

Vivien S. Piccin
Christiane Calciolari
Kelly Yoshizaki
Susimeire Gomes
Cláudia Albertini-Yagi
Marisa Dolhnikoff
Mariângela Macchione
Elia G. Caldini
Paulo H. N. Saldiva
Elnara M. Negri

Effects of different mechanical ventilation strategies on the mucociliary system

Received: 19 March 2010
Accepted: 9 September 2010
Published online: 28 October 2010
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This article is discussed in the editorial available at: doi:10.1007/s00134-010-2058-3.

Electronic supplementary material

The online version of this article (doi:10.1007/s00134-010-2056-5) contains supplementary material, which is available to authorized users.

V. S. Piccin (✉) · C. Calciolari ·
K. Yoshizaki · C. Albertini-Yagi ·
M. Dolhnikoff · M. Macchione ·
E. G. Caldini · P. H. N. Saldiva ·
E. M. Negri
Department of Pathology,
University of São Paulo School
of Medicine, São Paulo, Brazil
e-mail: vspiccin@uol.com.br

S. Gomes
Respiratory Intensive Care Unit,
University of São Paulo School
of Medicine, São Paulo, Brazil

Abstract Objective: To evaluate the effects of different mechanical ventilation (MV) strategies on the mucociliary system. **Design and setting:** Experimental study. **Subjects:** Twenty-seven male New Zealand rabbits. **Interventions:** After anesthesia, animals were tracheotomized and ventilated with standard ventilation [tidal volume (Vt) 8 ml/kg, positive end expiratory pressure (PEEP) 5 cmH₂O, flow 3 L/min, FiO₂ 0.4] for 30 min. Next, animals were randomized into three groups and ventilated for 3 h with low volume (LV): Vt 8 ml/kg, PEEP 5 cmH₂O, flow 3 L/min (*n* = 6); high volume (HV): Vt 16 ml/kg, PEEP 5 cmH₂O, flow 5 L/min (*n* = 7); or high pressure (HP): Ppeak 30 cmH₂O, PEEP 12 cmH₂O (*n* = 8). Six animals (controls) were ventilated for 10 min with standard ventilation. Vital signals, blood lactate, and respiratory system mechanics were verified. Tracheal tissue was collected before and after MV. **Measurements:** Lung and tracheal tissue sections were stained to analyze inflammation and mucosubstances by the point-counting method. Electron microscopy verified tracheal cell ultrastructure. In situ tracheal ciliary beating frequency

(CBF), determined using a video-scopic technique, and tracheal mucociliary transport (TMCT), assessed by stereoscopic microscope, were evaluated before and after MV. **Results:** Respiratory compliance decreased in the HP group. The HV and HP groups showed higher lactate levels after MV. Macroscopy showed areas of atelectasis and congestion on HV and HP lungs. Lung inflammatory infiltrate increased in all ventilated groups. Compared to the control, ventilated animals also showed a reduction of total and acid mucus on tracheal epithelium. Under electron microscopy, injury was observed in the ciliated cells of the HP group. CBF decreased significantly after MV only in the HP group. TMCT did not change significantly in the ventilated groups. **Conclusions:** Different MV strategies induce not only distal lung alterations but also morphological and physiological tracheal alterations leading to mucociliary system dysfunction.

Keywords Mucociliary clearance · Mucus · Cilia · Mechanical ventilation · Ventilator-induced lung injury

Introduction

Notwithstanding the life-saving potential of mechanical ventilation (MV) assistance, this procedure has some drawbacks and complications including the occurrence of ventilator-induced lung injury [1]. The mechanisms of injury include a cellular response to mechanical forces generated by MV (overextending or stretching of the lung parenchyma), polymorphonuclear cell recruitment and activation, and cellular apoptosis and necrosis events [2, 3]. Furthermore, upon occurrence of lung injury, the proteinaceous edema and inflammatory debris present in the airspace are subject to the pressure differences. These forces cycle tidally in both directions, increasing their potential to transport inflammatory products from one region of the lung to the other. Other factors, such as gravity, airway geometry, fluid rheological properties and quantities, as well as regional lung mechanics (local airway resistance and compliance) interact to influence the net movement of secretions [4].

Because the mucociliary system represents an important part of the respiratory defenses [5], the potential influence of MV on the tracheal epithelia and mucociliary system deserves close consideration. The mucociliary functions depend on the integration of ciliated epithelium, periciliary fluid, and mucus [6] and may be disturbed by several factors, such as increased mucus production, abnormal mucus rheology, abnormal ciliary activity, or loss of ciliated cells [7, 8]. Dynamic forces generated by MV could also affect the mucosal epithelium and respiratory secretions. However, while there are a considerable number of studies that have investigated the influence of different patterns of MV on pulmonary parenchyma [1, 9, 10], the influence of MV on mucociliary function has received little attention. Impaired mucociliary clearance [11] and mucus concentration [12] seem to be important factors that can influence the formation of bacterial colonies, which represent a potential threat for patients undergoing MV. We have hypothesized that MV exposure may cause not only distal lung alterations but also has an impact on the mucociliary system.

Materials and methods

This study was approved by the Ethics Committee of the University of São Paulo School of Medicine (FMUSP) and was performed according to the standards of care and use of laboratory animals.

Experimental animals and ventilation protocol

Twenty-seven male New Zealand rabbits (Central Biotherium of the FMUSP) of similar weight (3.3 ± 0.4 kg

bodyweight) were anesthetized by intramuscular administration of acepromazine (0.1 mg/kg) and ketamine chlorhydrate (2.5 mg/kg). The animals were then restrained in the supine position and intravenous and intra-arterial lines were installed at the ear vein and artery, respectively. A continuous infusion of midazolam (0.1 mg/kg), ketamine chlorhydrate ($1.8 \text{ mg kg}^{-1} \text{ h}^{-1}$), acepromazine ($0.1 \text{ mg kg}^{-1} \text{ h}^{-1}$) and pancuronium bromide ($0.2 \text{ mg kg}^{-1} \text{ h}^{-1}$) was delivered using the Infusomat[®] compact infusion pump (Laboratorios B. Braun, Brazil). The rabbits underwent a tracheotomy and were connected to a mechanical Newport e500 Wave ventilator (Newport Medical Instruments, USA) with an air humidifying system at 33°C [13, 14]. Animals were also connected to a noninvasive cardiac output NICO2TM Dixtal monitor system (Dixtal Biomedica, Brazil). The Capnostat[®] mainstream CO₂ Sensor (Novamatrix Medical Systems, USA) was connected between the tracheal and ventilation tubes. An 8F Foley catheter was passed through the rabbit's urethra to collect urine during the procedure.

The animals were randomized into either the control group ($n = 6$) or one of three ventilated groups according to the ventilation strategy: low volume (LV, $n = 6$), high volume (HV, $n = 7$), or high pressure (HP, $n = 8$). The LV group was used to study what happens to the mucociliary system in a conventional mechanical ventilation setting. The HV group was conceived to address the impact of cyclic stretch on the mucociliary apparatus. The HP group was adjusted to cause a continuous strain in lung tissue by maintaining a high mean airway pressure throughout the respiratory cycle. All animals were initially subjected to MV using a tidal volume (V_t) of 8 ml/kg of body weight, a flow of 3 L/min, a positive end expiratory pressure (PEEP) of 5 cmH₂O, a respiratory rate of 30/min, and FiO₂ sufficient to maintain SaO₂ over 90% (minimum 0.4 FiO₂) for 30 min (stabilization period), except the control group which only underwent this MV protocol for 10 min. After the stabilization period, the LV group was maintained with the same initial ventilatory parameters for an additional 3 h. The HV animals were maintained for 3 h with a V_t of 16 ml/kg of body weight, while the HP group was maintained for an additional 3 h with a PEEP of 12 cmH₂O at peak inspiratory pressures of 30 cmH₂O (in order to maintain the same V_t used for the LV group). At the end of protocol, animals were exsanguinated through the aorta. Afterwards, for all groups including the control, lung inflation was maintained by providing 20 cmH₂O of continuous positive airway pressure. The chest wall was opened, and the lungs and heart were removed en bloc. The right lung was clamped and immersed in a fixative solution for histological procedures.

Physiological measurements

After the MV stabilization period and at the end of the protocol, baseline arterial blood gases, lung mechanics,

and vital signs were recorded. Blood samples were immediately analyzed using the ABL 800 Flex blood gas analyzer (Radiometer, Denmark). Lung mechanics were recorded using Labview[®]. Vital signs were visualized on a DX 2020 portal monitor (Dixtal Biomedica).

Tracheal tissue collection

In ventilated groups, a small anterior wall portion of the proximal trachea (3 × 10 mm) was excised prior to the insertion of a tracheotomy tube. At the end of the protocol, a small portion of the trachea (3 × 10 mm) near the carina region was excised in these groups. Both procedures were performed to expose the epithelia for immediate measurements of ciliary beating frequency and tracheal mucociliary transport and were executed in a manner that avoided areas of tube contact.

Light microscopy

After 24 h of fixation, the tissue samples of the trachea and the right lung were collected as described and embedded in paraffin, cut into 5- μ m-thick sections, and mounted on glass slides. Lung sections were stained with hematoxylin and eosin (H&E) and examined microscopically for evidence of inflammatory infiltration by the point-counting method (400× magnification), which is expressed as the number of polymorphonuclear cells per square micrometer of tissue. Tracheal sections were also stained with H&E and a combination of periodic acid Schiff's and Alcian blue stains (PAS/AB) at a pH of 2.5 [15] to quantify mucosubstances. Using a microscope equipped with a video camera and an image analysis system, we digitized the microscopic images (400× magnification) into a high-resolution video of a known area. The areas of neutral and acid mucus contained within the tracheal epithelium were determined by point-counting [16] and converted to number/ μ m². Histological analysis was performed in a blinded fashion with respect to the groups and the ventilation protocol to which the animals were assigned.

Transmission electron microscopy

In all groups, specimens obtained from the tracheal epithelium (near the carina region) at the end of the protocol were fixed in 2% glutaraldehyde dissolved in 0.15 M phosphate buffer, pH 7.2, for 1 h, followed by post-fixation in 1% osmium tetroxide dissolved in 0.9% sodium chloride for 1 h. The fixed material was stained en bloc in 0.5% aqueous uranyl acetate overnight. The specimens were then dehydrated in a graded acetone

series and embedded in Araldite resin. Ultrathin sections (70 nm) were cut with the diamond knife of an LKB ultramicrotome, placed on 200-mesh copper grids, and double-stained with uranyl acetate and lead citrate. The grids were examined and photomicrographed under a Jeol 1010 transmission electron microscope operating at 80 kV.

Mucociliary function

In situ tracheal ciliary beating frequency (CBF)

The measurements were done immediately after the tissue excision, at room temperature (20–25°C). A stream of nebulized saline solution was continuously placed over the tissue to provide humidification [17, 18]. Focusing on a group of cilia using a light microscope (10× objective, 10× eyepiece) connected to a video camera (KODO, Model K-512EX, CCD Iris), the images captured by the microscope were sent to a monitor (Sony Trinitron). A stroboscopic light (Machine Vision Strobe, Model 5000, USA), which emits 0–30 Hz flashes, was placed in front of the tracheal ciliary epithelium. The incident light illuminating the ciliary epithelium was reflected from the grouped cilia and from the thin layer of mucus covering the cilia. Applying the stroboscopic light at the maximum frequency and reducing it continuously by using the computer, it was possible to manually determine the frequency of ciliary activity when the ciliary frequency was the same as the flash frequency and the observer could not distinguish the ciliary beat [18–22]. Values are reported in Hz.

In situ tracheal mucociliary transport (TMCT)

Following the excision procedure, the surface of the trachea (membranous fraction, collected as described before) was placed under a stereoscopic microscope (0.8×) equipped with a reticulated eyepiece (10×). Experiments were carried out at room temperature (20–25°C) in an acrylic chamber with 100% humidity (maintained by an ultrasonic nebulization of normal saline solution). The TMCT was monitored by direct observation of the movement of charcoal particles placed on the tracheal surface [18, 23, 24]. Values are reported in millimeters travelled per minute.

Statistical analysis

Results were generated using SPSS 15.0 for Windows[®] (SPSS, Chicago, IL, USA). All data were analyzed using the Kolmogorov-Smirnov normality test. The one-way

analysis of variance was performed with the Tukey post-hoc test for comparisons of physiological measurements, lung inflammatory infiltration, and for the comparison of fluid balance and respiratory mechanics data. Variables are expressed as the mean and standard deviation. Epithelial mucus-containing cells counts were tested using the Kruskal-Wallis and the Mann-Whitney test. To study differences between ventilated groups with respect to CBF and TMCT, the Kruskal-Wallis test and Wilcoxon's test were performed. Values are expressed as medians and quartiles. Statistical significance was set at $p < 0.05$.

Results

The physiological measurements, fluid balance, and ventilatory parameters collected throughout the study are shown in Tables 1 and 2.

After opening the chest wall, the lungs of the control and the LV groups appeared completely normal. HV lungs showed spotty areas of hemorrhage and localized areas of atelectasis, and lungs treated with high pressure ventilation appeared cherry red at necropsy with large areas of hemorrhagic consolidation. The number of polymorphonuclear cells per square micrometer on the lung parenchyma was significantly higher ($p < 0.001$) in the LV, HV, and HP groups compared to the control group ($1.61 \times 10^{-4} \pm$

0.36×10^{-4} , $1.36 \times 10^{-4} \pm 0.50 \times 10^{-4}$, $1.28 \times 10^{-4} \pm 0.35 \times 10^{-4}$, and $0.65 \times 10^{-4} \pm 0.14 \times 10^{-4}$, respectively).

In all ventilated groups the total and acid respiratory mucus on the rabbits' tracheas decreased significantly after MV, as shown in Fig. 1.

We observed no alterations of the cilia structure on the tracheal ciliary cells of all groups, as observed by electron microscopy. The descriptive electron microscopy analysis suggested a decrease in microvilli and also mitochondria on ciliary cells in the HP group. In this group there was no visual definition of the junctional constituents. In the HV and HP groups, there was an increased number of vesicles inside ciliary cells and also increased cytoplasm vacuum areas (Fig. 2c, d).

There was a significant decrease in the CBF in the HP group ($p = 0.05$) when comparing the tracheal tissue collected before the start of the protocol and after MV exposure (Fig. 3). The CBF in the LV, HV, and HP groups, before the protocol and after MV exposure, were 12.82 (11.23–13.69) to 14.92 (11.36–16.02) Hz, 12.34 (12.04–12.98) to 13.51 (11.90–14.81) Hz, and 13.51 (11.62–14.49) to 11.69 (10.12–14.18) Hz ($p = 0.047$), respectively.

There was no statistically significant difference in TMCT in the LV, HV, and HP groups before the protocol and after MV exposure: 0.35 (0.25–0.42) to 0.48 (0.38–0.53) mm/min, 0.34 (0.28–0.35) to 0.50 (0.29–0.54) mm/min, and 0.44 (0.36–0.52) to 0.83 (0.48–0.95) mm/min, respectively.

Table 1 Descriptive values of physiological measurements and fluid balance of all groups

Variables	Control	LV	HV	HP	<i>p</i>
Weight (g)	2,765 ± 161	3,373 ± 197	3,368 ± 146	3,426 ± 354	ns
ABP _{mean} (mmHg)					
Initial	62.0 ± 5.1	71.5 ± 21.5	64.6 ± 11.1	60.6 ± 11.2	ns
Final		54.0 ± 26.4	59.4 ± 9.1	47.9 ± 8.0	ns
Heart rate (bpm)					
Initial	232.7 ± 14.2	242.3 ± 7.8	234.9 ± 21.1	231.5 ± 18.9	ns
Final		258.3 ± 37.1*	198.7 ± 38.8*	219.6 ± 29.4	0.019*
Diuresis (ml)		133.3 ± 87.6	250.0 ± 141.4	196.4 ± 315.3	ns
Fluids (ml)		302.0 ± 26.8	421.7 ± 134.5	506.7 ± 229.4	ns
pH					
Initial	7.42 ± 0.07	7.41 ± 0.11	7.46 ± 0.14	7.43 ± 0.13	ns
Final		7.37 ± 0.10	7.43 ± 0.13	7.30 ± 0.07	ns
Lac (mmol/L)					
Initial	5.0 ± 0.76	2.4 ± 1.31	3.7 ± 1.25	3.0 ± 1.51	ns
Final		4.7 ± 2.00*	8.4 ± 3.06*	6.5 ± 2.19	0.04*
Bic (mmol/L)					
Initial	20.7 ± 2.45	19.6 ± 3.71	21.3 ± 3.11	20.8 ± 3.36	ns
Final		15.6 ± 3.24	11.0 ± 2.68	14.5 ± 4.76	ns

LV Low volume group, HV high volume group, HP high pressure group, Lac lactate, Bic bicarbonate

Initial values were verified at the end of the stabilization period and final values at the end of the protocol. Values are expressed as mean ± standard deviation. In the LV, HV, and HP groups, the ABP_{mean}, heart rate, lactate, and bicarbonate data were collected

immediately after the MV stabilization period and after an additional 3 h of MV exposure. In the control group, these data were collected before sacrifice. In all groups, weight was measured before the animal's manipulation, and diuresis and fluids data were collected before exsanguination

* Statistical significance between specified groups

Table 2 Ventilatory parameters

Variables	LV	HV	HP	<i>p</i>
FiO ₂ (%)				
Initial	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.1	ns
Final	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.1	ns
SaO ₂ (%)				
Initial	97.7 ± 3.0	98.4 ± 1.6	97.4 ± 1.9	ns
Final	97.8 ± 1.0	97.0 ± 2.3	96.6 ± 6.5	ns
EtCO ₂ (mmHg)				
Initial	26.0 ± 4.1	26.8 ± 2.9	27.6 ± 9.8	ns
Final	24.0 ± 5.5	14.5 ± 3.1	27.6 ± 16.2	ns
PaCO ₂ (mmHg)				
Initial	31.5 ± 8.41	31.0 ± 11.28	33.3 ± 13.04	ns
Final	28.6 ± 12.35*	16.3 ± 2.51*/**	29.9 ± 9.27**	0.054*/0.026**
PaO ₂ (mmHg)				
Initial	191.8 ± 18.54	168.6 ± 43.15	163.8 ± 48.88	ns
Final	160.8 ± 49.54	162.2 ± 46.23	155.7 ± 52.85	ns
Vt (ml)				
Initial	28.2 ± 5	35.1 ± 6	30.2 ± 7	ns
Final	27.8 ± 5	54.8 ± 8	27.7 ± 6	0.000†
Ppeak (cmH ₂ O)				
Initial	16.3 ± 2	19.1 ± 1	16.4 ± 2	ns
Final	17.4 ± 1	27 ± 3	30 ± 1	0.000†
Pmean (cmH ₂ O)				
Initial	8.6 ± 0.6	9.7 ± 2	8.8 ± 1	ns
Final	8.7 ± 0.5	11.6 ± 1	19.6 ± 3	0.000†
Pplat (cmH ₂ O)				
Initial	16.1 ± 2	18.5 ± 1	16.1 ± 2	ns
Final	16.9 ± 1	26.3 ± 3	29.4 ± 1	0.000†
Pflow (L/min)				
Initial	2.7 ± 0.6	3.4 ± 0.4	2.7 ± 0.3	ns
Final	2.6 ± 0.4	4.8 ± 0.8	8.6 ± 1	0.000†
PEEP (cmH ₂ O)				
Initial	5.3 ± 0.05	5.4 ± 0.1	5.3 ± 0.04	ns
Final	5.3 ± 0.03	5.3 ± 0.06	11.9 ± 2.66	0.000†
R _{ti} (cmH ₂ O L ⁻¹ s ⁻¹)				
Initial	45.2 ± 9.4	45.2 ± 7.1	52.8 ± 15.6	ns
Final	59.8 ± 12.9	52.4 ± 7.8	64.5 ± 25.4	ns
C _{dyn} (ml/cmH ₂ O)				
Initial	2.9 ± 0.3	3 ± 0.6	3.3 ± 0.9	ns
Final	2.9 ± 0.6*	3.1 ± 0.6**	1.6 ± 0.3*/**	0.001*/0.000**

LV Low volume group, HV high volume group, HP high pressure group

Initial values were verified at the end of the stabilization period and final values at the end of the protocol. Values are expressed as mean ± standard deviation. The data were collected immediately

before the end of the MV stabilization period and after an additional 3 h of MV exposure

* Statistical significances between specified groups and † among groups

Discussion

Our study conveys several new findings regarding tracheal mucociliary epithelium morphology and physiology in different strategies of MV. We demonstrated morphological and functional alterations in the tracheal epithelium after MV exposure. Light microscopy demonstrated reduced total mucus and acid mucus on the tracheal epithelium in all ventilated groups. Additionally, morphological alterations in the tracheal epithelium were verified by electron microscopy, revealing signals of injury on ciliated cells in the HV and HP groups. Finally, we observed a CBF reduction in the HP group, indicating impairment of

mucociliary clearance under the high pressure MV protocol. These findings will be explored below.

Tracheal mucociliary epithelial alterations

The LV, HV, and HP groups showed a significant decrease in total mucus and acid mucus in the trachea in relation to the control group. This result is indirect evidence that MV stimulated acid mucus extrusion. It is known that the epithelium expels mucus in specific situations [25, 26]. This mechanism, called exocytosis, occurs primarily in response

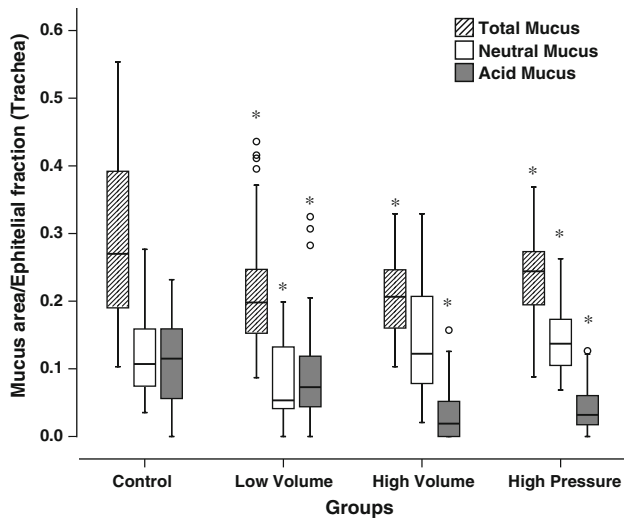


Fig. 1 Relationship between mucus area and tracheal epithelium fraction (tissue sample collected at end of protocol). * $p < 0.05$ compared with the control group. Values are presented as medians and quartiles

to an extracellular stimulus [27] and is probably a protective response to eliminate an offending agent (in our study, probably the agent was the forces generated by the MV). Some studies have also highlighted the role of oxidative stress in the lung epithelium upon exposure to mechanical forces generated by MV [28–30]. It seems reasonable to suppose that the ventilation is the stimulus for the production of reactive oxygen species, triggering an extrusion response from the tracheal epithelium. The decreased quantity of mucus could also be a consequence of goblet cell damage due to MV exposure [31, 32].

The sample analysis of the electron microscopy performed in this work demonstrated cellular damage in the HV and HP groups, but not in the control and LV groups. Although all ventilated animals maintained a good oxygenation relationship ($\text{FiO}_2/\text{PaO}_2$), the higher lactate levels observed in the HV and HP groups indicate partially reduced activated oxygen, which is an important mediator of cell death. Free radicals can cause lipid peroxidation and other deleterious effects on the cell structure. The early onset of cell death is characterized by cellular swelling, during which cells lose their microvilli and intercellular connections (as we observed in the HP group). To some extent these disturbances are reversible, but when ischemia persists the injury becomes irreversible and is accompanied by mitochondrial vacuolization, membrane injury, swelling of lysosomes, and calcium entry into cells. Moreover, a decrease in the pH (also observed in the HP group), in addition to changes in the ionic composition of the cell, leads to damage of the lysosomal membranes, which is followed by cytoplasmic leakage of enzymes and digestion of cellular components [33]. Additionally, the trachea is the area most susceptible

to impact forces generated by shear flow during the mechanical ventilation. In the HP group, in which there was also a greater impact of shear forces at high flow rates, the adaptive mechanisms do not appear sufficient, thus resulting in the cell damage.

Tracheal mucociliary functional alterations

We observed no alterations in CBF in the control, LV, and HV groups. The CBF decreased significantly in the HP group, in which the mean pressure was higher than that observed in other groups. We can speculate that this result could be due to decreased cardiac output with subsequent tissue hypoperfusion due to the high intrathoracic pressure. This theory is supported by the high levels of lactate observed in the HP group. However, this should not have been the only factor to influence the decrease in CBF because the HV group also demonstrated increased lactate levels. Another factor to have affected the CBF in the HP group may be the higher flow throughout the respiratory cycle during the mechanical ventilation. This flow effect may have led to direct damage of the ciliary epithelium by mechanical action.

The exposure to different protocols of MV resulted in no statistically significant differences in the TMCT in our study. This result is contrary to Trawoger et al. [34] who observed a significant decline in the mucociliary transport after 3 h of MV in sheep. Other studies also found a decrease in mucociliary transport under MV conditions due to the occurrence of dehydration [13, 35, 36], loss of cilia [37], and pulmonary infections [38]. However, it should be considered that the mucociliary transport velocity depends not only on the ciliary beat frequency but also on the movement pattern of the cilia [39], the perfect height and composition of the periciliary layer, and the ideal mucus viscoelastic properties [40].

Limitations

It is possible that other factors contributed to mucociliary alterations in our study, including anesthesia, oxygen toxicity, and body temperature. On the other hand, all animals received an identical anesthesia protocol. Moreover, the effects of oxygen toxicity in tracheal cells have been observed only after 24 h of exposure to 80% oxygen [31]. Normothermia and hypothermia, in contrast to hyperthermia, have not been shown to increase the detrimental effects of mechanical ventilation on the lung [41].

Electron microscope data of the tracheal epithelium were descriptive, and future quantitative studies are necessary. Additionally, our system of CBF measurement is limited and may convey imprecise data at frequencies

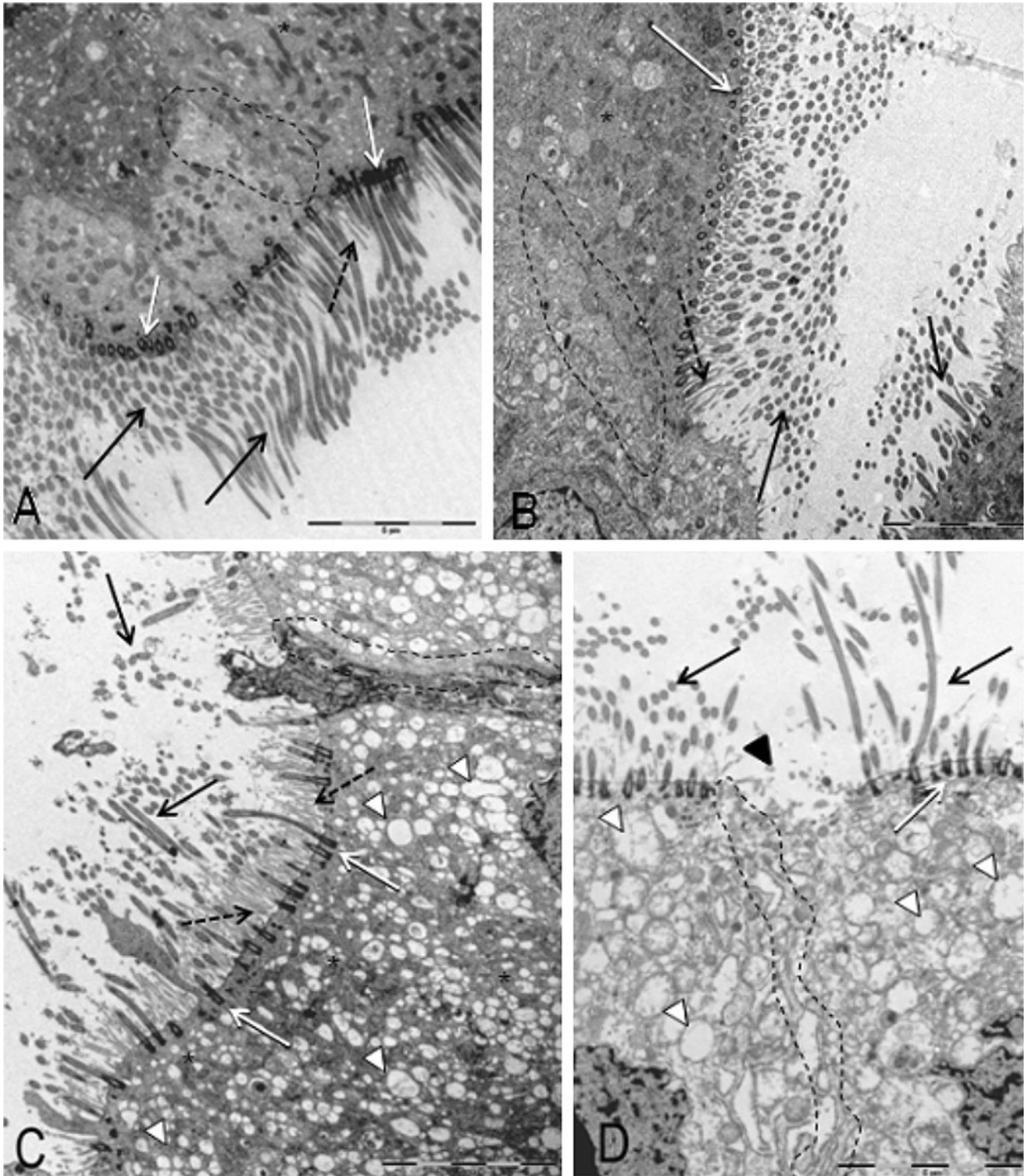


Fig. 2 Transmission electron microscopy of the rabbit tracheal epithelium (near carina region) of all groups. **a** Control, **b** low volume, **c** high volume, **d** high pressure groups. *Black arrows* indicate cilia, *dashed black arrows* microvilli, *white arrows* cilia anchorage, *asterisks* mitochondria, *white arrowheads* vesicles, *black arrowheads* loss of cilia, *encircled areas* cellular junction. 3,700 \times . Bar = 5 μ m

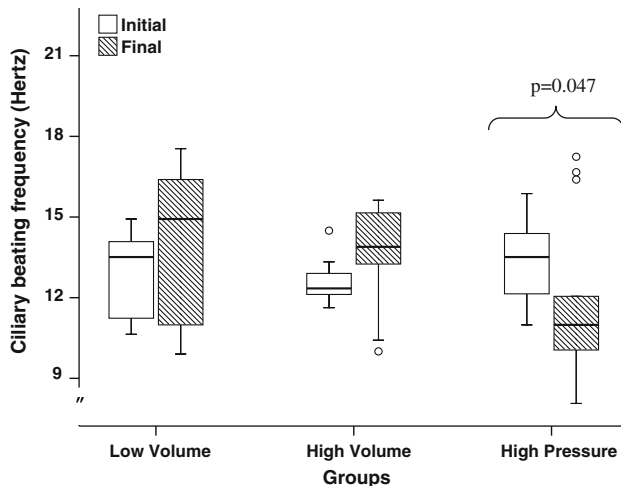


Fig. 3 Ciliary beating frequency was immediately measured on tracheal tissue samples collected at the *initial* (at the tracheotomy procedure) and *final* (at end of protocol) time points. There were no statistical differences among groups, but we observed a lower ciliary beating frequency in the high pressure group after mechanical ventilation exposure. Values are presented as medians and quartiles

above 15 Hz. However, the majority of our CBF results were below 15 Hz and therefore support our findings.

Conclusion

Different MV strategies may induce not only distal lung alterations but also morphological and physiological tracheal alterations leading to mucociliary system dysfunction. The damage is most noticeable when using an increased mean pulmonary pressure associated with an amplified air flow, resulting in cellular harm associated with mucociliary dysfunction of the ciliary beat frequency and loss of cilia. We speculate that these mucociliary alterations probably occur due to hyperperfusion and mechanical injury caused by high intrathoracic pressure and higher flow throughout the respiratory cycle.

Acknowledgments The authors wish to thank Maria Ires Amorim Mendes for her assistance with the histological procedures, Erick Darío León Bueno de Camargo for his technical support, and Dr. Geraldo Lorenzi-Filho for his critical comments on the manuscript. This research was supported by FAPESP, the Fundação de Amparo à Pesquisa do Estado de São Paulo (Sao Paulo State Foundation, Process 06/50725-8) and Medical Investigation Laboratories of the FMUSP (LIM 5, LIM 9, LIM 51, and LIM 59).

Conflict of interest The authors declare no conflict of interest.

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