Effects of mechanical ventilation at low lung volume on respiratory mechanics and nitric oxide exhalation in normal rabbits

EDGARDO D'ANGELO[1], MATTEO PECCHIARI[1], PATRIZIA DELLA VALLE[2], ANTONIA KOUTSOUKOU[3], and JOSEPH MILIC-EMILI[4]

[1]Istituto di Fisiologia Umana I, Università degli Studi di Milano, 20133 Milan, Italy, [2]Servizio di Coagulazione & Unità di Ricerca Trombosi, IRCCS Ospedale San Raffaele, 20112 Milan, Italy, [3]Critical Care Department, University of Athens, Greece, and [4]Meakins-Christie Laboratories, McGill University, Montreal, Quebec, Canada H2X 2P2

Running title: NITRIC OXIDE ELIMINATION

DOI: http://dx.doi.org/10.1152/japplphysiol.01368.2004

Address correspondence to:
Edgardo D'Angelo, MD
Istituto di Fisiologia Umana I
via Mangiagalli 32
20133 Milan, Italy
Tel. +39-02-50315440; Fax +39-02-50315430
E-mail edgardo.dangelo@unimi.it

ABSTRACT

Lung mechanics, exhaled NO (NOe), and tumor necrosis factor (TNF-α) in serum and bronchoalveolar lavage (BAL) fluid were assessed in 8 closed and 8 open chest, normal anesthetized rabbits undergoing prolonged (3-4 h) mechanical ventilation (MV) at low volume with physiologic tidal volumes (10 $ml \cdot kg^{-1}$). Relative to initial MV on positive end-expiratory pressure (PEEP), MV at low volume increased lung quasi-static elastance (Est; +267 and +281%), airway (Rint; +471 and +382%) and viscolelastic resistance (Rvisc; +480 and +294%), and decreased NOe (-42 and -25%) in closed and open chest rabbits, respectively. After restoration of PEEP, Rvisc returned to control, while Rint remained elevated (+120 and +31%), and NOe low (-25 and -20%) in both groups of rabbits. Est remained elevated (+23%) only in closed chest animals, being associated with interstitial pulmonary edema, as reflected by increased lung wet/dry ratio with normal albumin concentration in BAL fluid. In contrast, in 16 additional closed and open chest rabbits there were no changes of lung mechanics or NOe after prolonged MV on PEEP only. At the end of prolonged MV, TNF-α was practically undetectable in serum, while its concentration in BAL fluid was low and similar in animals subjected or not subjected to ventilation at low volume (62 vs 43 $pg \cdot ml^{-1}$). These results indicate that mechanical injury of peripheral airways due to their cyclic opening and closing during ventilation at low volume results in changes in lung mechanics and reduction in exhaled NO, and that these alterations are not mediated by a proinflammatory process as this is expressed by TNF-α levels.

Key words: lung elastance; interrupter resistance; viscoelasticity; proinflammatory cytokines; exhaled vapor condensate

In an *ex vivo* model of lavaged rat lung, Muscedere et al. (25) showed that ventilation with physiologic tidal volumes from zero end-expiratory pressure (ZEEP) resulted in a significant increase of histologic injury scores in the respiratory and membranous bronchioles relative to ventilation from positive end-expiratory pressure (PEEP) above the lower inflection point on the static inflation volume-pressure curve of the lung. Subsequently, it has been shown that also in normal open chest rabbits prolonged (3-4 h) mechanical ventilation at ZEEP induces histologic evidence of peripheral airway injury and parenchymal inflammation with a concomitant increase in airway resistance which persists after restoration of physiologic end-expiratory lung volume (11,12). In these studies, morphological and mechanical alterations have been attributed to shear stresses caused by cyclic opening and closing of peripheral airways with tidal ventilation at low lung volumes, as previously suggested by Robertson (31), possibly combined with increased surface tension due to surfactant depletion or inactivation.

Recruitment of polymorphonuclear leukocytes in the alveolar walls during ventilation at low volume (12) fits a recently described type of ventilator-induced lung injury called *biotrauma* (14). Under this condition, parenchymal overdistension and abnormal shear forces could represent the mechanical stimuli leading to release of mediators that prime polymorphonuclear leukocytes, which may represent the major effector cells in the generation of tissue injury and upregulation of the inflammatory response (14,39). Increased concentrations of proinflammatory cytokines, mainly tumor necrosis factor (TNF)-α and interleukin (IL)-6, have been in fact observed in bronchoalveolar lavage (BAL) fluid of excised rat lungs after prolonged ventilation at ZEEP (6,38). Because these cytokines have been shown to enhance the expression of inducible nitric oxide synthase (iNOS) in an *in vitro* preparation (2), nitric oxide concentration in expired air (NOe) could eventually increase also in normal rabbits ventilated at low lung volume, and thus serve as a marker of parenchymal inflammation. Other studies have shown, however, that injurious ventilation in initially intact rats does not affect *in vivo* proinflammatory cytokine production (18,30,41). Human alveolar macrophages and epithelial cells subjected to prolonged cyclic stretching release IL-8, which is involved in the recruitment of polymorphonuclear leukocytes, but not proinflammatory cytokines, such as TNF-α or IL-6 (29,42). Moreover, antiinflammatory mediators are also expressed during ventilation at low volume (38), thus making it difficult to recognize the effective orientation of cytokine balance (24). On this basis, increased concentration of NOe should not be expected to occur in normal rabbits during prolonged ventilation at low volume. In contrast, since most of the NO from the lungs is produced by small airway epithelium, a reduction in NOe levels could be a useful marker of the extent of the mechanical injury of the peripheral airways due to their cyclic opening and closing during tidal ventilation at low lung volumes (11,25). Moreover, NO

production could be reduced by prostaglandins E_2 and F_{2a} (21) released by alveolar macrophages, polymorphonuclear leukocytes, airway and alveolar epithelial cells activated by mechanical insults (23), as well as by the vasoactive intestinal peptide (13), which may be also involved in the inflammatory response of the lung (33).

The purpose of the present investigation in normal rabbits is therefore to assess the effects of 3-4 h ventilation at low lung volume on NO production from the tracheobronchial tree, also in relation to possible inflammatory reaction, as monitored by TNF-α levels in serum and BAL fluid. While previous studies (11,12) on the morphological and mechanical effects of ventilation at low lung volume were performed on open-chest rabbits only, the present experiments also included closed chest rabbits, in order to avoid confounding inflammatory responses elicited by the major surgical intervention required in open chest animals.

METHODS

Forty New Zealand white rabbits (weight range 2 to 3.1 kg) were anesthetized with an intravenous injection of a mixture of pentobarbital sodium (20 $mg \cdot kg^{-1}$) and urethane (0.5 $mg \cdot kg^{-1}$). A brass cannula and polyethylene catheters were inserted into the trachea, the carotid and femoral artery, and the external jugular vein, respectively. The animals were paralyzed with pancuronium bromide (0.1 $mg \cdot kg^{-1}$) and mechanically ventilated (respirator 660; Harvard Apparatus, Holliston, MA) with a pattern similar to that during spontaneous breathing. Anesthesia and complete muscle relaxation were maintained with additional doses of the anesthetic mixture and pancuronium bromide. Throughout the experiment, Ringer-bicarbonate solution was infused via the jugular vein at a rate of 4 $ml \cdot kg^{-1} \cdot h^{-1}$. Before the final mechanics and subsequent nitric oxide measurements, boluses of bicarbonate (1 M) solution and epinephrine were given intravenously to keep arterial pH and systemic blood pressure close to the initial values. Sixteen rabbits were studied with closed chest (*group A*), and sixteen animals with open chest (*group B*). The chest was opened via a median sternotomy, a coronal cut was made just above the costal arch, and a positive end-expiratory pressure of 2-2.5 cm H_2O was applied. In the latter animals and during the mechanics measurements, the ribs on the two sides and the diaphragm were pulled widely apart, in order to prevent contact between lung and chest wall, except in their dependent parts. The animals rested supine on a heating pad; rectal temperature was kept essentially constant under all conditions at 37.5±0.1(S.E.) and 36.3±0.1 °C in closed-chest and open-chest animals, respectively.

Airflow ($\dot{V}$) was measured with a heated Fleisch pneumotachograph no.00 (HS Electronics, March-Hugstetten, Germany) connected to the tracheal cannula and a differential pressure transducer (Validyne MP45, ±2 cm H_2O; Northridge, CA). The response of the pneumotachograph

was linear over the experimental range of $\dot{V}$. Tracheal (Ptr), esophageal (Pes), and systemic blood pressure (Pa) were measured with pressure transducers (model 1290A; Hewlett-Packard, Palo Alto, CA) connected to the side arm of the tracheal cannula, a latex balloon (2.5 cm long, 0.5 cm ID) suitably placed in the lower esophagus, and the femoral artery, respectively. There was no appreciable shift in the signals or alteration in amplitude up to 20 Hz. Nitric oxide (NO) was measured using a chemiluminescence analyzer (NOA 280i, Sievers, Boulder, CO), attached to a side port of the tracheal cannula through teflon tubing and set to draw air at a rate of 200 $ml \cdot min^{-1}$. Zeroing and calibration of the NO analyzer was verified repeatedly during the experiment using the Zero Air Filter and certified gas mixture provided by the manufacturer. The signals from the transducers were amplified (model RS3800; Gould Electronics, Valley View, OH), sampled at 200 Hz by a 12-bit A/D converter (AT MIO16E-10; National Instruments, Austin, TX), and stored on a desk computer, together with the signal from the NO analyzer. Volume changes (ΔV) were obtained by numerical integration of the digitized airflow signal. Arterial blood P_{O_2}, P_{CO_2} and pH were measured by means of a blood gas analyzer (IL 1620; Instrumentation Laboratory, Milan, Italy) on samples drawn from the carotid artery at the beginning and at the end of each test session, while the pH of the deareated, exhaled airway vapor condensate (Hunt et al., 2000) was measured using an Amersham Pharmacia C900 pH meter (Uppsala, Sweden).

After completion of the surgical procedure and instrumentation, the rabbits were ventilated with a specially designed, computer-controlled ventilator, delivering a NO free (NO concentration <0.5 ppb), water-saturated gas mixture from a high pressure source (4 atm) at constant flow of different selected magnitudes and duration. A three way stopcock allowed the connection of the expiratory valve of the ventilator either to the ambient (zero end-expiratory pressure; ZEEP) or to a drum in which the pressure could be made positive (positive end-expiratory pressure; PEEP) or negative (negative end-expiratory pressure; NEEP) by means of a flow-through system. A detailed description of the ventilator can be found elsewhere (12).

For all animals, the baseline ventilator settings consisted of fixed tidal volume (V_T; 10 $ml \cdot kg^{-1}$), inspiratory duration (T_I; 0.25 s), and cycle duration (1.8 s). An end-inspiratory pause of 0.35 s was applied in order to ensure a normal mean lung volume during the respiratory cycle. During NO measurements both the inspiratory and expiratory duration were set at 1 s and the end-inspiratory pause was removed. With the above settings, no intrinsic PEEP was present under any experimental condition, as evidenced by an end-expiratory pause (zero flow) and absence of Ptr changes with airway occlusion at end-expiration. While open chest rabbits were always ventilated with air, closed chest animals were intermittently ventilated with 70-80% oxygen during MV on

NEEP in order to limit the profound, life threatening hypoxia that occurred otherwise. However, measurements were always performed during air breathing.

Procedure and data analysis

Rabbits of *group A* and *B* were equally divided into two subgroups, *group Acontrol* and *Atest*, and *Bcontrol* and *Btest*, respectively. Rabbits of *group Atest* and *Btest* were subjected to the following sequence of PEEP and NEEP or ZEEP: *a)* 30 min of mechanical ventilation (MV) with PEEP ($PEEP_1$); *b)* 3-4 h of MV at NEEP or ZEEP; *c)* 1.5 h of MV with PEEP. Rabbits of *group Acontrol* and *Bcontrol* were subjected for the same cumulative time to MV with PEEP only. In open chest animals, the end-expiratory pressure was almost the same during the initial and final period of MV on PEEP, averaging 2.3±0.1 cmH_2O. In closed chest animals, an end-expiratory pressure of 1.2±0.1 cm H_2O was applied in order to limit or prevent the expected fall in the end-expiratory lung volume with anesthesia and paralysis, while NEEP was –7.7±0.1 cm H_2O. Upon completion of *in vivo* measurements, the animals were killed with an overdose of anesthetics, the lungs were isolated, the main right bronchus tied off, and the right lung was removed, weighed immediately, left overnight in an oven at 120 °C, and weighed again to compute the wet/dry ratio. The left lung was lavaged four times using 3-ml aliquots of normal saline, fluid recovery ranging from 40 to 50%. The effluents were pooled, centrifuged (Harrier 18/80, Sanyo Gallenkamp PLC, Loughborough, UK) at 2000 rpm for 10 min, and the supernatant frozen and stored at –20°C. The animals were from a single cohort and the experiments were done in random order.

In order to assess tumor necrosis factor levels in the bronchoalveolar lavage (BAL) fluid before the prolonged MV at low or normal end-expiratory lung, in an additional group of eight closed-chest rabbits (*group C*), instrumented as described above, BAL fluid was obtained after 15 min of MV with PEEP, while blood samples were taken before and after induction of anesthesia and paralysis, on completion of the surgical maneuvers, and at $PEEP_1$.

Mechanical characteristics were studied during $PEEP_1$, at start ($NEEP_1$ or $ZEEP_1$) and end of the NEEP or ZEEP period ($NEEP_2$ or $ZEEP_2$), and ~15 min after MV on PEEP had been restored ($PEEP_2$). Before all measurements on PEEP the lungs were inflated 3-4 times to Ptr of ~30 cm H_2O. Two types of measurements were carried out: *a)* while keeping V_T at baseline values, test breaths were intermittently performed with different $\dot{V}_I$ and T_I in the range 0.25 to 3 s to assess mechanics at end-inflation; and *b)* while keeping $\dot{V}_I$ at baseline values, test breaths were intermittently performed with different V_T to obtain quasi-static inflation volume-pressure curves. End-inspiratory occlusions lasting 5 s were made in all test breaths, which were performed in random order and repeated 3-5 times. During ventilation at NEEP or ZEEP, end-inspiratory occlusions were performed only for tidal volumes ≤ baseline V_T. In open chest rabbits and during ventilation with

PEEP, the expiratory valve was opened to the ambient in order to measure the difference between the end-expiratory and the resting lung volume (ΔEELV). In closed chest animals, ΔEELV was obtained as the volume exhaled with a Ptr of –20 cmH_2O. Mechanical parameters were assessed with the rapid airway occlusion method (3,10). The end-inspiratory airway occlusions were followed by a rapid initial drop in pressure (ΔP_1), and by a slow decay (ΔP_2) to an apparent plateau value (Pst). This pressure, computed as the mean pressure recorded during the last 0.5 s of occlusion, was taken to represent the quasi-static pressure, while ΔP_1 and ΔP_2 divided by $\dot{V}_I$ yielded the interrupter (Rint) and additional (ΔR) resistance, respectively. Viscoelastic parameters, Rvisc and τvisc=Rvisc/Evisc, were computed by fitting the values of ΔR and T_I with the function (10)

$$\Delta R = Rvisc \cdot (1 - e^{-T_I/\tau visc}) \quad (1)$$

while quasi-static elastance (Est) was obtained as (Pst-Pee)/V_T, Pee being the end-expiratory pressure. The parameters above referred to the respiratory system, lung and chest wall depending on whether tracheal, transpulmonary, or esophageal pressure was being used in the computations. In closed chest animals transpulmonary pressure (Ptp) was obtained as Ptr-Pes. The negative value of Ptr recorded after wide opening of the chest with closed airway opening was assigned to the esophageal pressure just before chest opening, and Pes values obtained under all other conditions were corrected accordingly.

Tracheal NO concentration was continuously measured for 15-20 min at the transition from PEEP ($PEEP_1$) to NEEP or ZEEP ($NEEP_1$ or $ZEEP_1$) and from NEEP or ZEEP ($NEEP_2$ or $ZEEP_2$) to PEEP ($PEEP_2$), and, for 10 min periods, ~60 and 90 min after restoration of MV on PEEP in order to check any possible deterioration of the preparation. Moreover, during this period, administration of epinephrine, boluses of bicarbonate (1M) solution, and/or short periods of hyperventilation and oxygen breathing were performed to keep the values of Pa, Pa_{O_2}, Pa_{CO_2} and pHa close to those with $PEEP_1$. For a given condition, ~60 breaths were ensemble averaged, and the mean concentration of NO during expiration was used as the exhaled NO concentration (NOe). Moreover, during $PEEP_1$, $NEEP_2$ or $ZEEP_2$, and $PEEP_2$, part of the tubing beyond the expiratory valve of the ventilator was immersed in ice cold water to obtain ~1 ml of exhaled airway vapor condensate (20).

Analysis of tumor necrosis factor (TNF-α) was carried out in a blinded fashion on BAL fluid and serum collected under conditions $PEEP_1$, $NEEP_2$ or $ZEEP_2$, and $PEEP_2$ in *group A* and *B*, and before and after induction of anesthesia and at the start and end of the 15 min period of MV on PEEP in *group C*, using a commercially available ELISA kit (BD Bioscience, Franklin Lakes, NJ), specific for rabbit. TNF-α color development was measured at 405 nm (Titertek Multiskan MCC,

Flow Laboratories, Milan, Italy), background absorbancy of blank wells being subtracted from the standards and samples prior to determination of the concentration. The lower limit of detection was 10 $pg \cdot ml^{-1}$, in which case TNF-α concentration was assumed to be nil. The albumin concentration of the BAL fluid supernatant and serum obtained shortly before lung lavage was determined with a clinical chemistry analyzer (Falcor 350, Menarini Diagnostics, Florence, Italy) at 630 and 700 nm using the BCG method (Menagent, Menarini Diagnostics) with bovine albumin as standard.

Statistics. Results are presented as means±SE, except for TNF-α measurements. The least-square regression method was used to assess the parameters in Eq.1 and of the pressure-volume relationship of the lungs. Comparisons among experimental conditions were performed using one-way analysis of variance (ANOVA); when significant differences were found, the Bonferroni test was performed to determine significant differences between different experimental conditions. Results from TNF-α measurements are expressed as median and range, and the statistical analysis was performed using the Mann-Whitney test. The level for statistical significance was taken at $P \leq 0.05$.

RESULTS

The group mean values of Pa_{CO_2} and pHa during $PEEP_1$ and $PEEP_2$ were similar in closed and open chest rabbits, whereas those of Pa_{O_2} were significantly lower in closed chest animals (Table 1). Relative to $PEEP_1$, with $NEEP_1$ or $ZEEP_1$ there was a similar increase of Pa_{CO_2} and decrease of Pa_{O_2} and pHa in both closed and open chest rabbits. Except for a significant decrease of pHa in open chest animals, no further changes of these parameters occurred with $NEEP_2$ or $ZEEP_2$.

The values of wet-to-dry ratio assessed at the end of the experiments were similar in both groups of open chest rabbits, as well as in closed chest rabbits ventilated on PEEP only, and not significantly different from those of freshly excised rabbits lungs (11). In contrast, the wet-to-dry ratio of closed chest animals ventilated on NEEP was significantly larger than that of all other groups of rabbits (Table 1), whilst albumin concentration in BAL fluid and the ratio of BAL to serum albumin concentration were similar in both *group A* and *B* rabbits, and similar to the corresponding values (0.2±0.1 $g \cdot l^{-1}$ and 1±0.3 %, respectively) found in closed chest animals not subjected to prolonged MV on ZEEP or PEEP (*group C*).

Mechanics

In closed chest animals, the quasi-static volume-pressure curve of the chest wall on $PEEP_1$ and $PEEP_2$ did not differ (Fig. 1). Moreover, chest wall viscous resistance and viscoelastic properties were the same under all conditions (Tables 3 and 4). Hence any change in the mechanical

properties of the respiratory system because of prolonged ventilation at low volume was the consequence of changes in lung mechanics.

Static inflation V-P relationships. In all groups of rabbits Ptp at end-expiration was similar during $PEEP_1$ and $PEEP_2$, averaging 2.2 cm H_2O, but the difference between the end-expiratory and the resting lung volume (ΔEELV) was significantly larger in open than in closed chest animals (Table 2). With $PEEP_1$ and $PEEP_2$, ΔEELV was the same in open chest rabbits and in closed chest rabbits ventilated on PEEP only, whilst in rabbits ventilated on NEEP, ΔEELV decreased significantly (-4.2±1.4 ml; $P<0.025$) with $PEEP_2$ (Table 2).

In closed chest animals, independent of ventilation on NEEP, the inflation V-P curve of the lungs on PEEP was s-shaped (Fig. 2). When the lower three data-points were disregarded, the V-P curve closely fitted ($r>0.95$) a function in the form $V_o-V_x \cdot e^{-K \cdot Ptp}$, where V_o is maximum volume above resting lung volume, V_x is a volume factor accounting for the rightward shift of the curve due to lung unit recruitment, and K (cmH_2O^{-1}) is a shape factor, that reflects the overall distensibility of the lung (9,16,34). In *group Acontrol* rabbits, all these parameters were essentially the same during $PEEP_1$ and $PEEP_2$ (Table 2). In *group Atest* rabbits V_o and V_x, decreased significantly with $PEEP_2$ (-11.6±3.1 and -15.3±3.4 ml; $P<0.001$), whilst K, though reduced, did not (-0.01±0.01 cmH_2O^{-1}; $P>0.5$).

In open chest animals, the entire inflation V-P curve on PEEP closely fitted ($r>0.97$) a function in the form $V_o \cdot (1-e^{-K \cdot Ptp},)$, with V_o and K as indicated above (Table 2). Because in all animals none of these values changed significantly after prolonged ventilation on ZEEP (*group Btest*) or PEEP (*group Bcontrol*), a unique relationship could be used to describe the quasi-static V-P curve above the end-expiratory lung volume with PEEP (Fig. 2). Finally, when compared to the corresponding value on $PEEP_1$ in closed chest animals, V_o in open chest rabbits was essentially the same (81.1±3.9 vs. 89±2.9 ml; $P=0.19$), while K was significantly larger (0.178±0.005 vs. 0.143±0.006 cmH_2O^{-1}; $P=0.001$).

On NEEP or ZEEP, the quasi-static inflation V-P curve of the lung shifted downwards both in closed-chest and open-chest animals (Fig. 2). Moreover, the V-P curve of open chest animals, which on PEEP was concave towards the pressure axis, became markedly s-shaped, as in closed chest animals. All these changes increased with $NEEP_2$ or $ZEEP_2$.

Elastance. On the basis of the V_o and ΔEELV values in Table 2, tidal ventilation with PEEP occurred in the range 25-50 and 33-65% V_o in closed and open chest animals, respectively, while during NEEP or ZEEP, tidal ventilation occurred in the range 0-26% V_o in both closed and open chest animals. The average values of quasi-static lung elastance (Est) obtained in the various groups of animals and conditions are reported in Table 3. With $PEEP_1$, Est was significantly larger in

closed than in open chest animals (209±11 vs. 144±5 $cmH_2O \cdot l^{-1}$; P<0.001). With $PEEP_2$, Est increased significantly only in *group Atest* rabbits. Est increased markedly and progressively from $NEEP_1$ or $ZEEP_1$ to $NEEP_2$ or $ZEEP_2$, more in closed than in open chest animals. Relative to $PEEP_1$, Est increased however, by a similar amount in *group Atest* and *Btest* rabbits both with $NEEP_1$ or $ZEEP_1$ (225±18 vs. 203±25%; P=0.5) and with $NEEP_2$ or $ZEEP_2$ (267±19 vs. 281±28%; P>0.5).

Rint. At the end-inspiratory volume of baseline ventilation, the pulmonary interrupter resistance (Rint) was independent of flow, at least in the range 10-100 $ml \cdot s^{-1}$, in all animals and conditions; hence the values of Rint obtained in each rabbit and condition were averaged (Table 3). With $PEEP_1$, Rint was similar in closed and open chest animals (11.3±0.9 vs. 10.4±0.5 $cmH_2O \cdot s \cdot l^{-1}$; P=0.38). With $PEEP_2$, Rint did not differ significantly from $PEEP_1$ values in animals ventilated on PEEP only (*group Acontrol* and *Bcontrol*), whereas in animals ventilated on NEEP or ZEEP (*group Atest* and *Btest*), Rint increased significantly, and more in closed than in open chest animals, both in absolute (14.6±3.5 vs. 2.9±0.6 $cmH_2O \cdot s \cdot l^{-1}$; P=0.005) and relative terms (120±32 vs. 31±6%; P=0.014). Rint increased progressively from $NEEP_1$ or $ZEEP_1$ to $NEEP_2$ or $ZEEP_2$, and more in closed than in open chest animals. Relative to $PEEP_1$, Rint increased, however, by a similar amount in *group Atest* and *Btest* rabbits both with $NEEP_1$ or $ZEEP_1$ (292±22 vs. 210±31%; P=0.11) and with $NEEP_2$ or $ZEEP_2$ (471±46 vs. 382±36%; P=0.15).

Viscoelastic properties. In all animals and conditions, a unique function in the form of *Eq. 1* adequately described the experimental ΔR-T_I data of the respiratory system, lung, and chest wall (r>0.92), allowing computation of the dependent Rvisc and $\tau visc$ values. Figure 3 depicts the group mean relationships of ΔR to T_I of the lung obtained under the various experimental conditions, while the group mean values of Rvisc and $\tau visc$ are reported in Table 4. With $PEEP_1$, both the Rvisc and $\tau visc$ values did not differ significantly between closed and open chest animals (72±6 vs. 65±7 $cmH_2O \cdot s \cdot l^{-1}$; P>0.5, and 1.30±0.11 vs. 1.34±0.13 s; P=0.09). With $PEEP_2$, neither Rvisc nor $\tau visc$ changed significantly relative to corresponding $PEEP_1$ values. Rvisc increased progressively from $NEEP_1$ or $ZEEP_1$ to $NEEP_2$ or $ZEEP_2$, and more in closed than in open chest animals (258±30 vs. 134±21 $cmH_2O \cdot s \cdot l^{-1}$; P=0.005, and 330±52 vs. 191±15 $cmH_2O \cdot s \cdot l^{-1}$; P=0.022). While in open chest animals $\tau visc$ did not change with ZEEP, in closed chest animal $\tau visc$ increased during NEEP ventilation ($\Delta \tau visc$=0.39±0.09 s; P<0.001).

NO production

The effects of prolonged MV at normal and reduced end-expiratory lung volume on exhaled NO (NOe) concentration in both closed and open chest rabbits are shown in Figures 4 and 5, respectively, together with the changes of a number of parameters that could potentially affect NOe concentration.

With $PEEP_1$, NOe concentration was 29.7±2.9 and 28.4±3.9 ppb in *group Acontrol* and *Atest* rabbits, and 20.4±1.5 and 21.3±1.9 ppb in *group Bcontrol* and *Btest* rabbits; thus NOe concentration did not differ significantly between the two subgroups of closed-chest and open-chest rabbits, whilst it was significantly larger in closed than in open chest rabbits (28.8±2.7 vs. 21±1.3 ppb; P=0.016). Mean systemic arterial pressure ($\overline{Pa}$), Pa_{O_2}, Pa_{CO_2}, pHa, and body temperature were similar in the two subgroups of closed and open chest rabbits, respectively. While $\overline{Pa}$, Pa_{CO_2}, and pHa were similar in open and closed chest animals, Pa_{O_2} was significantly lower (82±2 vs. 103±2 mm Hg; P<0.0001) and body temperature higher (37.4±0.1 vs. 36.8±0.1 °C; P=0.001) in closed chest animals.

With prolonged MV on PEEP only, there was a tendency for NOe to decrease; however, during the 5-5.5 h of MV the rate of decay of NOe concentration was not significant both in closed (-0.55±0.95 ppb·h^{-1}; P>0.5) and open chest animals (-0.37±0.47 ppb·h^{-1}; P=0.44). Similarly, none of the other parameters changed significantly with MV on PEEP only (Figs. 4 and 5).

On transition from PEEP to NEEP or ZEEP, NOe concentration increased both in closed (2.8±2.4 ppb) and open chest animals (3.9±0.2 ppb), the change being significant only in the latter group of rabbits (P<0.001). On NEEP or ZEEP, NOe concentration decreased more in closed (-12±2.7 ppb; P<0.001) than open chest animals (-5.7±1.9 ppb; P<0.025), whilst on transition to PEEP there was a small significant increase in NOe concentration in closed and open chest animals (2.4±0.5 and 1.4±0.1 ppb; P<0.001). On the other hand, no further changes in NOe concentration occurred during the subsequent 1.5 h of MV on PEEP both in closed and open chest rabbits (Figs. 4 and 5). During this period, the average NOe concentration was similar in closed and open chest animals (19.5±2.3 vs. 16.7±0.9 ppb; P=0.25) and markedly lower than that on $PEEP_1$ (Figs. 4 and 5), amounting to 69 and 78 % of $PEEP_1$ values in *group Ates* and *Btest* rabbits.

As shown in Figs. 4 and 5, no significant changes in Pa_{CO_2}, and pHa occurred on transition from PEEP to NEEP or ZEEP, whilst Pa_{O_2} decreased markedly both in closed and open chest animals (-40±3 and -23±5 mm Hg; P<0.001). With prolonged MV at NEEP or ZEEP, Pa_{CO_2} increased by ~10 mm Hg, pHa decreased by ~0.14, and Pa_{O_2} dropped by an additional ~15 mm Hg. while on transition from NEEP or ZEEP to PEEP ($PEEP_2$) only Pa_{O_2} showed a significant increase of ~15 mm Hg. With $NEEP_2$ and $ZEEP_2$, $\overline{Pa}$ decreased significantly both in closed and open chest

animals, and remained significantly lower than control values also on $PEEP_2$ (Figs. 4 and 5). During the final 1.5 h of MV on PEEP, administration of epinephrine, boluses of bicarbonate (1M) solution, and/or short periods of hyperventilation and oxygen breathing brought the values of all these parameters back to those with $PEEP_1$ (Figs. 4 and 5).

Airway vapor condensate

The mean pH of exhaled airway vapor condensate sampled in closed and open chest animals on $PEEP_1$, $PEEP_2$, $NEEP_2$, and $ZEEP_2$ are shown in Table 5. No significant differences were found between pH values of closed and open chest rabbits under all conditions, or among the pH values obtained under the various conditions in either closed or open chest rabbits. When pH values measured in all animals and conditions were pooled, the average pH of the exhaled airway vapor condensate was 6.89±0.05 (range: 6.15-7.61).

Tumor necrosis factor-α

Under all conditions, TNF-α concentration in BAL fluid was low, but significant. Although the largest median value occurred in animals subjected to prolonged MV on NEEP (*group Atest*), TNF-α levels did not differ significantly among all groups of animals (Table 6). Similarly, although the lowest median value of TNF-α concentration in BAL fluid occurred in animals not subjected to prolonged MV (*group C*), this concentration did not differ significantly from that of *group A* and *B* rabbits.

With $PEEP_1$, serum TNF-α concentration was high and similar in all groups of closed and open chest animals (Table 6). In contrast, serum TNF-α concentration was essentially nil during $NEEP_2$, $ZEEP_2$, and $PEEP_2$, as well as in *group C* rabbits before induction of anesthesia. In these rabbits, serum TNF-α levels during MV on $PEEP_1$ were, however, high and similar to those found under the same condition in *group A* and *B* rabbits (Table 6). Figure 6 provides a composite picture of the time course of serum TNF-α concentration in *group Atest, Btest*, and *C* rabbits. Serum TNF-α levels grew rapidly during the surgical interventions, peaking at their end, and then declined, becoming nil after 3-4 h, or likely earlier.

DISCUSSION

Lung injury during ventilation at low lung volumes with physiologic tidal volumes has been attributed to cyclic opening and closing of peripheral airways (11,31) with concurrent generation of abnormal, inhomogeneous shear stresses that are eventually responsible for mechanical and histologic damage in respiratory and membranous bronchioles (11,25), marked alterations of alveolar-bronchiolar coupling, inflammatory response, and increase in airway resistance (11,12). The present results indicate that prolonged mechanical ventilation at low lung volumes reduces NO

concentration in exhaled air without affecting proinflammatory cytokines, as indexed by TNF-α concentration in BAL fluid and serum, and that both the fall of NOe and the adverse mechanical effects on the lung (Tables 3 and 4, and Fig. 1) are greater in closed than open chest animals. In addition, in closed chest rabbits there is a significant increase in lung elastance, probably due to interstitial edema, as reflected by increased wet-to-dry ratio but normal albumin concentration in BAL fluid. On the other hand, when lung volumes are kept within the physiologic range with PEEP, prolonged mechanical ventilation does not result in significant changes in exhaled NO concentration, lung mechanics, and TNF-α levels in BAL fluid and serum.

Lung mechanics

In line with previous results (11,12), during mechanical ventilation with PEEP there was no evidence of airway closure in open chest animals, since the static inflation V-P curve of the lung was concave to the pressure axis (16), as shown in Figure 2. In closed chest animals, however, the initial part of the inflation V-P curve was slightly convex to the pressure axis, suggesting some progressive reopening of small airway (<1 mm in diameter; 19) during tidal ventilation. Indeed, small airway closure is likely to occur in the dependent zones of the lung in supine, anesthetized, intact rabbits at functional residual capacity, since under this condition pleural surface pressure in the lowermost part of the pleural space is nil (8). In contrast, at low end-expiratory volume the inflation V-P curve became markedly sigmoidal both in closed and open chest animals (Fig. 2), indicating that under this condition there was substantial cyclic airway opening and closing, which should be responsible for the histologic alterations previously observed in open chest animals (11,12), as well as the increase in Rint on $PEEP_2$ relative to $PEEP_1$. The presence of the vertical gradient of transpulmonary pressure in the closed chest rabbits probably explains the greater convexity of the initial part of the quasi-static inflation V-P curve from low end-expiratory volumes in closed than open chest rabbits (Fig. 2). Indeed, the ratio between Est with the lowest inflation volume (~4 $ml \cdot kg^{-1}$) and baseline V_T was significantly larger in *group Atest* than *Btest* both at the beginning (2.22±0.07 vs 1.18±0.06; $P<0.001$) and at the end of the NEEP and ZEEP period (2.83±0.16 vs 1.30±0.06; $P<0.001$), respectively. This should indicate that in closed chest animals more airways were involved in cyclic opening and closing with greater mechanical alterations (Table 3) and, possibly, histologic damage.

Both in closed and open chest rabbits, there was a significant increase of Est, Rint, and Rvisc relative to $PEEP_1$, which was significantly greater after 3-4 h ($NEEP_2$ and $ZEEP_2$) than after ~15 min ($NEEP_1$ and $ZEEP_1$) of ventilation at low lung volume (Tables 3 and 4). Similar results have been reported in previous studies on open chest rabbits (11,12,36), in which the increase of Est and Rvisc was attributed to a higher surface tension and a decrease of ventilated tissue due to airway closure and

dependent gas trapping, that of Rint to reduction of ventilated tissue, uncoupling between peripheral airways and lung parenchyma and, possibly, increased bronchomotor tone (11,12). The first mechanism should contribute mainly to the increase in Rvisc, especially on ZEEP$_2$, since most of Rvisc should reside in the air-liquid interface (1), while the second accounts for the proportional changes of Est and Rvisc with an essentially constant τvisc (Tables 3 and 4). Enhanced depletion or inactivation of lung surfactant and greater extent of airway collapse and cyclic opening and closing should explain the greater increase of Est, Rint, and Rvisc observed in closed chest animals, while an increased inequality of regional lung expansion with decreasing lung volume may account for the augmented τvisc and loss of proportionality between Rvisc and Est relative changes (Tables 3 and 4). Greater inhomogeneity of ventilation distribution in closed chest animals, poorly compensated by the P_{O_2}-dependent redistribution of pulmonary perfusion, is further supported by the lower values of P_{aO_2} (Table 1 and Figs. 4 and 5), in spite of intermittent oxygen administration.

Increased surface tension, differences in pulmonary vascular pressures, and release of vasoactive substances (see below) could explain the presence of pulmonary edema in closed chest animals with NEEP, as reflected by the significant increase of wet/dry ratio (Table 1), the downward shift of the inflation V-P curve (Figs. 1 and 2), and the reduction of ΔEELV (Table 2). Edema was, however, limited to the interstitium, because both the albumin concentration in BAL fluid and the ratio between albumin concentration in BAL fluid and serum were normal. Moreover, on dissecting the lungs no foam could be observed in peripheral units and airways.

After return to PEEP (PEEP$_2$), Rvisc and Est of open chest animals reversed to the initial (PEEP$_1$) values (Tables 3 and 4). In closed chest animals, however, Est, though partially restored, remained significantly larger (Table 3), likely because of interstitial edema and increased surface forces. In contrast, Rint was significantly increased both in closed and open chest animals (Table 3). The increase in Rint could not be related to changes in arterial blood gases or pH (Table 1), nor to changes in elastic recoil which, relative to PEEP$_1$, was either the same or moderately increased in open chest or closed chest rabbits, respectively (Table 3 and Fig. 2). This increase is due to damage of peripheral airway (11), changes in the mechanical coupling between peripheral airways and lung parenchyma (12), interstitial edema, and, possibly, increased bronchomotor tone. These mechanisms played a greater role in closed chest animals, as Rint increased more in closed (120±32%) than in open chest rabbits (31±6%). Based on greater wet/dry ratio (Table 1), small airway edema could have been more pronounced in closed chest animals. Similarly, because of the greater number of airways involved in cyclic opening and closing and higher airway opening pressures in closed than open chest animals during low-volume ventilation (Fig. 2), larger inhomogeneous shear stress should have occurred in the former group of rabbits, thus enhancing the functional alterations.

Exhaled NO concentration

In both closed and open chest animals ventilated for 3-4 h on PEEP, exhaled NO concentration did not change significantly (Figs. 4 and 5). Furthermore, NOe values on $PEEP_1$ were comparable with those of normal, anesthetized rabbits (15,17). They were, however, significantly higher in closed than open chest rabbits. This could reflect the lower body temperature in open than closed chest animals (36.3 vs 37.5°C), a difference that might have been larger at the level of airway epithelium. Moreover, the major surgery required to open the chest may also have played a role, though the concentration of the proinflammatory cytokine TNF-α did not differ significantly between open and closed chest animals (Table 6). At $ZEEP_1$ and $NEEP_1$ there was a small increase in NOe (Figs. 4 and 5), likely related to the decrease in pulmonary blood flow (4,7) with increasing pulmonary vascular resistance due to the reduction in lung volume.

Prolonged mechanical ventilation at low volume substantially lowered NOe both in closed and open chest animals, a reduction which persisted after restoration of the end-expiratory volume (Figs. 4 and 5). Since the tidal volume was the same under all conditions, the fall of NOe was the consequence of decreased elimination of NO from injured terminal and respiratory bronchioles, which in our preparation are the main source of exhaled NO (27). Indeed, prolonged mechanical ventilation at low lung volume causes small airway injury with epithelial necrosis and sloughing in open chest rabbits (11). Although peripheral airway injury has not been studied histologically in closed chest rabbits, it seems likely that the greater reduction of NOe found in these animals (Figs. 4 and 5) was related to greater histologic and mechanical damage.

In open chest animals ventilated on ZEEP, necrosis and epithelial sloughing involved 12% of peripheral airways (11). Considering that those rabbits were ventilated with lower inflation flows than the present ones, and that higher inflation flows cause greater functional and morphological damage (12), the percentage of injured airways might have been even larger in the present open chest animals ventilated on ZEEP. Interestingly, in these animals the fall in NOe at $PEEP_2$ averaged 22% relative to $PEEP_1$. However, the satisfactory correspondence between percentage of injured airways and relative decrease of NOe may be fortuitous, also because other factors can reduce NO production and elimination from airway epithelium. In isolated, blood perfused rabbit lungs ventilated with a fixed pattern, decreasing Pa_{O_2} to 33 mmHg caused a nearly 40% decrease in end-expiratory NO concentration (4). Therefore, hypoxia could explain the reduced NO elimination on NEEP and ZEEP (Figs. 4 and 5), as well as the larger decrease of NOe in closed than open chest animals. Hypercapnia can also depress NO formation: in intact, anesthetized rabbits an increase of Pa_{CO_2} from 30 to 75 mmHg decreased NOe by ~30% within a few minutes (35). On the other hand, the effect of hypoxia and hypercapnia are rapidly reversible (4,35), while in the present animals NOe

remained well below control values long after return to PEEP and initial Pa_{O_2}, Pa_{CO_2}, and pHa values (Figs. 4 and 5). It should be noted, however, that in the present experiments hypoxia was maintained for hours instead of minutes as in the isolated rabbit lung preparation.

Changes in the volume or composition of the fluid lining the lower airways may also affect NO formation. Exhaled NO is markedly decreased in isolated pig lungs with developing interstitial edema (7) and with administration of nebulized aqueous solutions in healthy subjects (22), whereas an increased hydrogen ions concentration in the lining fluid leads to an increased NOe (20). In the present animals, the acid-base balance of this fluid was essentially unaffected, since the pH of the condensate was the same under all conditions (Table 5). On the other hand, in animals ventilated at low volume interstitial edema (Table 1) could have contributed to the fall of NOe, which might have been also due to the depressant action exerted on NO production by prostaglandins E_2 and F_{2a}, VIP, and free radicals (13,21,26). Indeed, damage and shedding of small airway epithelium, by exposing sensory nerve endings, fibroblasts, and collagen, can eventually cause release of tachykinins and vasoactive intestinal peptide (VIP), activation of bradykinin with release of prostaglandins E_2 and F_{2a}, and formation of free radicals (28,40), while alveolar epithelial cells, macrophages and polymorphonuclear leukocytes activated by mechanical insults could represent an additional source of VIP and prostaglandins E_2 and F_{2a} (13,23). Moreover, because bradykinin causes bronchoconstriction of mainly peripheral airways and systemic vasodilation (28), it could have contributed to the persistent increase of Rint after NEEP or ZEEP ventilation (Table 3), as well as to the significant fall of $\overline{Pa}$ on $NEEP_2$, $ZEEP_2$ and $PEEP_2$ (Figs. 4 and 5). The latter effect was not related to reduced heart performance, since additional experiments in open chest rabbits showed that whilst $\overline{Pa}$ decreased progressively during the 3-4 h period of ventilation on ZEEP, mean pulmonary artery pressure remained essentially unchanged.

Proinflammatory cytokines

Serum TNF-α levels were essentially undetectable before and after induction of anesthesia and paralysis, peaked immediately after surgery ($PEEP_1$), and were back to initial values after 3-4 h of ventilation both at normal or decreased lung volume (Fig.6), consistent with the known kinetics of this cytokine (43). The time course of serum TNF-α could suggest that surgery was involved in this response; but peak levels were similar in closed and open chest rabbits, in spite of more extensive surgery in the latter animals. In contrast with serum TNF-α levels, those in BAL fluid, though higher with $PEEP_2$, did not differ significantly between $PEEP_1$ and $PEEP_2$, nor at $PEEP_2$ between animals undergoing prolonged ventilation at low (*group Atest* and *Btest*) and physiologic end-expiratory volume (*group Acontrol* and *Bcontrol*), independent of closed or open chest (Table 6). This indicates that *a)* the greater decrease of NOe production in animals subjected to prolonged

ventilation at low volume (Figs. 4 and 5) cannot be related to lower TNF-α levels; *b)* the mechanical alterations caused by this type of ventilation (Tables 2-4), as well as histologic damage of peripheral airway (11,12), are largely independent of cytokine production; and *c)* relative to ventilation at physiologic end-expiratory lung volume, prolonged ventilation at low volume does not induce any significant change in TNF-α production. Indeed, in most of the present rabbits, both serum and BAL fluid TNF-α levels were too low to produce injury (37), in line with recent results showing that in excised mouse lungs a positive cytokine response is elicited only with NEEP of -15 cm H_2O (5). Nevertheless, the higher TNF-α levels in rabbits ventilated at low volume (Table 6), possibly coupled with a secondary release of IL-8 (32), may account for the greater recruitment of polymorphonuclear leukocytes in the alveolar walls observed in a previous study (12). On the other hand, it has been suggested that ventilation at low volume can increase cytokine production (6,38); but this seems to depend mainly on the V_T used. While in excised rat lungs TNF-α, IL-6, and macrophage levels were higher during ventilation with ZEEP and large V_T (15 $ml \cdot kg^{-1}$) than with PEEP of 3 cmH_2O and low V_T (7 $ml \cdot kg^{-1}$), they were, however, similar to corresponding values with PEEP and large V_T (38). Similarly, inflammatory cytokines were higher in BAL fluid from lungs ventilated with low V_T on ZEEP than atelectatic lungs, but similar to those in BAL fluid from lungs ventilated with low V_T and PEEP of 5 cmH_2O (6).

In conclusion, the present results show that mechanical ventilation at low-volume with physiologic tidal volumes causes an increase in airway resistance in both open and closed chest normal rabbits. In closed chest animals, this increase is more pronounced and associated with an increase of lung elastance, likely due to interstitial edema and surfactant depletion or inactivation. Ventilation at low volume decreases NOe without affecting pH of exhaled vapor condensate and concentration of the proinflammatory cytokine TNF-α in BAL fluid and serum. The decreased NOe should mainly reflect necrosis and sloughing of the epithelium of the respiratory and membranous bronchioles due to the abnormal shear stress related to their cyclic opening and closing. On the other hand, the low TNF-α levels suggest that damage of small airways with ventilation at low volume is due to direct mechanical injury rather than *biotrauma* elicited by increased release of proinflammatory cytokines.

ACKNOWLEDGMENTS

This research was supported in part by Ministero dell'Istruzione, dell'Università e della Ricerca Scientifica (MIUR) of Italy, Rome. We thank Dr. E. Scurati for performing albumin concentration measurements.

REFERENCES

1. Bachofen H, Hildebrandt J, and Bachofen M. Pressure-volume curves of air- and liquid-filled excised lungs - surface tension in situ. *J Appl Physiol* 29: 422-431, 1970.
2. Barnes PJ, and Belvisi MG. Nitric oxide and lung disease. *Thorax* 48: 1034-1043, 1993.
3. Bates JHT, Bacconier P, and Milic-Emili J. A theoretical analysis of interrupter technique for measuring respiratory mechanics. *J Appl Physiol* 64: 2204-2214, 1988.
4. Carlin RE, Ferrario L, Boyd JT, Camporesi EM, McGraw DJ, and Hakim TS. Determinants of nitric oxide in exhaled gas in the isolated rabbit lung. *Am J Respir Crit Care Med* 155: 922-927, 1997.
5. Cheng KC, Zeng H, Slutsky AS. Ventilation with negative airway pressure induces a cytokine response in isolated mouse lung. *Anesthesia and Analgesia* 94:1577-1582, 2002.
6. Chu EK, Whitehead T, and Slutsky AS. Effects of cyclic opening and closing at low- and high-volume ventilation on bronchoalveolar lavage cytokines. *Crit Care Med* 32: 168-174, 2004.
7. Cremona G, Higenbottam T, Takao M, Hall L, and Bower EA. Exhaled nitric oxide in isolated pig lungs. *J Appl Physiol* 78: 59-63, 1995.
8. D'Angelo E, Bonanni MV, Michelini S, and Agostoni E. Topography of the pleural surface pressure in rabbits and dogs. *Respir Physiol* 8: 204-229, 1970.
9. D'Angelo E. Stress-strain relationships during uniform and non-uniform expansion of isolated lungs. *Respir Physiol* 23: 87-107, 1975.
10. D'Angelo E, Calderini E, Torri G, Robatto FM, Bono D, and Milic-Emili J. Respiratory mechanics in anesthetized paralyzed humans: effects of flow, volume, and time. *J Appl Physiol* 67: 2556-2564, 1989.
11. D'Angelo E, Pecchiari M, Baraggia P, Saetta M, Balestro E, and Milic-Emili J. Low-volume ventilation causes peripheral airway injury and increased airway resistance in normal rabbits. *J Appl Physiol* 92: 949-956, 2002.
12. D'Angelo E, Pecchiari M, Saetta M, Balestro E, and Milic-Emili J. Dependence of lung imjury on inflation rate during low-volume ventilation in normal open-chest rabbits. *J Appl Physiol* 97: 260-268, 2004.
13. Delgado M, Munoz-Elias EJ, Gomariz RP, and Ganea D. Vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide prevent inducible nitric oxide synthase transcription in macrophages by inhibiting NF-kB and IFN regulatory factor 1 activation. *J Immunol* 162: 4685-4696, 1999.

14. Dos Santos CC, and Slutsky AS. Cellular responses to mechanical stress. Invited Review: Mechanisms of ventilator-induced lung injury: a perspective. *J Appl Physiol* 89: 1645-1655, 2000.
15. Forsberg S, Ludwigs U, and Hedenstierna G. Effects of ventilatory pattern on exhaled nitric oxide in mechanically ventilated rabbits. *Acta Anesthesiol Scand* 43: 464-469, 1999.
16. Glaister DH, Schroter RC, Sudlow MF, and Milic-Emili J. Transpulmonary pressure gradient and ventilation distribution in excised lungs. *Respir Physiol* 17: 365-385, 1973.
17. Gustafsson LE, Leone AM, Persson MG, Wiklund NP, and Moncada S. Endogenous nitric oxide is present in the exhaled air of rabbits, guinea pigs and humans. *Biochem Biophys Res Comm* 181: 852-857, 1991.
18. Haitsma JJ, Uhlig S, Göggel R, Verbrugge SJ, Lachmann U, and Lachmann B. Ventilator-induced lung injury leads to loss of alveolar and systemic compartimentalization of tumor necrosis factor-α. *Intensive Care Med* 26:1515-1522, 2000.
19. Hughes JMB, Rosenzweig DY, and Kivitz PB. Site of airway closure in excised dog lungs: histologic demonstration. *J Appl Physiol* 29: 340-344, 1970.
20. Hunt JF, Fang K, Malik R, Snyder A, Malhotra N, Platts-Mills TAE, and Gaston B. Endogenous airway acidification. *Am J Respir Crit Care Med* 161: 694-699, 2000.
21. Kharitonov SA, Sapienza MM, Chung KF, and Barnes PJ. Prostaglandins mediate bradykinin-induced reduction of exhaled nitric oxide in asthma. *Eur Respir J* 14: 1023-1027, 1999.
22. Maniscalco M, Vatrella A, Cremona G, Carratù L, and Sofia M. Exhaled nitric oxide after inhalation of isotonic and hypotonic solutions in healthy subjects. *Cli Sci* 101: 645-650, 2001.
23. Moncada S, and Vane JR. Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A_2 and prostacyclin. *Pharmacol Rev* 30: 293-331, 1979.
24. Munford RS, and Pugin J. Normal responses to injury prevent systemic inflammation and can be immunosuppressive. *Am J Respir Crit Care Med* 163: 316-321, 2001.
25. Muscedere JG, Mullen JBM, Gan K, Bryan AC, and Slutsky AS. Tidal ventilation at low airway pressure can augment lung injury. *Am J Respir Crit Care Med* 149: 1327-1334, 1994.
26. Nadel JA. Decreased neutral endopeptidases: possible role in inflammatory diseases of airways. *Lung* 168: 123-127, 1990.
27. Persson MG, Wiklund NP, and Gustafsson LE. Endogenous nitric oxide in single exhalations and the change during exercise. *Am J Respir Crit Care Med* 148: 1210-1214, 1993.
28. Proud D. Kinins as mediators of lung disease. In: The lung. eds. Crystal RG, West JB, Weibel ER, and Barnes PJ. Lippincott-Raven Philadelphia-New York, chap. 7, 1997, pp.89-101.

29. Pugin J., Dunn I, Jolliet P, Tassaux D, Magnetat JL, Nicod LP, and Chevrolet JC. Activation of human macrophages by mechanical ventilation in vitro. *Am J Physiol Lung Cell Mol Physiol* 275: 1040-1050, 1998.
30. Ricard JD, Dreyfuss D, and Saumon G. Production of inflammatory cytokines in ventilator-induced lung injury: a reappraisal. *Am J Respir Crit Care Med* 163: 1176-1180, 2001.
31. Robertson B. Lung surfactant. In: *Pulmonary surfactant*, edited by Robertson B, Van Goulde L, Batenburg J. Amsterdam: Elsevier, 1984, p. 132-147.
32. Rolfe MW, Kunkel SL, Standiford TJ, Chensue SW, Allen RM, Evanoff HL, Phan SH, Strieter RM. Pulmonary fibroblast expression of interleukin-8: a model for alveolar macrophage-derived cytokine networking. *Am J Respir Cell Mol Biol* 5: 493-501, 1991.
33. Said SI. Vasoactive intestinal peptide and nitric oxide: divergent roles in relation to tissue injury. *Ann NY Acad Sci* 805:379, 1996
34. Salazar E, and Knowles JH. An analysis of the pressure-volume characteristics of the lung. *J Appl Physiol* 19: 97-104, 1964.
35. Strömberg S, Lönnqvist PA, Persson MG, and Gustafsson LE. Lung distension and carbon dioxide affect pulmonary nitric oxide formation in the anaesthetized rabbit. *Acta Physiol Scand* 159: 59-67, 1997.
36. Taskar V, John J, Evander E, Robertson B, and Jonson B. Surfactant dysfunction makes lungs vulnerable to repetitive collapse and reexpansion. *Am J Respir Crit Care Med* 155: 313-320, 1997.
37. Tracey KJ, Vlassara H, Cerami A. Cachectin/tumor necrosis factor. *Lancet* 1(8647): 1122-1126, 1989.
38. Tremblay L, Valenza F, Ribeiro S, Li J, and Slutsky AS. Injurious ventilatory strategies increase cytokines and c-*fos* m-RNA expression in an isolated rat lung model. *J Clin Invest* 99: 944-952, 1997.
39. Tschumperlin DJ, Oswari J, and Marguleis AS. Deformation-induced injury of alveolar epithelial cells. Effect of frequency, duration, and amplitude. *Am J Respir Crit Care Med* 162: 357-362, 2000.
40. Uddman R, Sundler F, Cardell LO, and Luts A. Neuropeptides in the lung. In: The lung. eds. Crystal RG, West JB, Weibel ER, and Barnes PJ. Lippincott-Raven Philadelphia-New York, chap. 8, 1997, pp.103-115.
41. Verbrugge SJC, Uhlig S, Neggers SJCMM, Martin C, Held HD, Haitsma JJ, and Lachmann B. Different ventilation strategies affect lung function but do not increase tumor necrosis factor-α and prostacyclin production in lavaged rat lungs in vivo. *Anesthesiology* 91: 1834-1843, 1999.

42. Vlahakis NE, Schroder MA, Limper AH, and Hubmayr RD. Stretch induced cytokine release by alveolar epithelial cells in vitro. *Am J Physiol Lung Cell Mol Physiol* 277: L167-L173, 1999.
43. Waage A. Production and clearance of tumor necrosis factor in rats exposed to endotoxin and dexamethasone. *Clin Immunol Immunopathol* 45: 348-355, 1987.

LEGENDS

Fig.1. Average relationships between volume above resting lung volume (ΔV) and tracheal (Ptr) or esophageal pressure (Pes) under quasi-static conditions obtained in 8 rabbits (*Group Atest; upper panels*) during ventilation with PEEP of 1.2 cmH_2O before ($PEEP_1$) and after 3-4 h of ventilation on NEEP ($PEEP_2$), and during the initial ($NEEP_1$) and final period ($NEEP_2$) of ventilation on NEEP (see key to symbols) and in 8 rabbits (*Group Acontrol; lower panels*) during ventilation with PEEP before ($PEEP_1$) and after 3-4 h of ventilation on PEEP ($PEEP_2$). Bars: SE. Continuous lines were visually fitted through all data points obtained in a given condition.

Fig. 2. *Left panels:* average relationships between volume above resting lung volume (ΔV) and quasi-static transpulmonary pressure obtained in 8 closed chest rabbits (*Group Atest; upper panel*) during ventilation with PEEP of 1.2 cmH_2O before ($PEEP_1$) and after 3-4 h of ventilation on NEEP ($PEEP_2$), and during the initial ($NEEP_1$) and final period ($NEEP_2$) of ventilation on NEEP (see key to symbols) and in 8 closed chest rabbits (*Group Acontrol; lower panel*) during ventilation with PEEP before ($PEEP_1$) and after 3-4 h of ventilation on PEEP ($PEEP_2$). Bars: SE. Continuous lines are visual fit through all data points obtained in a given condition; dotted lines are monoexponential fit in the form $V_o\text{-}V_x \cdot e^{-K.Ptp}$, after omission of the lower three data points of each curve. *Right panels:* same relationship obtained in 8 open chest rabbits (*Group Btest; upper panel*) during ventilation with PEEP of 2.3 cmH_2O before ($PEEP_1$) and after 3-4 h of ventilation on ZEEP ($PEEP_2$), and during the initial ($ZEEP_1$) and final period ($ZEEP_2$) of ventilation on ZEEP (see key to symbols) and in 8 open chest rabbits (*Group Bcontrol; lower panel*) during ventilation with PEEP before ($PEEP_1$) and after 3-4 h of ventilation on PEEP ($PEEP_2$). Bars: SE. On PEEP, all data fit a unique monoexponential function in the form $V_o \cdot (1\text{-}e^{-K.Ptp})$.

Fig. 3. Relationships of additional lung resistance (ΔR) to duration of inflation obtained at an inflation volume of 10 $ml \cdot kg^{-1}$ obtained in 8 closed chest (*Group Atest; upper left panel*) and 8 open chest rabbits (*Group Btest; upper right panel*) during ventilation with PEEP before ($PEEP_1$) and after 3-4 h of ventilation on NEEP or ZEEP ($PEEP_2$), at the beginning ($NEEP_1$ and $ZEEP_1$) and end of the 3-4 h period ($NEEP_2$ and $ZEEP_2$) of ventilation on NEEP or ZEEP (see key to symbols) and in 8 closed chest (*Group Acontrol; lower left panel*) and 8 open chest rabbits (*Group Bcontrol; lower right panel*) during ventilation with PEEP before ($PEEP_1$) and after 3-4 h of ventilation on PEEP ($PEEP_2$). Bars: SE. Under all conditions, the data fit a monoexponential function in the form of Eq. 1.

Fig. 4. Average values of concentration of nitric oxide in expired air (NOe), mean arterial pressure ($\overline{Pa}$), arterial pH (pHa), partial pressure of carbon dioxide ($PaCO_2$) and oxygen (PaO_2) in 8 closed chest rabbits (*Group Atest; hatched columns*) during ventilation with PEEP of 1.2 cmH_2O before

($PEEP_1$) and after 3-4 h of ventilation on NEEP ($PEEP_2$), and during the initial ($NEEP_1$) and final period ($NEEP_2$) of ventilation on NEEP and in 8 closed chest rabbits (*Group A$_{control}$; white columns*) during ventilation with PEEP before ($PEEP_1$) and after 3-4 h of ventilation on PEEP ($PEEP_2$). During the final 3-3.5 h on NEEP, the rabbits were ventilated with 70-80% oxygen. Bars: SE. *Values significantly different ($P<0.05$) from those on $PEEP_1$. Number in parentheses indicate the average time (minutes) elapsed from the initial measurements on PEEP.

Fig. 5. Average values of concentration of nitric oxide in expired air (NOe), mean arterial pressure ($\overline{Pa}$), arterial pH (pHa), partial pressure of carbon dioxide (Pa_{CO_2}) and oxygen (Pa_{O_2}) obtained in 8 open chest rabbits (*Group B$_{test}$; hatched columns*) during ventilation with PEEP of 2.3 cmH_2O before ($PEEP_1$) and after 3-4 h of ventilation on ZEEP ($PEEP_2$), and during the initial ($ZEEP_1$) and final period ($ZEEP_2$) of ventilation on ZEEP and in 8 closed chest rabbits (*Group B$_{control}$; white columns*) during ventilation with PEEP before ($PEEP_1$) and after 3-4 h of ventilation on PEEP ($PEEP_2$). Bars: SE. *Values significantly different ($P<0.05$) from those on $PEEP_1$. Number in parentheses indicate the average time (minutes) elapsed from the initial measurements on PEEP.

Fig. 6. Time course of serum TNF-α concentration (median values) in 8 closed chest rabbits not subjected to prolonged mechanical ventilation (*Group C*), and in 8 closed (*Group A$_{test}$*) and 8 open chest rabbits (*Group B$_{test}$*) during ventilation with PEEP of 2.3 cmH_2O before ($PEEP_1$) and after 3-4 h of ventilation on NEEP or ZEEP ($PEEP_2$), and during the final period of low-volume ventilation ($NEEP_2$ or $ZEEP_2$).

Table 1. Arterial blood gases and pH during mechanics assessment, wet-to-dry ratio of lung and ratio of albumin bronchoalveolar lavage (BAL) fluid to serum (S) concentration in closed and open chest rabbits with or without prolonged low-volume ventilation under various conditions

		Pa_{O_2} mmHg	Pa_{CO_2} mmHg	pHa	wet/dry	BAL/S %
Group A (closed chest)						
control	$PEEP_1$	80±4	39.0±1.7	7.34±0.01		
	$PEEP_2$	82±7	40.0±2.3	7.33±0.01	4.86±0.06	1.3±0. 3
test	$PEEP_1$	78±4	35.3±1.9	7.38±0.02		
	$NEEP_1$	34±1*	44.3±3.1*	7.32±0.03*		
	$NEEP_2$	34±2*	53.7±5.9*	7.29±0.03*		
	$PEEP_2$	75±3	40.5±2.3	7.33±0.02	5.23±0.13#	2.4±1.5
Group B (open chest)						
control	$PEEP_1$	92±3§	37.6±2.1	7.38±0.02		
	$PEEP_2$	91±4§	39.7±3.5	7.33±0.02	4.82±0.06	1.0±0.5
test	$PEEP_1$	98±2§	35.3±1.8	7.39±0.01		
	$ZEEP_1$	46±5*‡	45.7±4.0*	7.30±0.02*		
	$ZEEP_2$	52±3*§	48.6±3.9*	7.17±0.04*†‡		
	$PEEP_2$	94±4§	37.2±2.2	7.35±0.03	4.98±0.09	2.1±1.2

Values are means±SE. Pa_{O_2}, Pa_{CO_2}, and pHa: arterial P_{O_2}, P_{CO_2}, and pH, respectively; $PEEP_1$, ventilation with positive end-expiratory pressure (PEEP) at the beginning of the experiment; $NEEP_1$ and $NEEP_2$ or $ZEEP_1$ and $ZEEP_2$, initial and final part of the 3-4 h period of ventilation on negative (NEEP) or zero end-expiratory pressure (ZEEP); $PEEP_2$, ventilation with PEEP after 3-4 h of ventilation either on NEEP, ZEEP or PEEP. Significantly different from corresponding values on $PEEP_1$, *P<0.001; significantly different from corresponding values on $ZEEP_1$, †P<0.001; significantly different from corresponding values of *group A* rabbits, ‡P<0.05, §P<0.001; significantly different from corresponding control values, # P=0.017.

Table 2. Values of constants in equations $V_o - V_x \cdot e^{-K \cdot Pst}$ and $V_o \cdot (1 - e^{-K \cdot Pst})$ used to fit the lung inflation volume-pressure curve, Ptp_{EE} and ΔEELV at the beginning and end of the experiment in closed and open chest rabbits with (*test*) or without prolonged low-volume ventilation (*control*)

		V_o	V_x	K	Ptp_{EE}	ΔEELV
		ml	ml	cm H_2O^{-1}	cm H_2O	ml
Group A (closed chest)						
control	$PEEP_1$	90.1±4.4	123.8±6.6	0.152±0.007	2.3±0.2	17.5±1.7
	$PEEP_2$	90.8±3.7	123.8±5.2	0.147±0.011	2.1±0.1	19.7±1.6
test	$PEEP_1$	87.9±3.1	114.8±6.6	0.139±0.010	2.1±0.1	18.4±1.1
	$PEEP_2$	76.3±3.5*	99.5±5.2*	0.131±0.009	2.2±0.1	14.1±1.6*
Group B (open chest)						
control	$PEEP_1$	78.4±2.6		0.186±0.009†	2.3±0.1	24.2±1.7†
	$PEEP_2$	77.1±3.0		0.185±0.011†	2.2±0.1	25.9±1.3†
test	$PEEP_1$	83.9±5.7		0.174±0.006†	2.3±0.1	25.6±1.7†
	$PEEP_2$	79.1±5.2		0.172±0.008†	2.3±0.1	24.4±1.3†

Values are means±SE. V_o, maximum volume above resting lung volume; V_x and K, volume and shape factors (see text); Ptp_{EE}, end-expiratory transpulmonary pressure; ΔEELV, difference between end-expiratory and resting volume; $PEEP_1$, ventilation with positive end-expiratory pressure (PEEP) at the beginning of the experiment; $PEEP_2$, ventilation with PEEP after 3-4 h of ventilation on either NEEP, ZEEP or PEEP. Significantly different from corresponding values on $PEEP_1$, *P<0.001; significantly different from corresponding values of *group A* rabbits, †P<0.01.

Table 3. Values of Est and Rint in closed and open chest rabbits with (*test*) or without prolonged low-volume ventilation (*control*) under various conditions

		Lung		Chest Wall	
		Est	Rint	Est	Rint
		$cmH_2O \cdot l^{-1}$	$cmH_2O \cdot s \cdot l^{-1}$	$cmH_2O \cdot l^{-1}$	$cmH_2O \cdot s \cdot l^{-1}$
Group A (closed chest)					
control	$PEEP_1$	177±5	9.7±1.1	44±4	8.4±0.6
	$PEEP_2$	163±9	8.9±1.3	47±6	8.6±0.5
test	$PEEP_1$	241±13	12.9±1.2	50±6	7.9±3.2
	$NEEP_1$	768±19†‡	49.3±3.2†‡	123±22†	8.4±0.8
	$NEEP_2$	869±23†‡	72.5±7.1†‡	114±15†	8.4±0.8
	$PEEP_2$	294±20*	27.5±3.6†	58±5	7.3±0.8
Group B (open chest)					
control	$PEEP_1$	148±9§	11.2±0.9		
	$PEEP_2$	157±11	10.7±0.9		
test	$PEEP_1$	140±10§	9.7±0.6		
	$ZEEP_1$	422±33†‡	29.6±2.8†‡		
	$ZEEP_2$	529±34†‡	45.9±3.5†‡		
	$PEEP_2$	160±11	12.6±0.9†		

Values are means±SE. Est, quasi-static elastance; Rint, interrupter resistance; $PEEP_1$, ventilation with positive end-expiratory pressure (PEEP) at the beginning of the experiment; $NEEP_2$ and $ZEEP_2$, final part of the 3-4 h period of ventilation on negative (NEEP) or zero end-expiratory pressure (ZEEP); $PEEP_2$, ventilation with PEEP after 3-4 h of ventilation on either NEEP, ZEEP or PEEP. Significantly different from values on $PEEP_1$,* $P<0.05$, † $P<0.01$; significantly different from corresponding values on PEEP, ‡ $P<0.01$; significantly different from corresponding values of *group A* rabbits on $PEEP_1$, § $P<0.01$.

Table 4. Values of Rvisc and τvisc computed according to Eq.1 in closed and open chest rabbits with (*test*) or without prolonged low-volume ventilation (*control*) under various conditions

		Lung		Chest Wall	
		Rvisc $cmH_2O \cdot l^{-1}$	τvisc s	Rvisc $cmH_2O \cdot l^{-1}$	τvisc s
Group A (closed chest)					
control	$PEEP_1$	74±5	1.29±0.14	29±8	1.31±0.08
	$PEEP_2$	75±4	1.24±0.12	29±8	1.31±0.11
test	$PEEP_1$	70±8	1.31±0.15	27±4	1.25±0.20
	$NEEP_1$	328±35*	1.79±0.18*	24±4	1.03±0.16
	$NEEP_2$	401±57*	1.61±0.15*	23±4	1.01±0.17
	$PEEP_2$	91±15	1.24±0.18	26±4	1.20±019
Group B (open chest)					
control	$PEEP_1$	62±19	1.33±0.16		
	$PEEP_2$	57±15	1.28±0.15		
test	$PEEP_1$	66±5	1.34±0.17		
	$ZEEP_1$	200±25*‡	1.25±0.15†		
	$ZEEP_2$	257±19*†	1.31±0.14†		
	$PEEP_2$	69±8	1.20±0.13		

Values are means±SE. Rvisc, viscoelastic resistance; τvisc, viscoelastic time constant; $PEEP_1$, ventilation with positive end-expiratory pressure (PEEP) at the beginning of the experiment; $NEEP_2$ and $ZEEP_2$, final part of the 3-4 h period of ventilation on negative (NEEP) or zero end-expiratory pressure (ZEEP); $PEEP_2$, ventilation with PEEP after 3-4 h of ventilation on either NEEP, ZEEP or PEEP. Significantly different from values on PEEP, *P<0.001; significantly different from corresponding values of *group A* rabbits, †P<0.05, ‡P<0.01.

Table 5. pH of exhaled airway vapour condensate in closed and open chest rabbits under various conditions

	$PEEP_1$	$NEEP_2$	$ZEEP_2$	$PEEP_2$
Group A (closed chest)	6.97±0.08	6.86±0.15		6.82±0.14
Group B (open chest)	6.96±0.08		6.87±0.11	6.86±0.11

Values are means±SE. $PEEP_1$, ventilation with positive end-expiratory pressure (PEEP) at the beginning of the experiment; $NEEP_2$ and $ZEEP_2$, final part of the 3-4 h period of ventilation on negative (NEEP) or zero end-expiratory pressure (ZEEP); $PEEP_2$, ventilation with PEEP after 3-4 h of ventilation on either NEEP, ZEEP or PEEP.

Table 6. Tumor necrosis factor-α concentration (pg/ml) of bronchoalveolar lavage (BAL) fluid and serum under various conditions in closed and open chest rabbits

	Closed chest rabbits			Open chest rabbits	
	Group A		Group C	Group B	
	control	test		control	test
BAL fluid					
$PEEP_1$			18 (0-234)		
$PEEP_2$	55 (0-234)	80 (0-2200)		64 (0-191)	36 (0-205)
Serum					
awake			0		
$PEEP_1$	2345 (0-20000)	1194 (53-20000)	2251 (150-20000)	1183 (0-7299)	1106 (0-20000)
$NEEP_2$-$ZEEP_2$		0 (0-142)			8 (0-433)
$PEEP_2$	0 (0-62)	0 (0-456)		0 (0-51)	30 (0-290)

Values are median with range in parentheses. $PEEP_1$, ventilation with positive end-expiratory pressure (PEEP) at the beginning of the experiment; $NEEP_2$ and $ZEEP_2$, final part of the 3-4 h period of ventilation on negative (NEEP) or zero end-expiratory pressure (ZEEP); $PEEP_2$, ventilation with PEEP after 3-4 h of ventilation on either NEEP, ZEEP or PEEP.

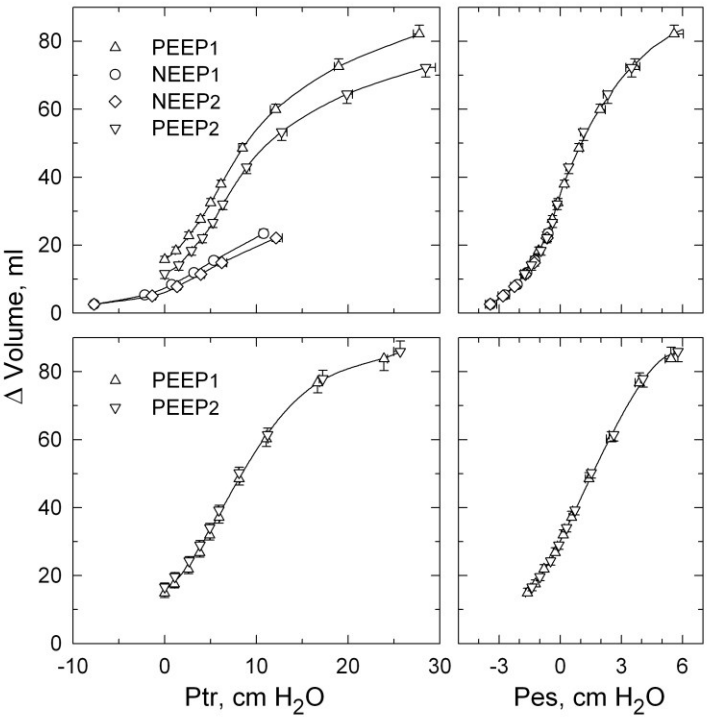
PEEP1
NEEP1
NEEP2
PEEP2
PEEP1
PEEP2
Δ Volume, ml
Ptr, cm H2O
Pes, cm H2O

Figure 1

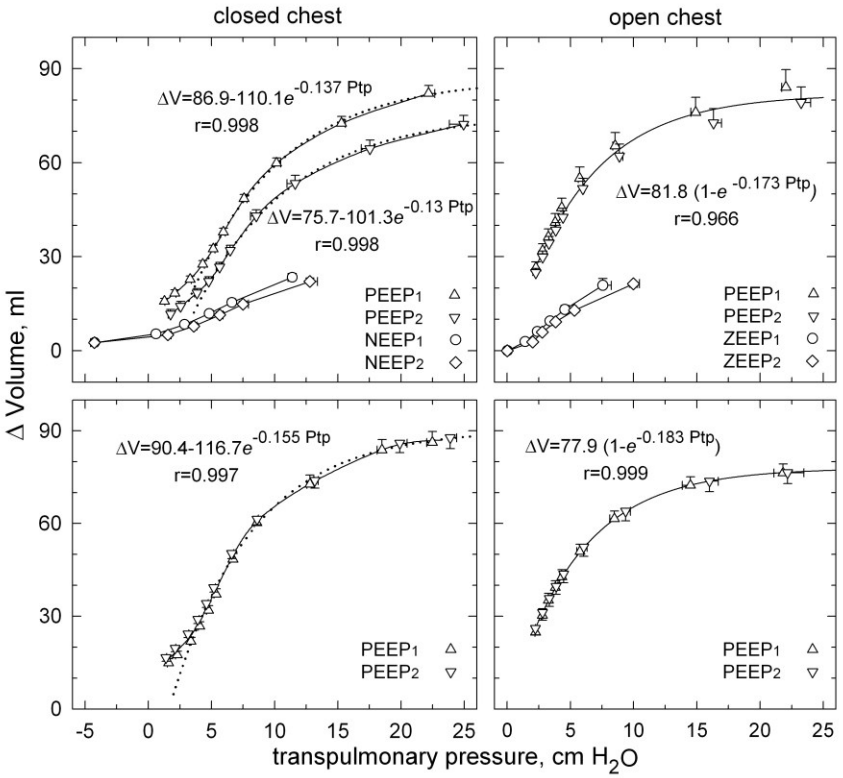
closed chest
open chest
ΔV=86.9-110.1e^-0.137 Ptp
r=0.998
ΔV=75.7-101.3e^-0.13 Ptp
r=0.998
ΔV=81.8 (1-e^-0.173 Ptp)
r=0.966
ΔV=90.4-116.7e^-0.155 Ptp
r=0.997
ΔV=77.9 (1-e^-0.183 Ptp)
r=0.999
PEEP1
PEEP2
NEEP1
NEEP2
ZEEP1
ZEEP2
Δ Volume, ml
transpulmonary pressure, cm H2O

Figure 2

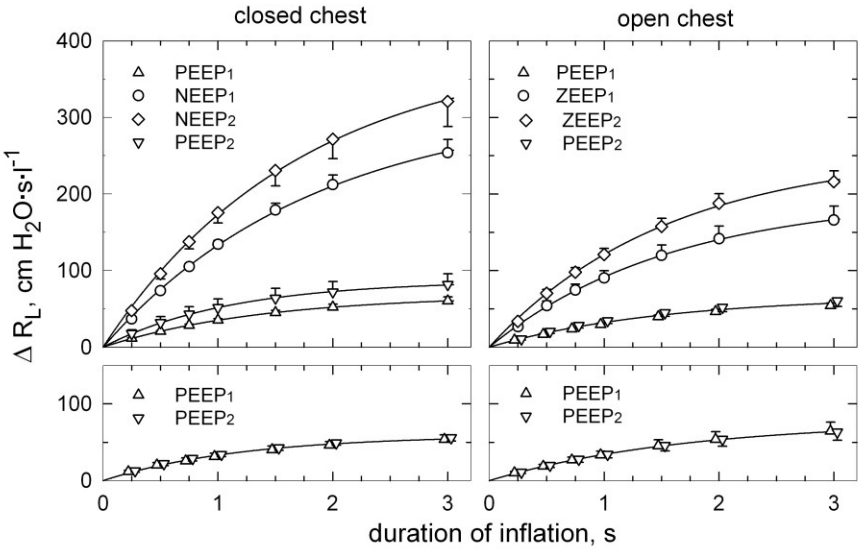
closed chest
open chest
PEEP1
NEEP1
NEEP2
PEEP2
ZEEP1
ZEEP2
Δ RL, cm H2O·s·l-1
duration of inflation, s
0
100
200
300
400
1
2
3

Figure 3

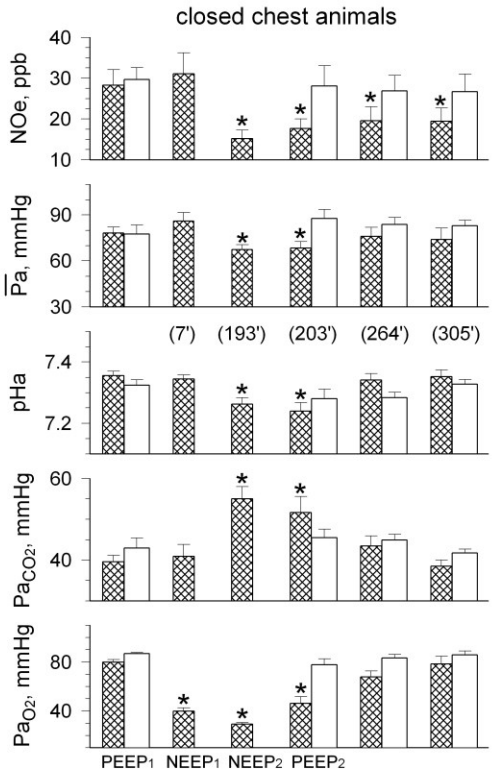
closed chest animals
NOe, ppb
40
30
20
10
$\overline{Pa}$, mmHg
90
60
30
(7')
(193')
(203')
(264')
(305')
pHa
7.4
7.2
Pa_{CO_2}, mmHg
60
40
Pa_{O_2}, mmHg
80
40
PEEP1
NEEP1
NEEP2
PEEP2

Figure 4

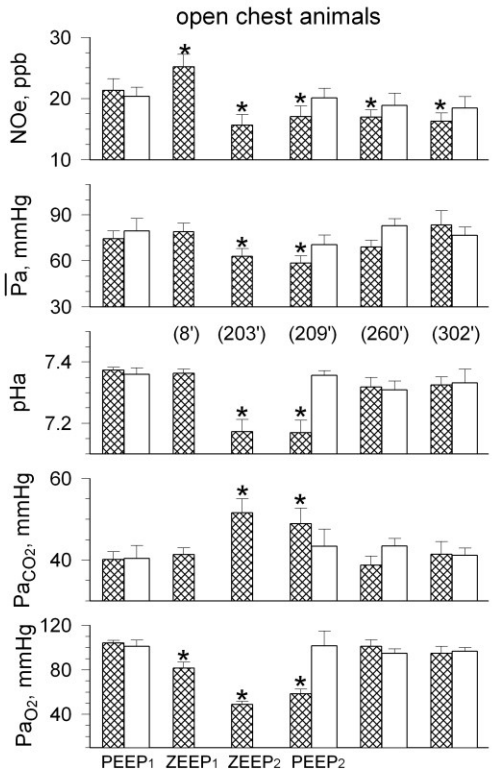
open chest animals
NOe, ppb
30
20
10
$\overline{Pa}$, mmHg
90
60
30
(8')
(203')
(209')
(260')
(302')
pHa
7.4
7.2
Pa_{CO_2}, mmHg
60
40
Pa_{O_2}, mmHg
120
80
40
PEEP1
ZEEP1
ZEEP2
PEEP2

Figure 5

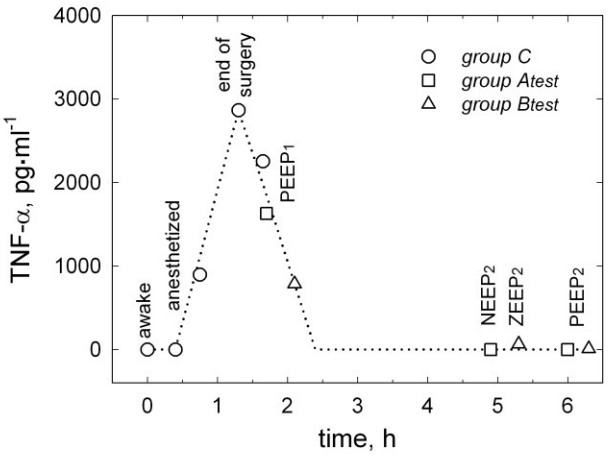
4000
3000
2000
1000
0
TNF-α, pg·ml-1
0
1
2
3
4
5
6
time, h
awake
anesthetized
end of surgery
PEEP1
NEEP2
ZEEP2
PEEP2
group C
group Atest
group Btest

Figure 6