformable (e.g., dogs [27] and humans [19]), the chest wall may be less compliant with negative pressure, and for a given distending pressure may therefore cause greater diaphragmatic displacement and more even aeration and better lung compliance.

An important practical point is whether simply increasing PEEP in positive pressure would result in the same net effect as NPV; however, this issue cannot be resolved with the current experimental design.

Finally, despite a large clinical experience with the technique, practical difficulties, especially where the respiratory system compliance is low, have to date precluded widespread use.

Conclusions
The current study provides compelling evidence that, in certain circumstances, ventilation with negative pressure may fundamentally differ to that achieved with positive pressure in terms of
transmission of applied pressure, development of transpulmonary distending pressure, volumes of recruited lung, and distribution of lung aeration during inspiration and expiration. The observations suggest that clinical reliance on a single transpulmonary pressure measurement (e.g., esophageal manometry) may be inadequate to describe the overall forces acting on the lung, and that use—or incorporation—of NPV should be reinvestigated in the clinical context in which oxygenation is problematic or lung injury is likely.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

References