Benefits of physiologically variable ventilation in improving respiratory function in healthy and diseased lungs

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Doctoral Thesis

BENEFITS OF PHYSIOLOGICALLY VARIABLE VENTILATION IN IMPROVING RESPIRATORY FUNCTION IN HEALTHY AND DISEASED LUNGS

A thesis submitted in fulfilment of the requirements

for the degree of Doctor in Medical Sciences,

MD-PhD

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"...an opening must be attempted in the trunk of the trachea, into which a tube of reed or cane should be put; you will then blow into this, so that the lung may rise again and take air"

Andreas Vesalius

DE HUMANI CORPORIS FABRICA, 1543

Physiologically variable ventilation

– when mechanical ventilation mimics natural breathing –

Thesis Statement

While conventional mechanical ventilation is manifestly a source of lung injury, physiologically variable ventilation, a modality which reproduces the variability of natural breathing, has shown promise in reducing the harm of prolonged mechanical ventilation, preventing the deterioration of lung function.

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Summary (EN/FR)

English

Background ¦ Prolonged mechanical ventilation has been recognised to trigger multiple detrimental phenomena in the lung tissue, including excessive stretch, inflammation, derecruitment and atelectasis. These damaging mechanisms, globally known as ventilator-induced lung injury, deteriorate lung function both in healthy and injured lungs. A ventilation modality that mimics spontaneous breathing by incorporating tidal variations in volume and respiratory rate, termed *physiologically variable ventilation* (PVV), has been proposed to reduce the deleterious effects of mechanical ventilation.

Aim ¦ We sought to compare PVV with a conventional mode of ventilation (pressure-controlled ventilation, PCV) using animal models of healthy and diseased lungs, with acute respiratory distress syndrome (ARDS), asthma and chronic obstructive pulmonary disease (COPD). The primary outcomes were oxygenation ratio and lung tissue elastance, while secondary outcomes included gas exchange, respiratory mechanics, lung volumes, intrapulmonary shunt fraction (Qs/Qt), pulmonary imaging and pathological findings.

Methods ¦ Experimental models of ARDS, asthma exacerbation and COPD were induced in New-Zealand White rabbits. Thereafter, animals were anaesthetised and ventilated during six hours with either PCV or PVV. Respiratory function and structure were assessed by blood gas analysis, oscillometry, gas dilution methods and several techniques of *in vivo* imaging. Post-mortem histology, cytology and cytokine quantifications were performed.

Results ¦ In comparison to conventional ventilation (PCV), PVV demonstrated significant benefits in both primary and secondary outcomes for all the four respiratory conditions that were studied. PVV prevented the deterioration of respiratory tissue mechanics and oxygenation that was observed with PCV. Additionally, PVV prevented the increases in driving pressure and in Qs/Qt that were observed with PCV. Alveolar derecruitment and lung inflammation (measured by imaging, histology and protein assays) were significantly lower in the groups ventilated with PVV.

Conclusion ¦ Prolonged ventilation with PVV, applied to experimental models of healthy and diseased lungs (ARDS, asthma and COPD), prevented deterioration in respiratory function in comparison to conventional (monotonous) ventilation. A reduction in alveolar derecruitment and lung tissue stress leading to better aeration and gas exchange may explain the benefits of physiologically variable ventilation. This modality could become clinically useful to mitigate the impact of ventilator-induced lung injury.

Français

Introduction ¦ La ventilation mécanique prolongée déclenche de multiples phénomènes néfastes dans le tissu pulmonaire, par étirement et cisaillement excessifs, inflammation, dérecrutement et atélectasie. Ces mécanismes dommageables, globalement reconnus sous le terme de lésions pulmonaires induites par le ventilateur (VILI, de l'anglais *´ventilator-induced lung injury´*), détériorent la fonction pulmonaire aussi bien dans les poumons malades que dans les poumons sains. Une nouvelle modalité ventilatoire imitant la respiration spontanée en incorporant des variations dans le volume courant et la fréquence respiratoire, appelée ventilation physiologique variable (PVV), a été proposée pour réduire les effets délétères de la VILI.

Objectif ¦ Nous avons comparé la PVV à un mode de ventilation conventionnel (ventilation à pression contrôlée, PCV) en utilisant des modèles animaux de poumons sains ou atteints de syndrome de détresse respiratoire aiguë (ARDS), d'asthme et de bronchopneumopathie chronique obstructive (BPCO). Les critères de jugement principaux étaient le taux d'oxygénation et l'élastance des tissus pulmonaires; les critères de jugement secondaires comprenaient les échanges gazeux, la mécanique respiratoire, les volumes pulmonaires, la fraction de shunt intrapulmonaire, l'imagerie pulmonaire et les résultats anatomo-pathologiques.

Méthodes ¦ Le SDRA, l'exacerbation d'asthme ou la BPCO ont été induits expérimentalement chez des lapins *New-Zealand White*. Les animaux ont été anesthésiés et randomisés pour recevoir six heures de PCV ou PVV. La fonction et la structure pulmonaires ont été évaluées par analyse des gaz du sang, oscillométrie, méthodes de dilution de gaz et plusieurs techniques d'imagerie *in vivo*. En post-mortem, de l'histologie, cytologie et des quantifications de cytokines ont été réalisées.

Résultats ¦ Par rapport à la ventilation conventionnelle (PCV), la PVV a démontré des avantages significatifs dans les critères de jugement primaires et secondaires pour les quatre modèles respiratoires étudiées. La PVV a empêché la détérioration de la mécanique des tissus respiratoires et de l'oxygénation, contrairement à la PCV. De plus, la PVV a empêché les augmentations de pression motrice et de Qs/Qt observées avec le PCV. Le dérecrutement alvéolaire et l'inflammation pulmonaire (mesurées par imagerie, histologie et dosages protéiques) étaient significativement plus faibles dans les groupes ventilés par PVV.

Conclusion ¦ La ventilation mécanique avec PVV, appliquée à des modèles expérimentaux de poumons sains et malades (ARDS, asthme et BPCO), a empêché la détérioration de la fonction respiratoire par rapport à la ventilation conventionnelle (monotone). Une réduction du dérecrutement alvéolaire et du stress tissulaire pulmonaire conduisant à une meilleure aération et à un meilleur échange gazeux peut expliquer les bénéfices de la ventilation variable physiologique. Cette modalité pourrait devenir cliniquement utile pour atténuer l'impact des lésions pulmonaires induites par le ventilateur (VILI).

Abbreviations

ARDS	acute respiratory distress syndrome
BALF	broncho-alveolar lavage fluid
CO_2	carbon dioxide
COPD	chronic obstructive pulmonary disease
CT	computed tomography
FiO_2	fraction of inspired oxygen
FRC	functional residual capacity
G	respiratory tissue damping
H	respiratory tissue elastance
I_{aw}	airway inertance
ICU	intensive care unit
IL-6	interleukin 6
MV	minute volume
O_2	oxygen
P_{aw}	airway pressure
P_{crit}	critical closing pressure
PaO_2	partial pressure of oxygen in the arterial blood
$PaCO_2$	partial pressure of carbon dioxide in the arterial blood
PCV	pressure-controlled ventilation
PEEP	positive end-expiratory pressure
PET	positron-emission tomography
PVV	physiologically variable ventilation
Qs/Qt	intrapulmonary shunt fraction
R_{aw}	airway resistance
RR	respiratory rate
SIRS	systemic inflammatory response syndrome
SPECT	single-photon emission computed tomography
VCV	volume-controlled ventilation
VILI	ventilator-induced lung injury
V_T	tidal volume
V/Q	ventilation / perfusion

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INTRODUCTION

Background

Principles of breathing and ventilation

- **Respiration – Lung Physiology and gas exchange**

Respiration is the main function of the lung. By providing oxygen (O_2) and removing carbon dioxide (CO_2), it supports cell metabolism in all the tissues of the body. Amongst all the functions of respiration, this brief introductory section will mainly focus on the physical and chemical processes taking place during *pulmonary ventilation* and *gas exchange*. The transport and exchange of O_2 and CO_2 in the blood and body tissues, as well as the regulation of ventilation are extensively revised elsewhere (1-3).

Pulmonary ventilation is the process of inflow and outflow of air connecting the atmosphere and the lung alveoli through the airways. Ultimately, ventilation's purpose is to renew the air in the gas exchange areas of the lungs. In these areas, the air is in close contact with the pulmonary blood: alveoli, alveolar sacs, alveolar ducts, and respiratory bronchioles (**Figure 1**).

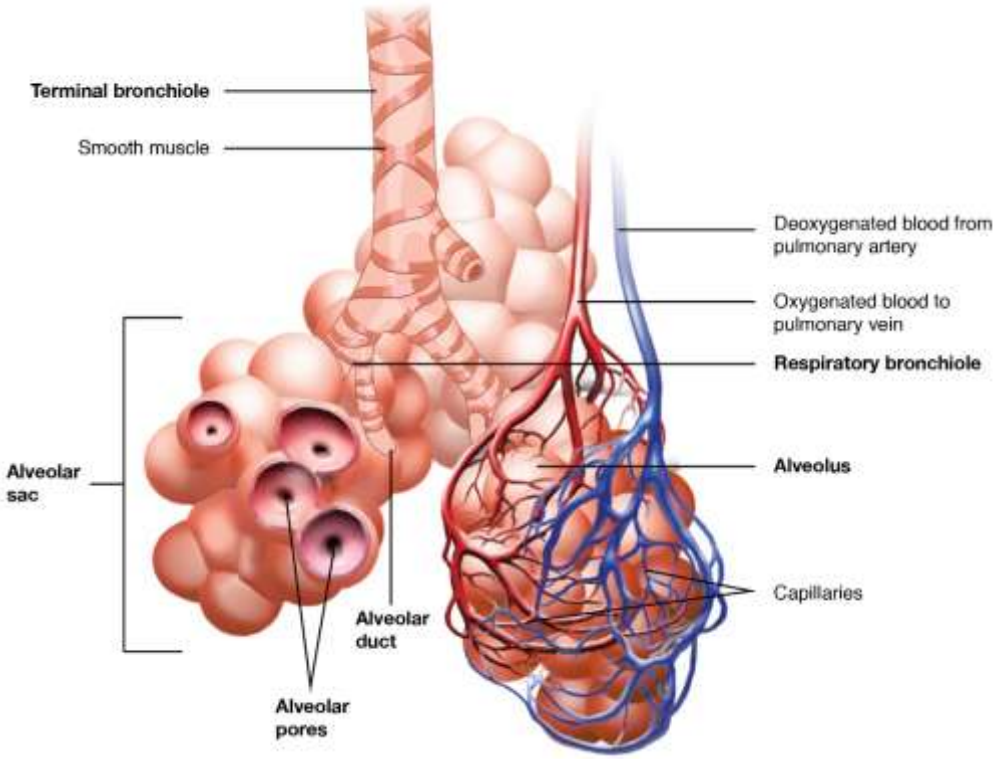

Figure 1. The respiratory unit. Reprinted from (4).

For pulmonary ventilation to happen, pressures need to be created in the respiratory system, causing air movement in and out of the lungs. The understanding of such pressures is essential in respiratory physiology, as well as to interpret the principles of mechanical ventilation and its potential of harm (*see chapter below*).

The interaction of two specific pressures explains the tidal movement of air during spontaneous breathing: **pleural pressure**, a slightly negative pressure in the layer between the lung and the chest wall, that keeps the lungs open; and **alveolar pressure**, the pressure of the air inside the lung alveoli. The latter falls below zero during inspiration, causing air inflow, and becomes slightly positive during expiration, forcing the air out of the lungs. The movements of the chest wall and diaphragm are the driving force creating the tidal differences in alveolar pressure.

The difference between the alveolar pressure and the pleural pressure is called **transpulmonary pressure**. It is a measure of the elastic forces tending to collapse the lung at each instant of respiration (recoil pressure). The volume of air that expands the lung for each unit increase in transpulmonary pressure is termed **lung compliance** (1).

It is important to note that the compliance of the entire respiratory system (the lungs and chest wall together), in physiological conditions, is approximately half of that of the lungs alone (1). Moreover, in pathological conditions or when ventilation conditions are extreme (high or low volumes) the constraints provoked by the chest are even more drastic. Such consideration is paramount when one is evaluating the lung mechanical conditions through measurements of the entire lung-thorax system. Indeed, this physiological principle will be relevant while analysing the results of this thesis.

In regard to pulmonary volumes, some terminology is worth noting, in order to define the terms used hereafter. **Tidal volume** (V_T) is the volume of air inspired (or expired) within one breath cycle. **Respiratory minute volume** (MV) is the total amount of air inhaled (or exhaled) during one minute; quantified in L/min, MV is equal to the V_T times the respiratory rate (RR). The **functional residual capacity** (FRC) is determined by the equilibrium between the expanding thoracic forces and the recoil forces of the lung and equals the volume of air remaining in the lungs at the end of a normal expiration. Under mechanical ventilation, the volume remaining at the end of the expiration is expressed as **end-expiratory lung volume** (EELV), accounting also for the lung volume that results from the pressure applied in end-expiration.

Once the fresh air arrives to terminal bronchiole and reaches the alveoli, another process of respiration takes place: **gas exchange**. This process includes the diffusion of O_2 from the alveolar air into the blood, and of CO_2 in the opposite direction. Chemically speaking, it consists on the motion of gaseous molecules through the respiratory membrane and adjacent fluids.

All the gases that are exchanged in the lungs are simple molecules, which can diffuse freely in the alveoli, fluids and tissues of the body. The rate of diffusion of each gas is directly proportional to the pressure caused by that gas alone, and this is called *partial pressure* (1). In fluids (e.g. blood), gas diffusion depends on two other parameters: 1) the *solubility* of the gas; in this regard, it is noteworthy that CO_2 is 20 times as soluble as oxygen; 2) the distance through which the gas must diffuse (cross-sectional area).

The alveolar walls are extremely thin, and they are surrounded by a very dense network of interconnecting capillaries, as shown in **Figure 1**. Additionally, the mean diameter of pulmonary capillaries is only 5 μm, meaning that erythrocytes are in contact with the respiratory membrane during circulation (1). Thus, if the respiratory membrane is not thickened by oedema or any pathological process, the alveolar O_2 and CO_2 are in very close proximity to the blood.

Even though there is an ideal anatomical matching between the respiratory units and the capillary network, the gas exchange is not homogeneous throughout the lung. Even normally to some extent, and more marked in respiratory diseases, some areas of the lungs do not have a good ventilation/perfusion (V/Q) matching. When respiratory units are well ventilated but have low/no perfusion, these imbalanced zones are called **dead space**; conversely, zones that are well perfused with low/no ventilation are termed **shunt**.

All the aforementioned physiological principles explain the healthy functioning of the respiratory system, under spontaneous breathing. However, several physical aspects of breathing are altered during mechanical ventilation (i.e., "artificial breathing"). The next section describes the principles and modalities of mechanical ventilation.

▪ Mechanical ventilation – Principles, modes, settings and application

Mechanical ventilation is a life-support technique where mechanical means are used to *replace* or *assist* the spontaneous breathing using a ventilator. It is usually applied for

short periods during general anaesthesia (e.g., surgical procedure) or in a prolonged manner in Intensive Care Units (ICU).

The usual indications for mechanical ventilation in the ICU were described in a multinational study including 1'638 patients (5) and are summarised in **Table 1**. Likely, the most common reason for applying mechanical ventilation is to decrease the work of the respiratory muscles (6). In some patients with respiratory failure, the respiratory muscles account for as much as 50% of the total O_2 body consumption (7). By carefully selecting the ventilator parameters, the inspiratory effort can be reduced to normal levels (8).

Reason for mechanical ventilation	**Percentage of cases**
Acute respiratory failure	66 %
Chronic Obstructive Pulmonary Disease	13 %
Coma	15 %
Neuromuscular disorder	5 %
Cause of acute respiratory failure	
ARDS	12 %
Post-operative	15 %
Heart failure	12 %
Aspiration	3 %
Pneumonia	16 %
Sepsis	16 %
Trauma	12 %
Others	13 %

Table 1. Indications for the initiation of mechanical ventilation in the ICU. Adapted from (5).

Mainly, two types of mechanical ventilation exist: *positive pressure ventilation*, where gases are pushed into the respiratory system, and *negative pressure ventilation*, where gases are pulled into the lungs through negative pressures outside the chest. The latter type has been abandoned since the 1950's *(see chapter 'History and pitfalls of mechanical ventilation')*, so our current practice relays on **positive pressure ventilation**. The fundamental operation of positive pressure ventilation is to apply a pressure in the lung passageways that will move a certain gas volume (9). Although this definition might look trivial, there are over 150 specific modalities to ventilate in positive pressure (10), and its number tend to grow as technology evolves. However, succinctly, there are two

conventional methods of positive pressure ventilation, based on different inspiratory flow and are as follows:

- **Pressure-controlled ventilation** (PCV)

In this mode, the user sets the inflation pressure. The ventilator keeps the preselected pressure during the whole inspiration, resulting in a *decelerating* inspiratory flow (**Figure 2**). If the inspiratory time is appropriately adjusted, the flow rate is zero by the end of inspiration; at this point, in the absence of flow in end-inspiration, the airway pressure equals the peak alveolar pressure (1) (Palv, Figure 2). Palv reflects the alveolar volume at the end of lung inflation.

- **Volume-controlled ventilation** (VCV)

In this mode, the inflation volume (V_T) is set by the user. The lungs are inflated at a *constant* flow until the preselected volume is reached (**Figure 2**). Differently from PCV, there is airflow at end-inspiration; thus, the peak airway pressure (Paw) is higher than the Palv. To measure Palv in VCV, a short *inflation-hold manoeuvre* is typically added after the preselected V_T is reached in each cycle. This steady occlusion pressure corresponds to Palv and is called **plateau pressure**.

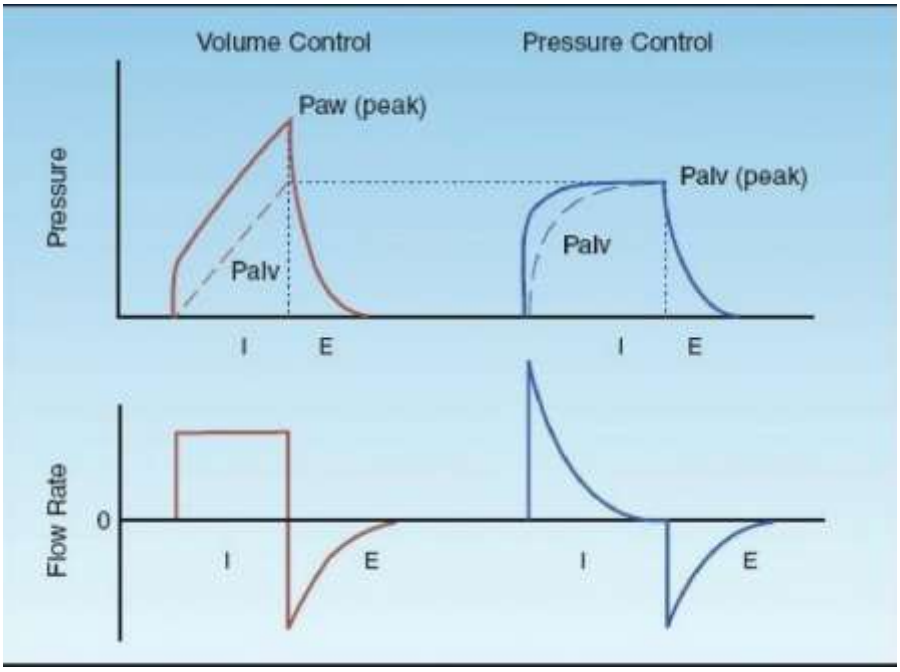

Figure 2. Pressure and flow curves of a single breath represented during inspiration (I) and expiration (E), for both volume control ventilation (red, *left*) and pressure-controlled ventilation (blue, *right*). Changes are represented for similar tidal volumes in the 2 modes. Solid lines indicate changes in airway pressure (Paw) while dashed lines represent the changes in alveolar pressure (Palv). Reprinted from (1).

In summary, these two main modalities allow the user to set either an inspiratory volume (VCV) or an inspiratory pressure (PCV). Besides that, both modes equally allow the preselection of a desired FiO_2, RR and end-expiratory pressure, among other parameters.

The end-expiratory pressure is the *minimum pressure in the alveoli* (1). At the end of expiration, narrowing of the airways might lead to collapse of airspaces (**atelectasis**), especially in dependent lung zones (gravitational effect). The level of transpulmonary pressure that leads to airspace collapse in healthy lungs is about 3 cmH2O (11) and is called *critical closing pressure* (P_{crit}). This phenomenon is extremely detrimental to the lung, since atelectasis impairs gas exchange and cyclic collapse and re-opening of airspaces leads to **atelectrauma** *(see chapter 'ventilator-induced lung injury').*

To avoid alveolar collapse at end-expiration, all modes of ventilation permit the preselection of a **positive end-expiratory pressure** (PEEP), preventing the airway pressure from falling below the P_{crit}. Since the P_{crit} cannot be measured in clinical setting (1), the optimal level of PEEP is very difficult to define. Indeed, PEEP has been a subject of research for more than 50 years and is still today a topic of much debate.

Other than PCV and VCV, many other ventilation modalities exist, including *mandatory* and *assisted* ventilation modes, with patient-synchronised algorithms and non-invasive strategies, which are extensively revised in textbooks (12, 13) and do not belong to the scope of this introduction.

Independently of the specific mode of ventilation, the objectives of mechanical ventilation are similar for all modalities, as listed in the table below:

Improve pulmonary gas exchange
Reverse hypoxemia
Relieve acute respiratory acidosis
Relieve respiratory distress
Decrease oxygen cost of breathing
Reverse respiratory muscle fatigue
Ameliorate pressure-volume relations
Prevent and reverse atelectasis
Improve compliance
Prevent further injury
Permit lung and airway healing
Avoid complications

Table 2. Objectives of mechanical ventilation. Adapted from (14).

All the objectives listed in **Table 2** must be carefully reappraised in light of the last objective (avoid complications). *Primum non nocere*. In this regard, suboptimal parameters for gas exchange and lung aeration should be considered rather than striving for normal values that aggravate lung injury (15). This approach, currently called '*protective ventilation'*, shall provide the lung tissue and airways the opportunity to heal while mechanical ventilation is delivered (14).

Measurements used to evaluate the mechanical properties of the respiratory system (i.e., airway pressures and lung distensibility) are of utmost importance to guide the ventilatory strategy and avoid harm during mechanical ventilation. Such measurements are in-built features of all commercial ventilators and are invaluable tools to guide the clinician.

History and pitfalls of mechanical ventilation

The very first existing reference about the study of ventilation comes from the second century A.D., when Galen, the prominent Greek scientist and physician, stated that breathing was required to maintain the circulation (16). No records exist of any contribution to the understating of respiration in the following 1'500 years, during the Dark Age. It is during the mid-16th century, with Andreas Vesalius, professor of Anatomy in Padua (Italy), that some light was shed in this field. He is responsible for the first definitive reference to artificial ventilation, in his treatise *'De Humani Corporis Fabrica'* in 1543 (**Figure 3**) (17). Astonishingly, the principles of positive pressure ventilation, as they currently stand, are still consistent with his description back then. To cite: "[...] an opening must be attempted in the trunk of the trachea, into which a tube of reed or cane should be put; you will then blow into this, so that the lung may rise again and take air" (17). Although strikingly precise, this description of mechanical ventilation did not lead to its medical practice for several centuries.

Figure 3. Frontispiece of Vesalius' *De humani corporis fabrica* (1543). Reprinted from (18).

In 1667, Robert Hooke, the bright English scientist who inspired the use of microscopes and conceived the term "cell" in biology, performed a cunning experiment in the Royal Society of London to test the role of the lungs and breathing (19). He used bellows to generate a continuous flow of air through the lungs of a dog, in which he made cuts in the chest wall and pleura, to allow the constant airflow to be washed out. Aside from keeping the animal alive, he was able to demonstrate that ceasing the airflow would provoke lung collapse and subsequently cause "[...] dying convulsive fits; but be as soon revived again by renewing the fullness of his lungs with the constant blast of fresh air [...]" (19). Such inventive experiment highlighted the importance of breathing as a vital function; however, at this time, it was still not clear to physicians why people breathe (20).

Another 100 years were passed until more light was shed in this field, when Joseph Priestly and Wilhelm Scheele discovered oxygen in 1774 (21). Another step forward was made in 1775, when Antoine-Laurent Lavoisier clarified the importance of oxygen in respiration, as he came to agree that room air contained this element which supported life (21).

Mechanical ventilators were finally created in the second half of the 19th century, when body-enclosing devices applying negative pressures were created. Such negative pressure boxes, known as *iron lungs*, were built upon physiological principles that we still currently accept: negative pressure causes the lungs to inhale; the opposite produces exhalation. In 1864, Alfred Jones patented one of the first body-box ventilators (22) and in 1876, the first operable iron lung was built by Alfred Woillez (23). However, it was only in 1929 that an iron lung was widely applied: it was developed by Drinker and Shaw in Boston, to treat patients with poliomyelitis (24).

The deadly polio outbreak was the definitive turning point in the history of mechanical ventilation. Usually, great medical advancements take place during wars and epidemics, when a deep need for therapeutic options drive medical research and innovation. Mechanical ventilation was no exception.

In 1950's, when respiratory failure was identified as the cause of death during the polio epidemics, the benefits of artificial ventilation were found to be tremendously life-saving. In 1953, during the polio outbreak in Copenhagen, Bjorn Ibsen brilliantly demonstrated that poliomyelitis mortality dropped by the half with the usage of mechanical ventilation (25, 26). Nevertheless, one aspect of the available device for ventilation, the iron lung, quickly became problematic: it was extremely difficult to nurse and to access the patient's body (**Figure 4**). This scenario paved the way for a paradigm shift, leading to the approach we still use today in mechanical ventilation: intermittent positive pressure. During this period, the demand for mechanical ventilation outgrew the supply of ventilators. To overcome this technical hurdle in Denmark, approximately 1'500 medical students were

engaged in hospital practice in 8-hour shifts as "human ventilators", having provided hand-bag ventilation of afflicted patients, for the equivalent of 165'000 hours (9, 27).

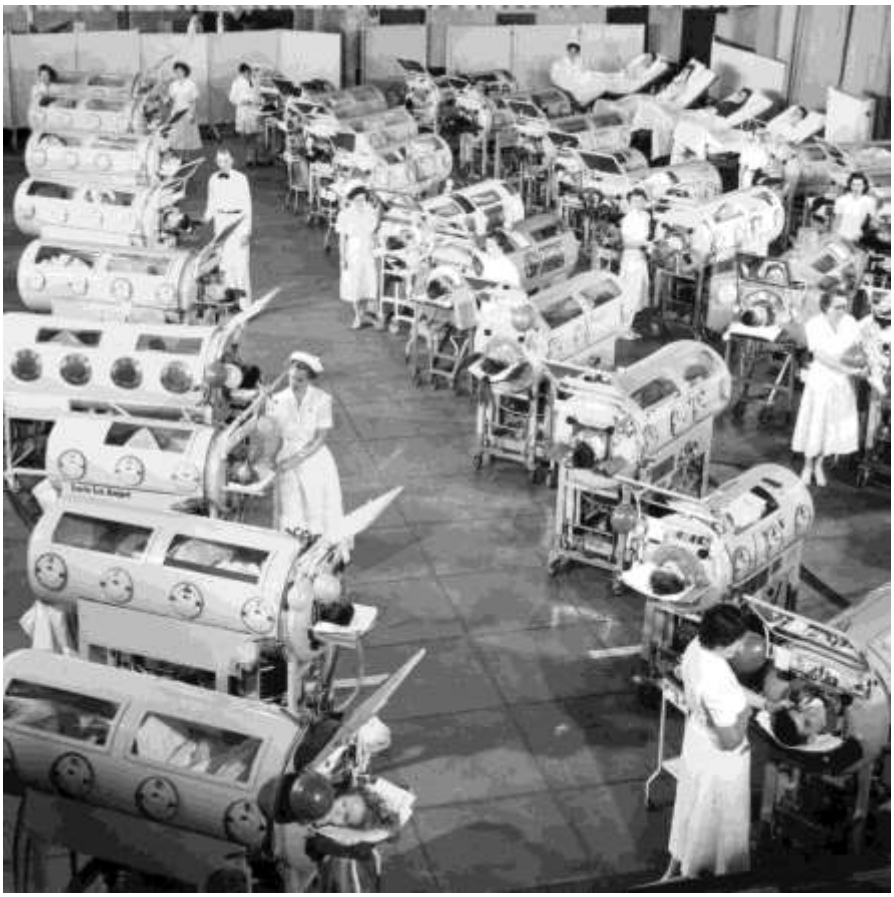

Figure 4. Hospital respiratory ward during the polio outbreak in Los Angeles (1952). Reprinted from (28). Source: Centers for Disease Control and Prevention.

Mechanical ventilation in the context of poliomyelitis was essentially introduced to replace the failing neuromuscular pump. These patients' otherwise healthy lungs required low pressures that were rather safe to apply. Over the ensuing decades, as ventilation started to be applied to severely injured lungs, the focus switched mainly to the oxygenation failure (20). This more challenging context definitely led physicians to abandon negative pressure ventilators, less efficient in pressure delivery.

The recent era of positive pressure ventilation also witnessed some great paradigm shifts, as the progressive awareness of the harmful effects of mechanical ventilation has had a serious impact on the strategies applied in ventilation. In 1967, with the identification of the Acute Respiratory Distress Syndrome (ARDS) (29), several new concepts came into play: the advantages of using PEEP and the understanding of atelectasis in supine patients. In 1981, Darioli, Domenighetti and Perret recommended low tidal volumes and low

frequencies to ventilate patients with acute respiratory failure (30). This 'new strategy' was expected to decrease the risk of barotrauma by avoiding high airway pressures. This rationale paved the way for the concept of permissive hypercapnia in *status asthmaticus* (31), by relieving hypoxemia without seeking rapid correction of hypercapnia (30). This subject was further developed in 1982, when Pepe and Marini describe the concept of auto-PEEP (32). In 1990, Hickling et al. described for the first time the use of permissive hypercapnia also in ARDS (33), by using lower V_T (more physiologic). The aforementioned principles proposed by Darioli and Hickling, suggesting a decrease of ventilatory pressures to avoid barotrauma, paved the way to a more modern concept of ventilation (Lung-protective ventilation, *see chapter below)*. Finally, in 2000, a milestone work demonstrated a significant decrease in mortality of ARDS (34), following the use of a lung-protective strategy, with the aim of minimizing the ventilator-induced lung injury (VILI, *see chapter below)*. The ventilation strategy proposed in this work revolutionized the treatment of ARDS, however, it does not avoid all the harmful effects of mechanical ventilation and still requires improvement. As of today, 20 years after the introduction of protective ventilation, VILI is still commonly seen in clinical practice. Despite the application of protective ventilation strategies at a global scale, during the COVID-19 global pandemic, we are still witnessing a substantial amount of VILI with deleterious effects in patients' outcomes.

On a final note, it must be acknowledged that extraordinary improvements were made in the technical features of ventilators over the past 70 years. High-performance valves, detectors and microprocessors permitted the development of better flow delivery, improved triggering and many new modes of ventilation. Ventilatory support has been improved ever since to ameliorate the interaction between the patient's respiratory drive and the ventilator's flow delivery, with the aim to promote a less artificial, more physiological ventilation.

The slow-moving history of mechanical ventilation was marked by several pitfalls and hazardous approaches. In the 18th century, when fundamental knowledge in respiratory physiology was lacking, the potential for harm of mechanical ventilation was acknowledged early on, even before the discovery of oxygen. In 1744, John Fothergill recognised that the bellows used to resuscitate patients could be harmful by generating high airway pressures (35). In this regard, he suggested instead a safer usage of mouth-to-mouth insufflation of the lungs during resuscitation manoeuvres: "[...] the lungs of one man may bear, without injury, as great a force as those of another man can exert; which by the

bellows cannot always be determined." His early understanding of what would be termed 'barotrauma' some 200 years later, warranted him the designation of father of VILI.

Fothergill observations were made following what appears to be the first clinical description of mouth-to-mouth rescue breathing, performed by William Tossach to a suffocated coal miner in 1732 (36). By 1740, the Paris Academy of Sciences had officially recommended mouth-to-mouth resuscitation to save drowning victims (37). Even so, regrettably, the discovery of oxygen 30 years later led to disapproval of mouth-to-mouth ventilation because of the belief that expired air ventilation was lacking in oxygen and was, thus, futile (20).

During the 19th and 20th centuries, reports on side effects of ventilation put many scientists on guard and likely set the field back for decades (20). In 1829, d'Etioles described fatal pneumothoraxes after ventilation with bellows (38). Such reports led the Royal Humane Society, in 1837, to remove the recommendation on ventilation during resuscitation manoeuvres (38). It was only much later, in the 1940's, that the underlying mechanisms explaining pneumothoraxes and barotrauma during mechanical ventilation, through broncho-alveolar tears, were described by Macklin and Macklin (39).

Supplementary concerns on the side effects of mechanical ventilation arose in the 1960's with reports of increased mortality and serious complications while using a fraction of inspired oxygen (FiO_2) of 100% (20). By that time, reluctant to use high FiO_2, physicians treated hypoxemia with very high V_T, superior to 20 mL/Kg (40). This approach temporarily improved oxygenation but, unsurprisingly, promoted further VILI.

Another concept that was not explored for several decades was the PEEP. Although Barach described the benefits of PEEP in the treatment of a number of diseases in 1938 (41), it was only in 1967 that the utility of PEEP was established by Ashbaugh and colleagues, with the observation of a significantly decreased mortality in ARDS patients (29).

All these pitfalls in the history of mechanical ventilation led to involuntary iatrogenic injury, delaying its (safer) implementation. However, the comprehension of the mechanisms by which mechanical ventilation causes harm has greatly increased in the past 40 years. We now know that high airway pressures cause injury through lung overdistention, a mechanism called volutrauma by Dreyfuss and Saumon in 1998 (42) (*see chapter below*). Additionally, we currently recognize that injurious forms of ventilation can have systemic repercussions, leading to the release and translocation of mediators, toxins and microorganisms (43-45).

Our better understanding of the ventilatory pathophysiology, including both positive and negative features of positive pressure ventilation, led to a better care of patients requiring mechanical ventilation in recent times.

In recent times, even considering the substantial technical improvements in modern devices, it is most likely that the greatest advance in delivering mechanical ventilation has been in minimising its collateral damage, known as VILI.

Ventilator-induced lung injury

Mechanical ventilation is a life-support technique and a salvage treatment for respiratory failure of undeniable value. However, as every powerful treatment, it has adverse effects that are globally named **ventilator-induced lung injury** (VILI). The term VILI regroups all the deleterious effects that the ventilation itself triggers (or aggravates) in the pulmonary airways, lung tissue and in the blood-gas barrier. Ultimately, the structural damage caused by VILI will impair the respiratory function, both lung mechanics and gas exchange. (46).

During physiological breathing, the air is ***pulled in*** the lungs *(see chapter 'Respiration – lung Physiology and gas exchange')*. Through this mechanism, very high volumes of air can be inhaled without noticeable harm to the lung (9). However, during positive pressure ventilation, the air is ***pushed in*** the respiratory system. The transition from spontaneous awake breathing to the anaesthetised state under mechanical ventilation introduces profound physiologic changes. In fact, the positive pressure can disrupt the lung architecture, which is maintained by a "skeleton" of elastin and collagen, embedded in the extracellular matrix that anchors the lung cells (47). During mechanical ventilation, the lung microstructures create a counterforce per unit of area that balances and reacts to the external load (***stress***). The clinical equivalent of *stress* is the *transpulmonary pressure*. The associated deformation of the structure is called **strain**. Positive-pressure ventilation generates stress and strain even in healthy lungs but more dramatically in injured lungs. In diseased lungs, VILI can arise with a physiologic or even smaller V_T (42, 48).

The direst repercussions of VILI result from damage to the respiratory membrane, the alveolar-capillary interface. This allows fluid and proteins to leak into the airspaces (46). The resulting "flooding" of alveolar regions is not an innocuous pulmonary oedema; instead, it leads to an inflammatory infiltration of the lung and to a clinical condition similar to ARDS (49).

In fact, VILI has been studied primarily in patients with ARDS. Classically, 4 mechanisms of VILI have been described: barotrauma, volutrauma, atelectrauma and biotrauma.

Early since the creation of mechanical ventilation, it was recognised that excessive pressure could result in rupture and air leaks both in airways and airspaces (**barotrauma**). The leakage of air from the broncho-alveolar space can drain into all adjacent anatomical structures, causing life-threatening conditions (*pneumothorax*, *pneumomediastinum* and *pneumoperitoneum*). In the 1970's, it was shown that high inflation pressures could lead to diffuse pulmonary oedema (50). Subsequently, in the 1980's, a turning point on the comprehension of VILI arrived with the demonstration that *overdistention* (high inflation volumes) rather than high inflation pressures was the source of lung injury produced by

mechanical ventilation (51). This mechanism was called **volutrauma**. It is of particular importance in situations of lung infiltrative diseases (e.g. pneumonia, ARDS), where functional lung volume is greatly reduced (9), and "normal" V_T will overdistend the remaining normal lung.

Both volutrauma and barotrauma arise from injurious lung inflation; however, other VILI mechanisms occur during lung emptying. The small airways tend to collapse during expiration (*atelectasis*), especially in conditions of low compliance and in dependent lung regions. The next inspiratory pressure might be able, or not, to recruit those collapsed airspaces. This repetitive closure and opening phenomena, termed **atelectrauma** (48), can injure the airway epithelium, possibly through excessive shear stress (52). In order to mitigate atelectrauma, clinicians often apply a certain level PEEP, which is expected to be above the P_{crit}.

Furthermore, it has been demonstrated that lung injury occurs at tidal inflation volumes that do not produce structural damage (53). This rather unsettling observation was due to the ability of positive pressure to trigger the release of proinflammatory cytokines, a process defined as **biotrauma**. Remarkably, the mechanical ventilation itself can induce or amplify lung injury. In fact, VILI can synergistically potentiate pre-existing lung injury (54) through promotion of tissue oedema, alveolar disruption and cytokine release (55). Additionally, it was shown that mechanical ventilation could induce both a pulmonary and systemic cytokine response (53). Oddly enough, this means that mechanical ventilation itself can be the cause of a Systemic Inflammatory Response Syndrome (SIRS) and multiorgan failure, similar to those caused by septic shock, for example.

The differences between the four types of VILI are to some extent rather theoretical, as they represent the multiple facets of excessive mechanical energy. A summary variable which englobes all measurable causes of VILI has been proposed: **damaging power** (56). Indeed, all the parameters implied in ventilation (V_T, RR, driving pressure, flow, resistances and PEEP) are different components of a unique physical variable, which is the energy delivered over time, that is, the *mechanical power* (57). As a matter of fact, modifications of the tidal inflation pattern and RR may increase or decrease the risk of VILI (58). Such mechanical power acts in neighbouring respiratory units with different elasticity, through simultaneous mechanisms of overdistension and collapse. Stress and strain are amplified by repetitive opening and closure of alveoli (**atelectrauma**) with consequent overdistention of the remaining open alveoli (**volutrauma**). The tissue damage occurs ultimately through mechanisms of **biotrauma**, namely "microfractures" of the extracellular matrix, cytokine signalling, inflammatory cascades and vascular injuries

that lead to tissue wounding and (local and systemic) inflammatory activation (43, 53, 59).

The progressive understanding of the iatrogenic repercussions of mechanical ventilation, namely the VILI mechanisms described above, paved the way for one of the great advances in ventilation management over the past few decades: **lung-protective ventilatory strategies.**

Lung-protective ventilation

These ventilation strategies, derived from the hallmark clinical trial published by the ARDS Network in 2000 (34), are designed to limit the risk of all forms of VILI described above (9). A full description of this ventilation protocol can be found in (60). Briefly, it recommends the use of:

i. V_T = 6 mL/kg (ideal body weight)
ii. Plateau pressure ($P_{plateau}$) ≤ 30 cmH_2O
iii. PEEP ≥ 5 cmH_2O
iv. increasing PEEP with increasing FiO_2 according to a sliding scale - *see* (60)
v. 7.30 ≤ pH ≤ 7.45

Recommendations i) and ii) are intended to prevent **barotrauma** and **volutrauma**. Since $P_{plateau}$ is equivalent to the peak alveolar pressure (9), $P_{plateau}$ ≥ 30 cmH_2O is a surrogate measure of alveolar overdistention. Recommendation iii) serves the purpose of preventing **atelectrauma**, by applying PEEP to avoid collapse of airspaces during expiration. Finally, a slight respiratory acidosis is permitted, as long as the pH does not fall below 7.30 (**permissive hypercapnia**).

The lung protective ventilation is currently recommended for all patients who require mechanical ventilation in the context of ARDS. Moreover, there is also growing evidence that lung-protective strategies are useful for patients without ARDS, for instance, to prevent the development of ARDS (61), to prevent respiratory complications in anaesthetised patients undergoing surgery (62) and to preserve lungs for transplantation in patients with brain death (63).

As a matter of fact, mechanical ventilation does efficiently restore the respiratory function and normalises arterial gases. However, way beyond the short-term success of ventilatory support, patient survival and outcomes deeply depend on the settings used for ventilation. As an example, in patients with ARDS, even though mechanical ventilation is able to normalise arterial gases, mortality remains high due to subsequent multiorgan failure (64). Thus, protective ventilation strategies (34), as well as non-invasive and assisted modes (65, 66) have been adopted in recent times to decrease the risk of VILI. However, there is still room for improvement; to quote: "mechanical ventilation: we have come a long way but still have a long road ahead (67)."

In conclusion, there is still a need for modalities of mechanical ventilation that ensure both a satisfactory gas exchange and a minimal level of VILI.

Variable ventilation

During mandatory mechanical ventilation, both RR and V_T are fixed (constant). However, both these parameters vary considerably during spontaneous breathing (68). Thus, there has been recent interest in a mode of mechanical ventilation that resembles spontaneous breathing by incorporating tidal variations in both volume and respiratory rate. This mode, termed **variable ventilation** or sometimes *'noisy' ventilation*, has the potential to be less injurious (i.e. promote less VILI).

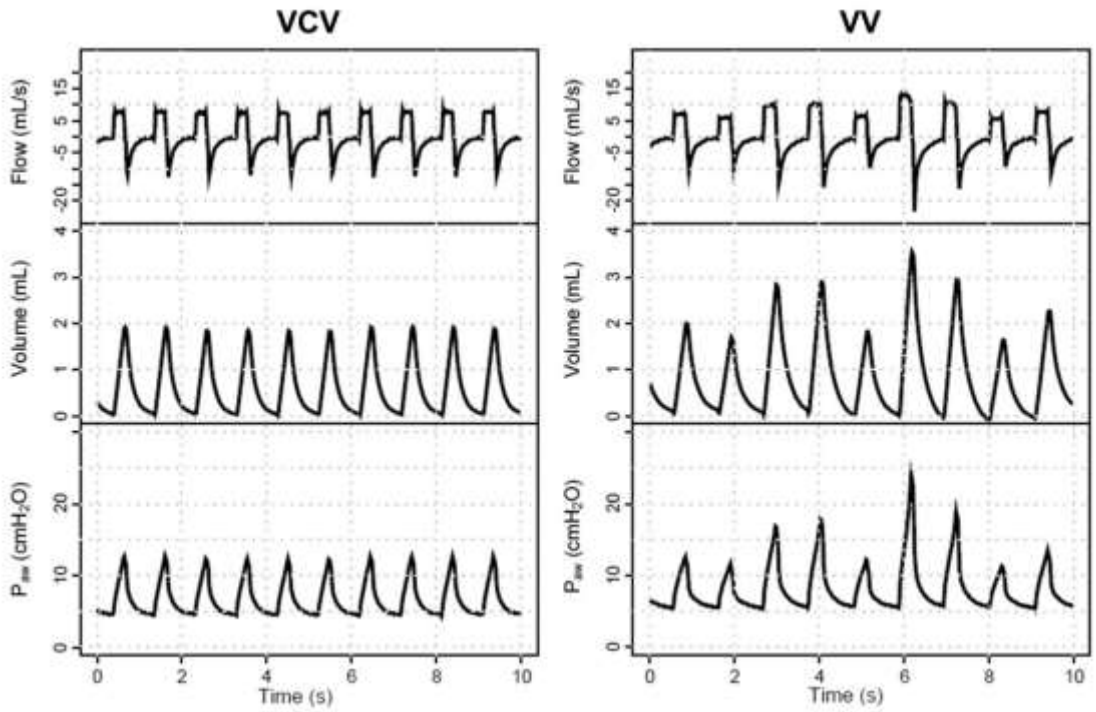

Figure 5. Representative tracings of flow (upper), volume (middle), and pressure (P_{aw}, bottom) during conventional volume-controlled ventilation (VCV, left column) and variable ventilation (VV, right column). Reproduced from (69).

The rationale for this mode of ventilation is rooted in the fact that *variability*, or *'noise'*, defines the pace of several organic phenomena. Frequencies that appear to be random underlie many natural processes (70, 71), in particular the respiratory system function and structure. For example, the airway branching architecture is a scale-free process, with complex fractal dimension (72). Likewise, spontaneous breathing demonstrates appreciable tidal variation in rate and volume, even in steady-state conditions and at rest (68, 73). Aside from the constant adjustment to internal and external conditions, it was shown that these natural fluctuations in the breathing pattern are beneficial for structural and functional reasons (74), given the nonlinear behaviour of the lungs.

Interestingly, a significant loss of this intrinsic fluctuation (*variability*) has been documented in ageing and disease (75, 76). To list some examples: a reduced heart rate variability was shown in patients suffering from coronary heart disease or sleep disorders (77, 78); diminished blood pressure variability was observed during pathological sleep and pre-eclampsia (78, 79); reduced variability both in V_T and RR occurs in patients with COPD (80) and during prolonged ventilatory weaning (81). Indeed, during mechanical ventilation, reduced breathing variability has been shown to be a predictor of unsuccessful weaning (81). It has been argued that elimination of the inherent variability of respiration during conventional ventilation may be detrimental and contribute to the morbidity related to this life-support technique (82).

The aforementioned observations led to great interest and research in **variable ventilation**, a mechanical ventilation mode which mimics the natural variability of breathing.

The first reference describing in 1996 a *"computer-controlled mechanical ventilation programmed for biological variability"* (82), paved the way for many experimental attempts to deliver variable ventilation. Despite a considerable number of promising reports being published over the past 20 years (69, 82-128), a variable ventilation mode is still not commercially available in clinical practice.

In-depth knowledge is lacking on the precise mechanisms underlying the improvements caused by variable ventilation in lung function; however, two main epiphenomena were advanced as likely explanations (129): 1) recruitment and stabilisation of airspaces, contributing to gas exchange; and 2) improvement in V/Q matching. A graphic representation of these mechanisms is depicted in **Figure 6**.

Recruitment and stabilisation of airspaces, contributing to gas exchange

A proposed explanation for the benefits of variable ventilation on lung mechanics is rooted on the *Jensen's inequality* (130), which postulates that the addition of 'noise' (variability) to the airway pressure, on a convex airway pressure-volume curve, leads to an amplification in the mean tidal volume. This occurs by virtue of the nonlinearity of the pressure-volume curve (74). Otherwise said, for the same average inspiratory pressure, the 'gain' of volume during higher-than-average pressure cycles exceeds the 'loss' of volume during lower-than-average pressure cycles. Consequently, during variable ventilation, the driving pressure for a given tidal volume would be theoretically reduced (129).

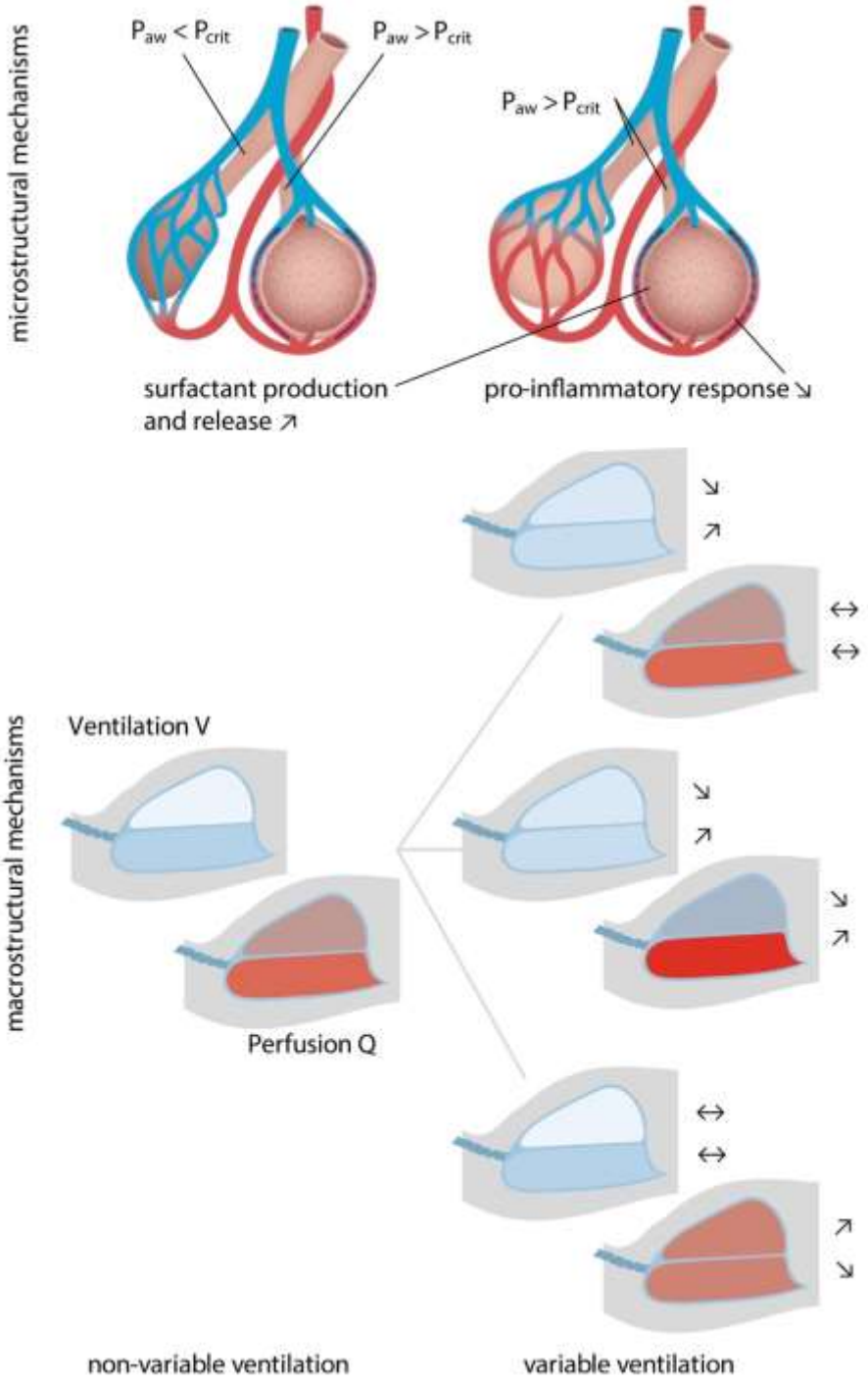

Figure 6. Distinct microstructural (*top,* alveolar and cellular level) and macrostructural mechanisms (*bottom,* pulmonary system) can explain the potential benefits of variable ventilation. The mechanisms underlying **conventional** ventilation (*left side*) are compared to those of **variable** ventilation (*right side*). Blood perfusion (*red*) and distribution of ventilation (*white/blue*) are represented for dependent and non-dependent lung regions. V/Q matching is represented with arrows (↗ increase; ↘ decrease; ↔unchanged). P_{aw}: airway pressure; P_{crit}: critical closing pressure. Reprinted from (129). Illustration by Peter Ernst.

The recruitment of previously collapsed alveoli occurs by exceeding the P_{crit}. It was demonstrated by Suki et al. (131) that both the timing and magnitude of pressure delivery is crucial for triggering alveolar recruitment. In fact, once the P_{crit} of collapsed airspaces has been overcome, all the downstream airways/alveoli with lower P_{crit} will open 'like an avalanche' (131). Thus, the 'avalanche process' of airspace recruitment is *stochastic* (random probability distribution). Therefore, since P_{crit} of collapsed regions and the timing to achieve P_{crit} are heterogenous across the lung, the probability of airspace recruitment and stabilisation could be maximised with a variable ventilation pattern, as compared to conventional (monotonous) patterns (104).

In the short term, these mechanisms lead to an increase on the surface area for gas exchange and, hence, to an increase in PaO_2. Long-term benefits could also arise as recruitment of atelectatic regions would occur without an increase in mean airway pressure (74, 107).

Improvement in V/Q matching

Variable ventilation leads intermittently to inspiratory pressures that exceed P_{crit} of airspaces in dependent lung zones, resulting in alveolar recruitment. Following that, aeration increases in the dependent lung and, as blood perfusion preferentially flows in dependent lung zones, local (and global) V/Q matching improve (129). Additionally, it has been observed that oxygenation increases with variable ventilation even with unchanged aeration. In experimental ARDS, a perfusion redistribution from dependent to non-dependent lung zones was also documented (119), resulting in improvement of the V/Q matching.

To summarise, the benefits of variable ventilation proceed from multiple macrostructural changes in the lung, leading to improved recruitment, oxygenation and lung mechanics. In addition, it has been shown that **microstructural effects** also play a role in the improvement of respiratory function during variable ventilation.

Worthy of note, the variable stretch in the respiratory epithelium (resulting from a variable respiratory pattern) has been shown to ameliorate alveolar stability, surfactant production and inflammation.

Once recruitment has occurred, **surfactant** production is crucial for stabilisation of recruited lung regions (132). Surfactant secretion increases exponentially with stretch in alveolar type II cells (133). Therefore, periodical high tidal volume cycles during variable ventilation may stimulate surfactant release to a higher degree than the average stretch

during conventional ventilation (129). This hypothesis has been tested and confirmed in experimental settings using variable ventilation (84, 85, 116).

Finally, the protective effects of variable ventilation could also attenuate the release of inflammatory mediators in comparison to conventional ventilation.

A considerable amount of experimental data has documented the beneficial effects of variable ventilation, due to the above-mentioned mechanisms of improvement in lung function and structure. Nonetheless, a considerable amount of conflicting evidence exists on the effects of variable ventilation.

While most studies have demonstrated an improvement in **gas exchange** (69, 82-85, 88, 89, 92, 95, 96, 99, 101, 103, 105-109, 114, 117-120, 123, 124), **ventilatory** and/or **mechanical parameters** (69, 82, 83, 85-90, 92, 95-97, 99-103, 105-109, 111, 113, 114, 116-118, 123-125, 127, 134) in comparison to conventional ventilation modes, a certain number of studies did not observe differences in gas exchange (87, 90, 91, 93, 97, 98, 100, 102, 110, 116, 122, 125, 128) nor differences in respiratory mechanics (84, 91, 93, 110, 122, 128). Noteworthy, no study has reported detrimental effects of variable ventilation on gas exchange, ventilatory pressures or mechanical parameters.

Concerning lung **pathology**, the research findings are less consensual. Several studies reported favourable effects of variable ventilation in tissue oedema, leading to decreased wet-to-dry ratio (82, 87, 88), improved histological injury scores (69, 91, 102, 114, 117, 125), lower bronchoalveolar lavage fluid (BALF) cells (86), reduced inflammatory cytokine concentration, namely IL-6, IL-8, IL-1β, TNFα, among others (69, 84, 85, 88, 102, 112, 123, 124, 126) and increased surfactant proteins (85, 116, 123). Nonetheless, a considerable number of studies found no significant difference between ventilation modes, regarding tissue findings, surfactant proteins, cytokines and cell quantifications (90, 93, 95, 97, 99, 105, 111, 120, 123, 128). Importantly, no study has reported detrimental effects of variable ventilation on pathological findings, in comparison to conventional ventilation.

At least to some extent, the inconsistent findings in previous reports are due to very distinct **experimental settings** and **variability patterns**. As a matter of fact, the type of '*variability'* in the breathing pattern applied in previous research is not homogeneous, ranging from biologically derived patterns to randomly generated computer signals, with several fold change in terms of degree of variability (coefficient of variation).

In conclusion, there is still a lack of detailed knowledge on the outcomes of variable ventilation. Discordant data is available on gas exchange, mechanical outcomes, and pulmonary inflammation. Moreover, only a minority of studies used structural or functional imaging methods to assess the effects of variable ventilation. And finally, most of the research on variable ventilation has been performed on acute lung injury. Few studies have been conducted on the potential of variable ventilation in the context or *chronic and obstructive diseases* like asthma or COPD. Therefore, further research was needed to clarify the benefits of variable ventilation both in healthy and diseased lungs. Hence, it was the aim of this thesis.

Aim

The present translational research aimed at characterising the effects of a variable ventilation mode, based on a physiological breathing pattern. To be able to accomplish this aim, we sought to:

1. develop a ***physiologically*** variable pattern for mechanical ventilation, generated from ***spontaneous breathing*** recordings of awake subjects;

2. assess the benefits of ***prolonged*** application of physiologically variable ventilation in ***healthy lungs*** and in experimental models of ***lung diseases*** associated with airway obstruction (asthma) and/or tissue injury (chronic obstructive pulmonary disease (COPD) and acute respiratory distress syndrome (ARDS)).

The primary outcomes were the oxygenation ratio and respiratory tissue elastance after 6h of application of PVV compared to PCV, while secondary outcomes included gas exchange, respiratory mechanics, lung volumes, intrapulmonary shunt fraction, pulmonary imaging and pathological findings.

Ultimately, the aim of the present work is to initiate the first move towards a safe implementation of this mode of ventilation in the clinical setting.

METHODS – general description

This section comprises a general description of the methods. Supplementary details on the methods of each experimental protocol are provided in the full-text publications in the Appendix of this thesis.

1. Ethical statement

All animal procedures (including animal housing, handling and surveillance before, during and after interventions) were performed according to the best standards in animal care and the Swiss animal protection Laws (LPA, RS455).

The author of this thesis underwent theoretical and practical training in animal wellbeing, handling and surgery, as well as in legal and ethical principles of animal research. He obtained a certificate in Laboratory Animal Science (*Réseau des Animaleries Lémaniques – Module 1*) in June 2017, prior to the commencement of the experimental work. This course is accredited by the Federation of European Laboratory Animal Science Associations (FELASA B personal identification number: RESAL 1/927).

All experimental protocols were submitted and approved by the Animal Welfare Committee of the Canton of Geneva and the Experimental Ethics Committee of the University of Geneva, according to current standards and regulations. The experimental work of this thesis was performed under the following authorisations:

- GE 144/17, August 21st, 2017, modified from GE 64/17, May 2nd, 2017
- GE 7/18, February 8th, 2018
- GE 71/19, April 29th, 2019, modified from GE 99/18, July 19th, 2018
- GE 99/19, June 24th, 2019, modified from GE 184/18, January 2nd, 2019

2. Animal procedures

2.1. Animal species, housing, and handling

All experiments have been conducted in New Zealand White Rabbits. This species has been extensively used for research in respiratory medicine. The rabbit has considerable anatomical and physiological resemblance to humans in regards of the respiratory system. Furthermore, it allowed repeated measurements and sampling, greatly reducing the number of animals required for research.

Rabbits were purchased from the farm of University of Geneva (Arare, Geneva) and were housed in groups in the animal facility of the Faculty of Medicine, University of Geneva, where all experiments were conducted (*Centre Médical Universitaire*). Animal cages were enriched with playing material and straw in order to refine the housing conditions. During experiments with juvenile rabbits, the pups were transported and housed with their respective mothers until weaning age. Animals were regularly assessed, weighted, and provided with water and food *ad libitum*.

2.2. Animal preparation and anaesthesia

A detailed description of the animal surgical preparation, sedation, equipment, and monitoring is provided for each experimental work, in the Appendix of this thesis. In all experiments, anaesthesia was induced by intramuscular injection of ketamine and xylazine followed by maintenance with total intravenous anaesthesia with propofol, fentanyl and midazolam. An appropriate depth of anaesthesia was ensured before neuromuscular blockade with atracurium was added during the experimental period of mechanical ventilation. Animals were equipped with invasive vascular catheters for repeated blood sampling and continuous blood pressure monitoring. Mechanical ventilation was delivered through a tracheal tube, using ventilators with supplementary research software, permitting the application of the experimental ventilation modes.

2.3. Animal welfare scoring system

The research experiments in animal models of healthy lungs and ARDS were conducted in rabbits under terminal anaesthesia (sedation was maintained during the whole research protocol, followed by sacrifice under the same general anaesthesia). On the contrary, the research protocols in animal models of chronic respiratory diseases, asthma and COPD, both required recurrent anaesthesia procedures over several weeks. To ensure animal welfare and to screen for any potential distress, we created a welfare score sheet that was approved by the Animal Welfare Committee of the Canton of Geneva, to be repeatedly applied over the course of the experimental protocols. A detailed description of the scoring system can be found in page 128.

The scoring system was designed to allow early identification and to prevent respiratory distress in the rabbits during the experimental period. It follows the rationale of the 3R principles in animal research (135) which advocates for a more ethical use of animals for research purposes, through the reduction, refinement and replacement (3R) of experimental animals. The design and implementation of the welfare scores in this

research work were recognized by the Swiss 3R Competence Centre, who attributed the Best Young Scientist Poster Award to the author (see Declarations section, Prizes).

3. Experimental protocols

Overview

The experiments were conducted in animal models of different pulmonary conditions, namely in healthy lungs and diseased lungs (ARDS, Asthma and COPD). For each of these pulmonary conditions, the research subjects received prolonged mechanical ventilation (five to six hours) under general anaesthesia, with either conventional ventilation (pressure-controlled ventilation, PCV) or physiologically variable ventilation (PVV).

The effects of PCV and PVV (during and after the period of mechanical ventilation) were compared using several techniques for the assessment of respiratory function, namely 1) **blood gas analyses**, which were performed hourly to evaluate oxygenation and carbon dioxide; 2) **oscillometry** (or forced oscillation technique), by applying a multifrequency oscillatory signal during 10-second periods of apnoea, to assess the mechanical properties of the respiratory system (airway resistance and inertance, respiratory tissue elastance and damping); 3) **airway pressure** monitoring, continuously measuring the ventilatory driving pressure; 4) end-expiratory lung volume, which was directly assessed by **helium dilution method** and indirectly by 2-dimension imaging (**X-ray fluoroscopy**); 5) **lung clearance index**, assessed through the helium dilution method to evaluate the ventilation heterogeneity; 6) **intrapulmonary shunt fraction** (or venous admixture) which was estimated from the oxygen content in the arterial and central venous blood; 7) *in vivo* imaging to assess lung aeration, tissue metabolism and perfusion through **computed tomography** (CT), **positron-emission tomography** (PET) and **single-photon emission computed tomography** (SPECT), respectively; 8) **histological injury** and **morphological analysis**, measured *post-mortem* in stained lung tissue sections, using a scoring system validated for experimental acute lung injury in animals; and 9) **inflammation markers**, assessed through quantification of protein and cell content in the blood plasma, broncho-alveolar lavage fluid and frozen lung tissue.

Detailed information on the methodology for each aforementioned technique are provided in the Appendix of this thesis.

The research protocol for each experimental condition is graphically represented below.

The effects of PVV were assessed in experimental models of:

Healthy lungs

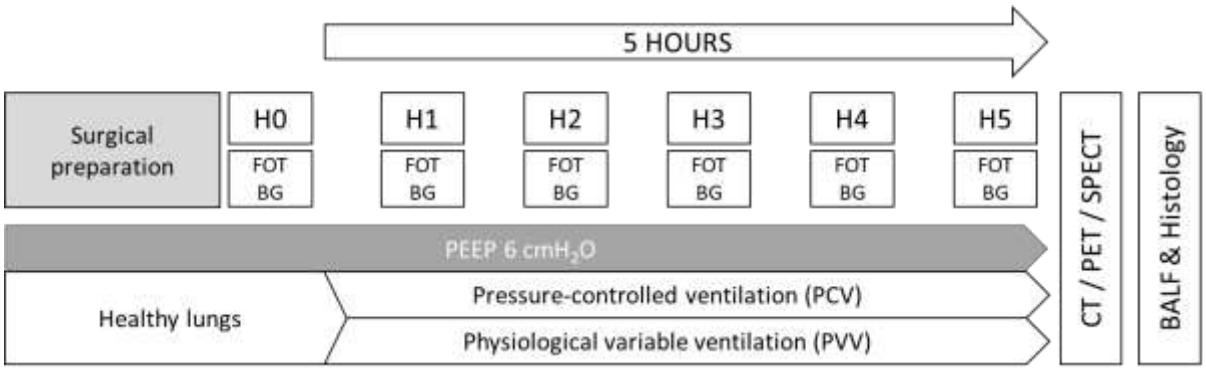

Figure 7. Schematic representation of the experimental protocol in healthy lungs. H0–H5: measurement at hour zero to hour five; FOT: forced oscillation technique (impedance of respiratory system); BG: blood gas; PEEP: positive end-expiratory pressure; CT: computed tomography; PET: positron-emission tomography; SPECT: single-photon emission computed tomography; BALF: bronchoalveolar lavage fluid.

Acute Respiratory Distress Syndrome

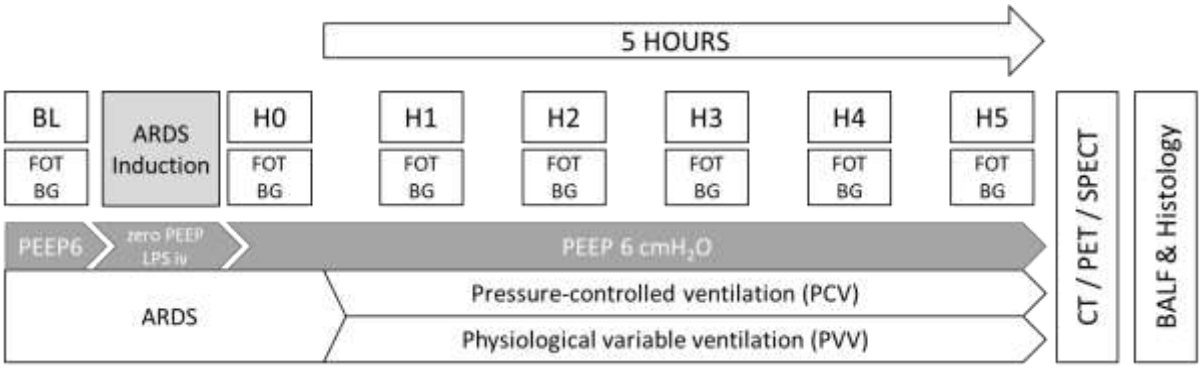

Figure 8. Schematic representation of the experimental protocol in ARDS. BL: baseline; H0–H5: measurement at hour zero to hour five; FOT: forced oscillation technique (impedance of respiratory system); BG: blood gas; PEEP: positive end-expiratory pressure; LPS iv: lipopolysaccharide intravenous; CT: computed tomography; PET: positron-emission tomography; SPECT: single-photon emission computed tomography; BALF: bronchoalveolar lavage fluid.

Asthma

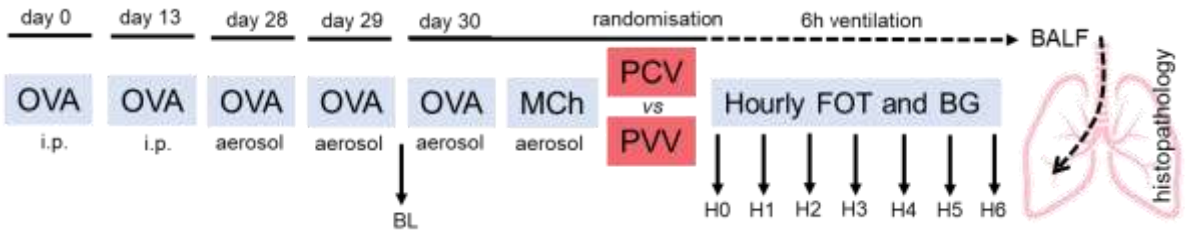

Figure 9. Schematic representation of the experimental protocol in Asthma. i.p.: intraperitoneal; OVA: ovalbumin; MCh: methacholine; PCV: pressure-controlled ventilation; PVV: physiologically variable ventilation; FOT: forced oscillation technique; BG: blood gas; BALF: bronchoalveolar lavage fluid; BL: baseline; H0 to H6: measurement time points at hour zero to hour six.

Chronic Obstructive Pulmonary Disease

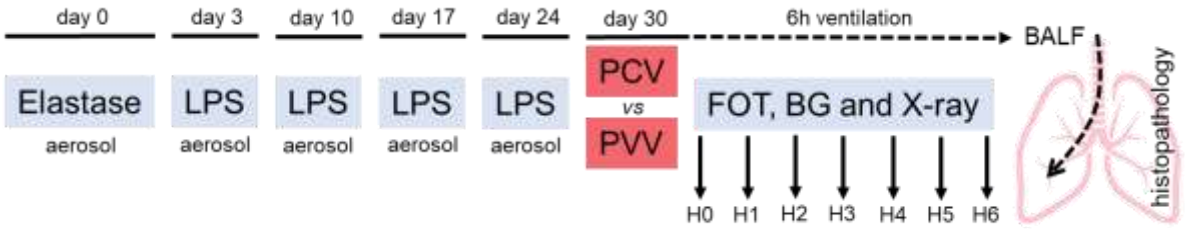

Figure 10. Schematic representation of the experimental protocol in COPD. LPS: lipopolysaccharide PCV: pressure-controlled ventilation; PVV: physiologically variable ventilation; FOT: forced oscillation technique; BG: blood gas; BALF: bronchoalveolar lavage fluid; H0 to H6: measurement time points at hour zero to hour six.

RESULTS

This section describes the main findings regarding the primary and secondary outcomes of the experimental protocols. Further details on the experimental results, including ancillary and supplemental data are presented in the three full-text publications that are provided in the Appendix of this thesis.

1. Benefits of PVV in healthy lungs and ARDS

PRIMARY OUTCOMES:

- **Respiratory mechanics**

Prolonged ventilation (5 hours) with PCV worsened respiratory mechanics both in healthy and ARDS models; however, this trend was improved by PVV (Figure 11). Applying PCV for 5 h led to significant increases in tissue elastance (T1–T5, $p < 0.01$) in the control animals and in Raw in the ARDS model (T1–T5, $p < 0.03$). Conversely, ventilating the lungs with PVV resulted in a significant decrease in tissue damping in control animals (T1–T5, $p < 0.01$), whereas no change in respiratory mechanics was detected in the ARDS model. The comparison of the two ventilation modes revealed significantly lower relative changes with PVV in tissue damping for the control animals (T4-T5, $p < 0.03$) and tissue

elastance for the ARDS model (T1-T5, $p < 0.01$), parameters that reflect a reduced lung volume loss and stiffening of the lung tissue in the PVV groups.

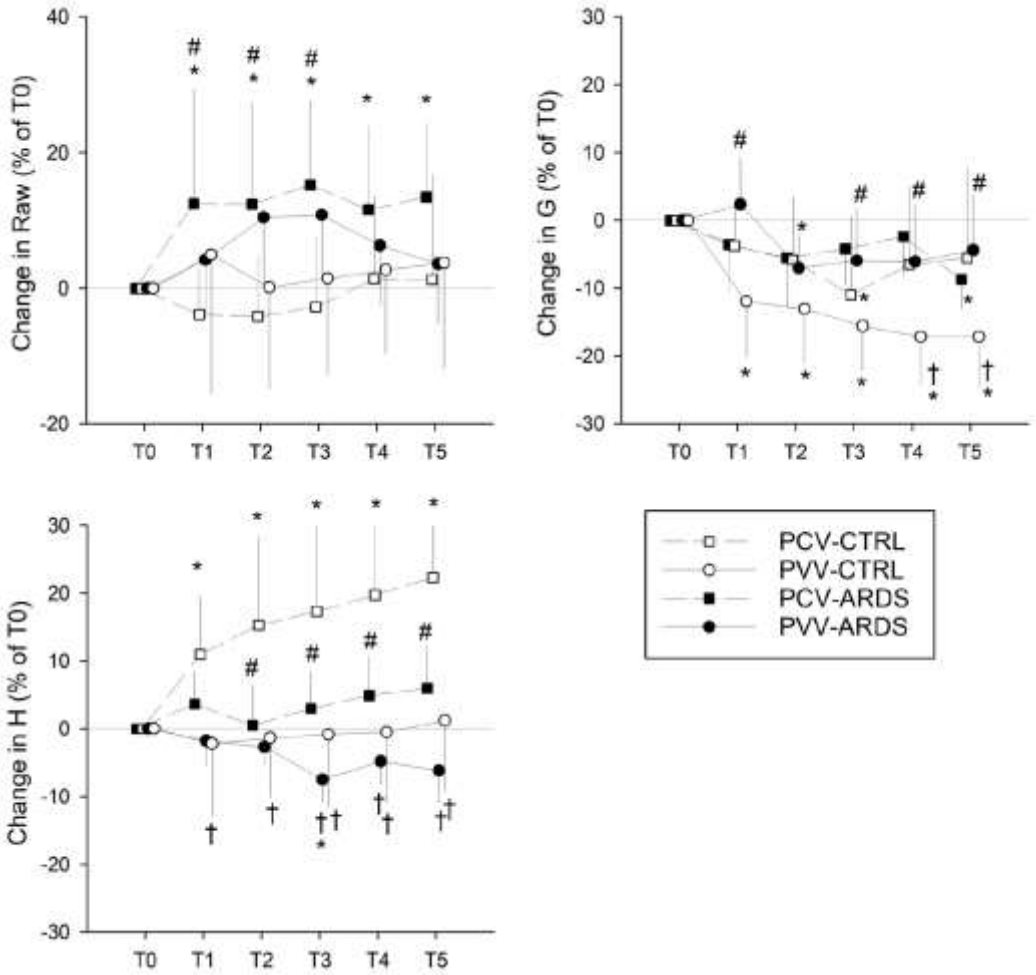

Figure 11. Changes in respiratory mechanical parameters relative to those obtained immediately after induction of lung injury (T0). Values expressed as mean ± half-width of 95% confidence interval. Raw: airway resistance; G: respiratory tissue damping; H: respiratory tissue elastance, T0: immediately after induction of lung injury; T1–T5: time points at the end of the corresponding hour of the 5-h long ventilation period; PCV: pressure-controlled ventilation; PVV: physiological variable ventilation; ARDS: presence of lung injury; CTRL: absence of lung injury. *$p < 0.05$ vs. T0, #$p < 0.05$ vs. CTRL, †$p < 0.05$ vs. PCV

- **Gas exchange**

The hourly measurements of blood gas parameters are represented in Figure 12. The induction of ARDS led to significant impairment of the blood oxygenation index (PaO_2/FiO_2), resulting in a mild to moderate ARDS, according to the Berlin definition (136). There was no evidence for a statistical difference in the oxygenation ratio between PCV and PVV in the ARDS model after 5 hours of ventilation.

Applying variable ventilation for 5 h in an animal model with healthy lungs (CTRL) had no systematic effect on gas exchange, whereas higher $PaCO_2$ levels (T1–T5, $p < 0.05$) were observed in animals with ARDS (T1–T5, $p < 0.01$).

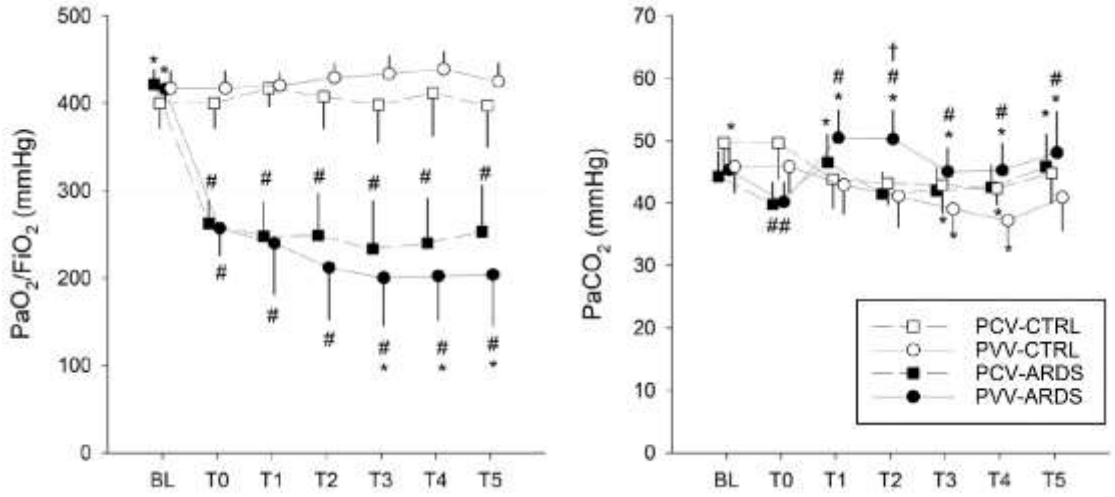

Figure 12. Blood gas parameters obtained before and during the 5-h ventilation period. Values expressed as mean ± half-width of 95% confidence interval. PaO_2: partial pressure of arterial oxygen concentration; FiO_2: fraction of inspired oxygen; $PaCO_2$: partial pressure of arterial carbon dioxide concentration; BL: baseline; T0: immediately after induction of lung injury; T1–T5: time points at the end of the corresponding hour of the 5-h long ventilation period; PCV: pressure-controlled ventilation; PVV: physiological variable ventilation; ARDS: presence of lung injury; CTRL: absence of lung injury. *$p < 0.05$ vs. T0, #$p < 0.05$ vs. CTRL, †$p < 0.05$ vs. PCV

- **Lung imaging**

Representative CT, PET and SPECT images with the corresponding regional aeration maps in control and ARDS conditions are shown in Figure 13. More heterogeneous lung structure, as indicated by heterogeneous regional distribution of 18^F-FDG uptake and ^{99m}Tc-labeled albumin macroaggregates, was observed in the presence of ARDS. The PET uptake values calculated for the total lung and at regional levels are summarized for the study groups in the left panels of Figure 14. When averaging the entire lung, significantly lower mean ^{18}F-FDG uptake was evidenced for the lungs in the animals ventilated with PVV, regardless of the presence of lung injury. This difference was also detected at the regional level in rabbits with healthy lungs ventilated with PVV ($p < 0.04$). Characterizing the differences in ^{18}F-FDG uptake among the various aeration zones, defined by CT density, revealed the highest

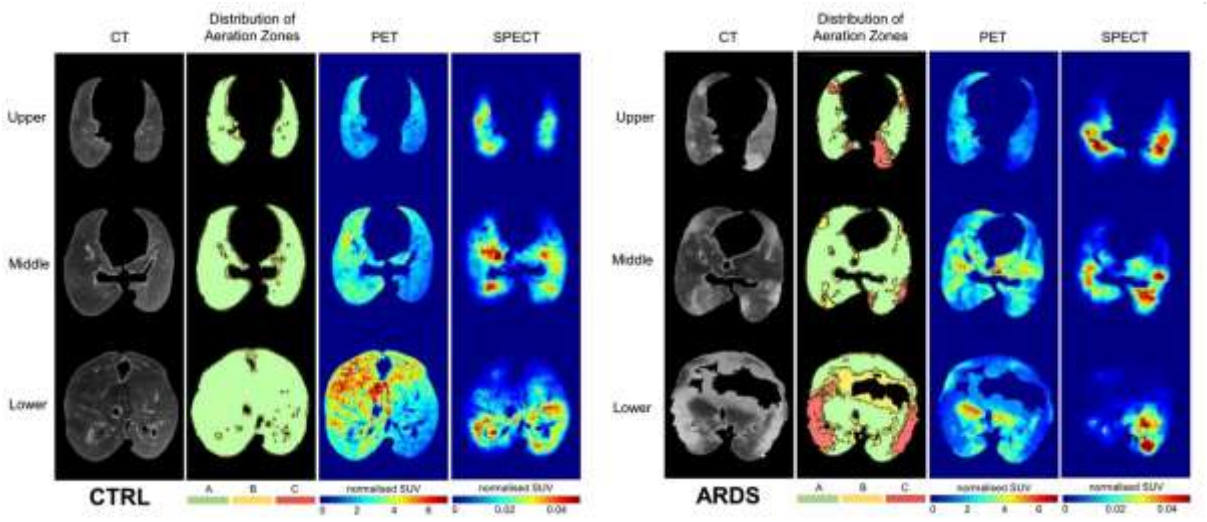

Figure 13. Representative images of CT, aeration maps, PET and SPECT (from left to right) in upper, middle and lower sections of the lung (from top to bottom) in the different experimental groups (CTRL and ARDS). Aeration zones A, B and C represent the well, poorly and non-aerated lung zones, respectively. PET and SPECT heating maps are represented in SUV normalized for tissue fraction for fludeoxyglucose and 99mTc-labeled albumin macroaggregates, respectively.

activity in the well aerated zones, with 2 to 3-fold differences compared to the non-aerated zones ($p < 0.01$, well aerated vs. poorly aerated or non-aerated). Likewise, ventral (non-dependent) regions presented significantly higher ^{18}F-FDG uptake compared to dorsal (dependent) regions in both ventilation modes. Furthermore, significantly decreased mean ^{18}F-FDG uptake was observed in the control animals ventilated with PVV compared to those with PCV ($p < 0.01$). No evidence for a difference in SPECT activity was detected between the protocol groups (Figure 14, right panels). However, regional perfusion was significantly and consistently higher in the well aerated zones and the dorsal zones of the lung, without differences between the experimental groups.

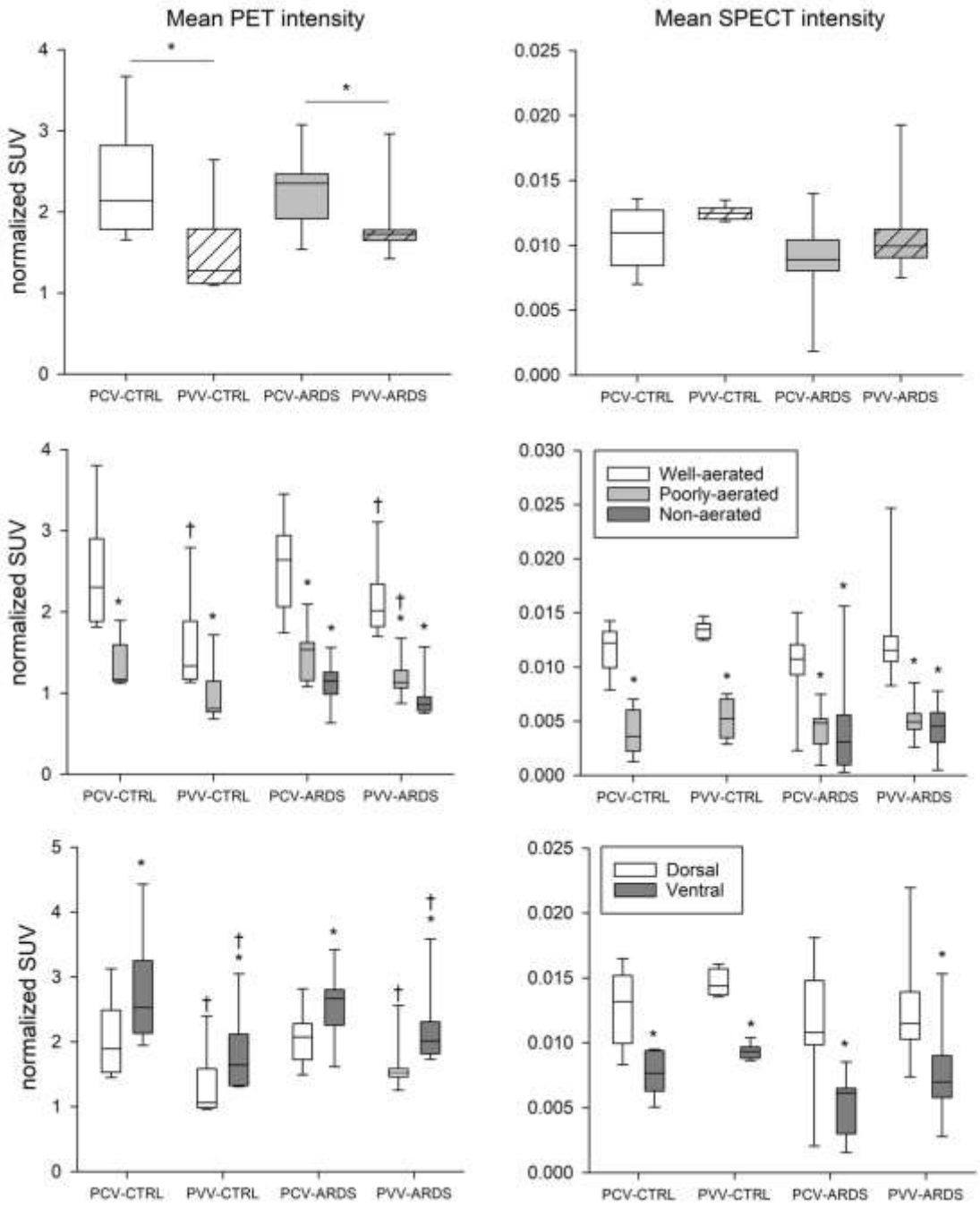

Figure 14. Left panels depict pulmonary inflammation characterized by PET imaging normalized to the tissue fraction. Right panels show pulmonary circulation characterized by SPECT imaging, normalized to tissue fraction. Upper panels represent mean PET and SPECT intensities averaged for the entire lung. Middle panels demonstrate the regional distribution based on aeration zones. Bottom panels represent the regional distribution based on the dependent (dorsal) and non-dependent (ventral) zones. SUV: standardized uptake value; PCV: pressure-controlled ventilation; PVV: physiological variable ventilation; ARDS: presence of lung injury; CTRL: absence of lung injury. *p < 0.05 vs. well-aerated or vs. dorsal, †p < 0.05 vs. PCV

SECONDARY OUTCOMES:

The detailed results on ventilation and hemodynamic parameters, cytokine levels and lung injury histological indices can be found on the Appendix of this thesis.

In the presence of ARDS, significantly higher driving pressure was required to maintain the same minute ventilation than in healthy animals ($p < 0.01$, ARDS vs. CTRL, Figure 15). In healthy animals, a progressive reduction in driving pressure was observed with PVV ($p < 0.01$ vs. T0, Figure 15), which was not observed in animals ventilated with PCV.

No differences were detected between the two ventilation modes in regards of the hemodynamic parameters, lung injury score, cytokine and cell content of the BALF.

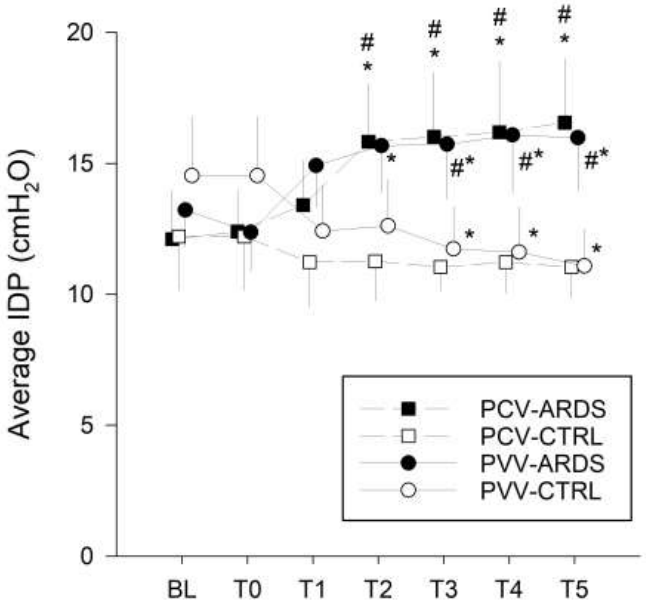

Figure 15. Inspiratory driving pressure (IDP) obtained before and during the 5-hour ventilation period. Values expressed as mean ± half-width of 95% confidence interval., BL: baseline, T0: immediately after induction of lung injury, T1-T5: average value during the corresponding hour of the 5-hour long ventilation period. PCV: pressure-controlled ventilation, PVV: physiological variable ventilation, ARDS: presence of lung injury, CTRL: absence of lung injury.
*: $p < 0.05$ vs. T0, #: $p < 0.05$ vs. CTRL, †: $p < 0.05$ vs. PCV.

2. Benefits of PVV in Asthma

PRIMARY OUTCOMES:

- **Gas exchange**

At the study baseline, PaO_2/FiO_2 levels were in the physiological range (Figure 16). Inducing bronchoconstriction led to comparable decreases in PaO_2/FiO_2 in both experimental groups (H0). The ventilation mode significantly affected oxygenation, with higher PaO_2/FiO_2 at H5 and H6 in the PVV group (p=0.018). No differences between PCV and PVV groups were observed after 6 h of ventilation in arterial pH (7.42 ± 0.02 *vs* 7.43 ± 0.03, respectively) or $PaCO_2$ (5.7 ± 0.3 kPa vs 5.6 ± 0.4 kPa, respectively). Values are expressed as mean ± half-width of 95% confidence interval.

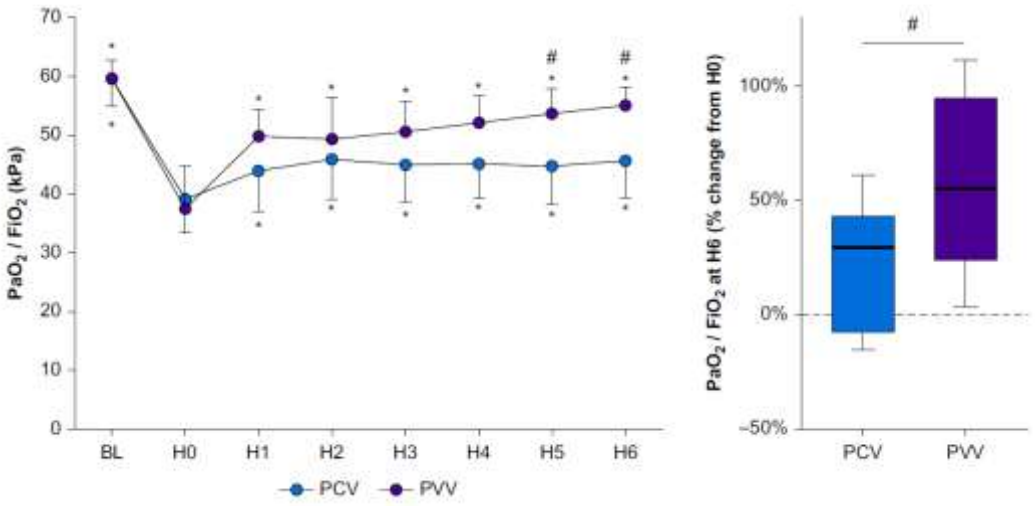

Figure 16. Absolute values of oxygenation index (left, PaO_2/FiO_2) measured at baseline (BL), immediately after induction of bronchoconstriction by ovalbumin and methacholine (H0) and during 6 h of ventilation (H1-H6). Results from physiologically variable ventilation (PVV; n=12) and pressure-controlled ventilation (PCV; n=10) are represented with filled and empty symbols, respectively. Values are expressed as mean (half-width of 95% confidence interval). Relative changes compared with H0 after the application of 6 h PCV or PVV are reported on the right graph. FiO_2, fraction of inspired oxygen; PaO_2, arterial partial pressure of oxygen. *p<0.05 *vs* H0; #p<0.05 *vs* PCV.

- **Respiratory mechanics**

The mechanical parameters of the respiratory system are summarized in Figure 17. The application of PVV decreased respiratory tissue elastance (p<0.001) throughout the 6 hours of ventilation, whereas PCV further increased the elastance (p=0.011; Figure 17, lower panel). Ovalbumin and methacholine nebulisations approximately doubled the R_{aw} and induced increases in G (Fig. 4). R_{aw} remained elevated in both experimental groups throughout the 6-h ventilation, independent of the ventilatory mode. In comparison with H0, PCV did not alter G whereas PVV reduced G (p=0.002) throughout the 6-h of ventilation.

SECONDARY OUTCOMES:

- **Airway driving pressure**

Nebulisation of ovalbumin and methacholine increased $P_{driving}$ similarly in each group (Figure 18). From H2 to H6, animals ventilated with PVV received lower $P_{driving}$ than those ventilated with PCV (p=0.045 and p=0.002 for H2 and H6, respectively). The continuous monitoring of expiratory pressure and flow revealed no evidence for intrinsic PEEP or air trapping during the study period.

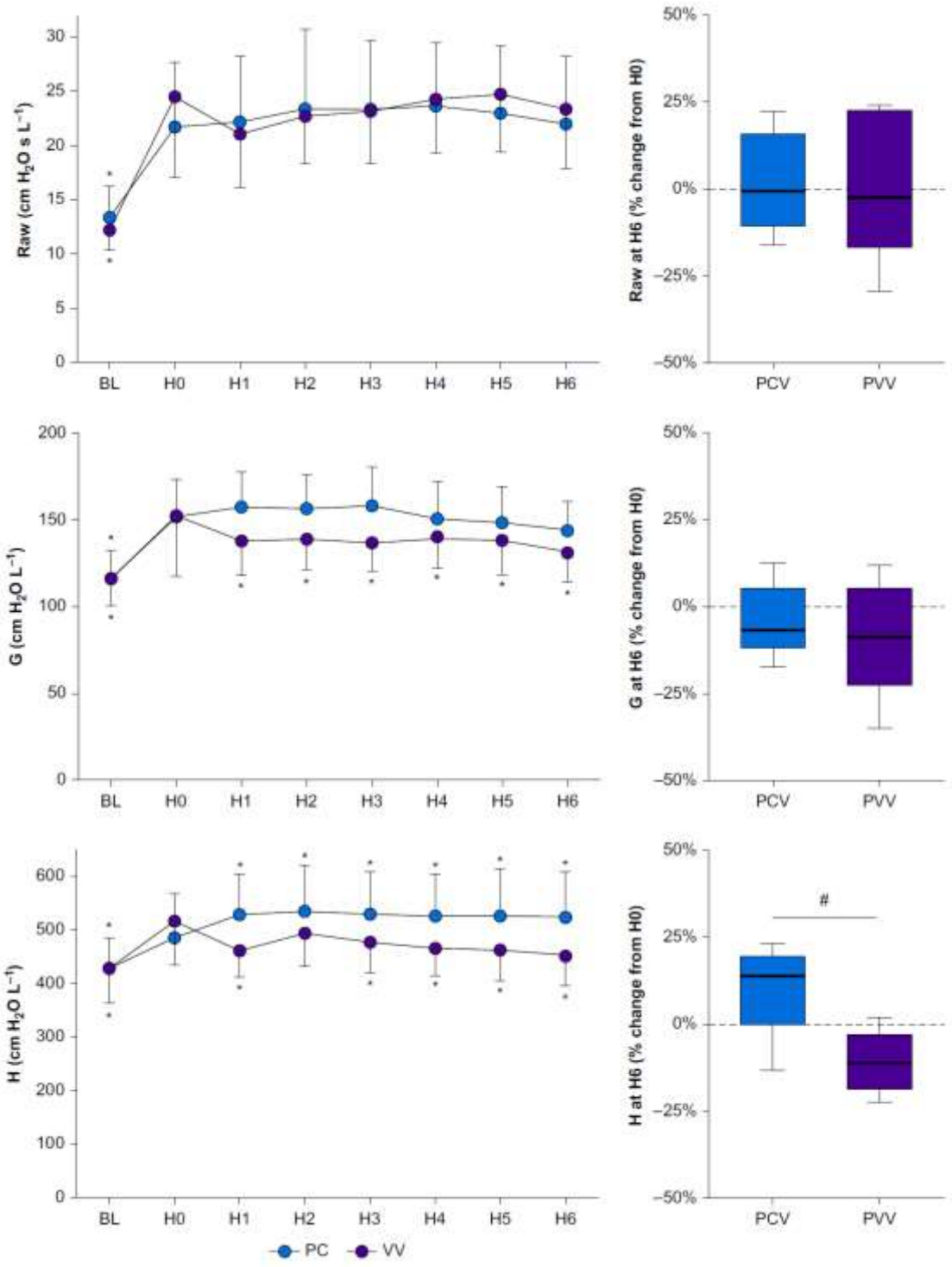

Figure 17. Absolute values of respiratory mechanical parameters (left) at baseline (BL) in rabbits, after bronchoconstriction with ovalbumin and methacholine (H0) and during 6 h of ventilation (H1-H6) with physiologically variable ventilation (PVV; n=12) or pressure-controlled ventilation (PCV; n=10). In the right panels, relative changes after 6 h of ventilation compared with those immediately after induction of bronchoconstriction (H0). Values are expressed as mean (half-width of 95% confidence interval). R_{aw}, airway resistance; G, respiratory tissue damping; H, respiratory tissue elastance. *p<0.05 vs H0; #p<0.05 vs PCV.

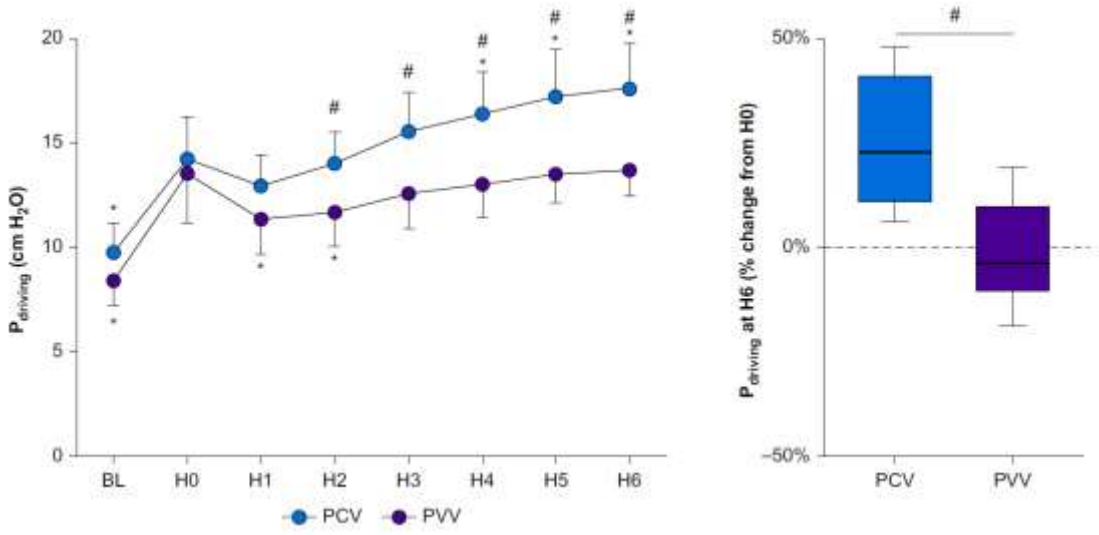

Figure 18. Absolute values of driving pressure (left, $P_{driving}$) measured at baseline (BL), immediately after induction of bronchoconstriction by ovalbumin and methacholine (H0) and during 6 h of ventilation (H1-H6). Results from physiologically variable ventilation (PVV; n=12) and pressure-controlled ventilation (PCV; n=10) are represented with filled and empty symbols, respectively. Values are expressed as mean (half-width of 95% confidence interval). Relative changes of $P_{driving}$ compared with H0 after the application of 6 h PCV or PVV (right). *p<0.05 vs H0; #p<0.05 vs PCV.

- **Lung injury**

Rabbits receiving PVV had less lung injury, as adjudged by histological score compared with rabbits receiving PCV (Figure 19; p=0.003). The total protein (p=0.035) and cell count in BALF (p=0.005) were also lower in animals ventilated with PVV (Figure 19). Total and differential WBC counts in the blood were similar between the ventilation modes throughout the experimental protocol (see Appendix). Levels of inflammatory cytokines, surfactant proteins and E-cadherin were similar in each experimental group (see Appendix).

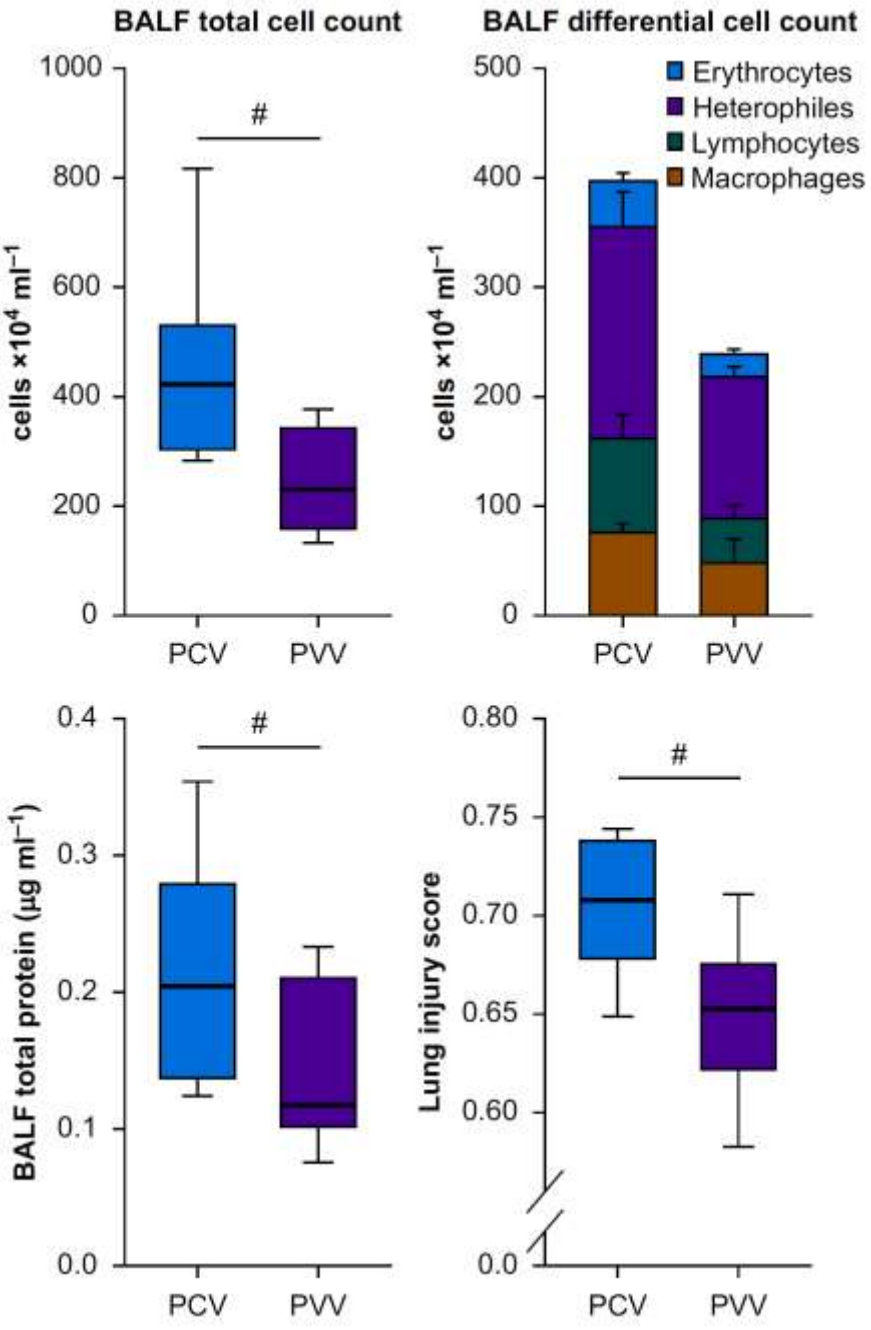

Figure 19. Total and differential cell counts (upper left and right, respectively) and total protein concentration (bottom left) in the bronchoalveolar lavage fluid (BALF) after 6 h of mechanical ventilation with physiologically variable ventilation (PVV; n=12) or pressure-controlled ventilation (PCV; n=10). On the bottom right, lung injury score assessed in the lung histological sections after 6 h application of PVV or PCV. #p<0.05.

3. Benefits of PVV in COPD

PRIMARY OUTCOMES:

- **Gas exchange**

Changes in PaO_2/FiO_2 and $PaCO_2$ during the study protocol are summarized in Figure 20. Despite COPD induction, O_2 and CO_2 levels at H0 were in the physiological range in both groups. While PaO_2/FiO_2 remained constant throughout the 6-h ventilation with PVV, it progressively decreased under PCV, and this decrease became significant after 2 h of ventilation ($p < 0.001$). Subsequently, animals in the PVV group exhibited significantly higher PaO_2/FiO_2 in the second half of the ventilation period ($p < 0.001$). Despite a lack of difference in respiratory rate between the study groups (Figure 22), the animals in the PCV group presented a significantly higher $PaCO_2$ after 6 h of mechanical ventilation than those in the PVV group ($p < 0.001$).

- **Respiratory mechanics**

All mechanical parameters (R_{aw}, I_{aw}, G, and H) progressively increased after 6 h of mechanical ventilation, irrespective of the ventilation mode (Figure 21). Nevertheless, increases in H were significantly lower after 6 h of mechanical ventilation with PVV compared to PCV ($p=0.002$). Moreover, there was a significant correlation between H and oxygenation index during the 6 h of ventilation ($r = –0.47$, $p < 0.001$, see Appendix).

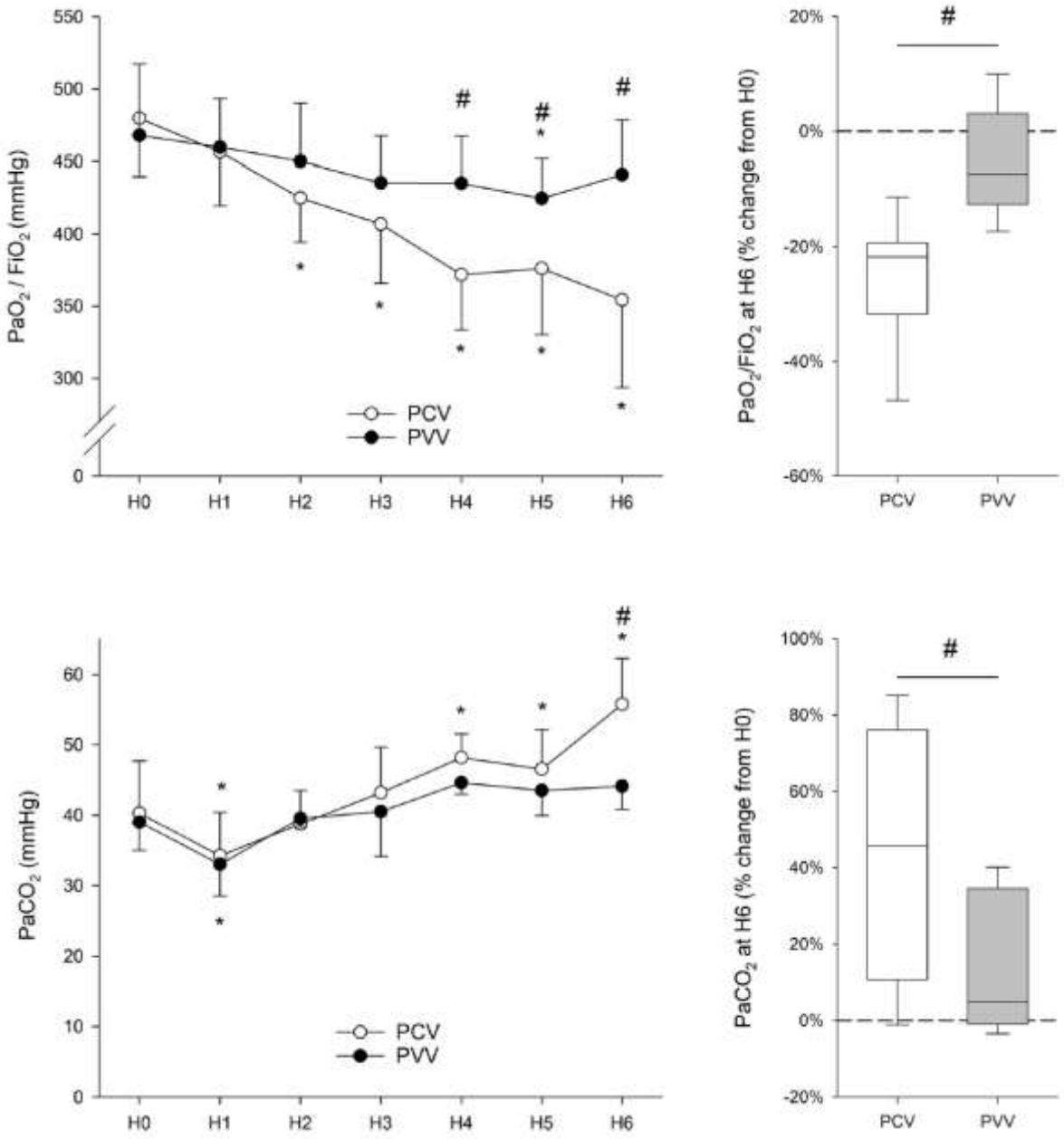

Figure 20. Gas exchange parameters expressed as oxygenation index (PaO_2/FiO_2) and partial pressure of CO_2 in the arterial blood ($PaCO_2$), measured at the onset (H0) and throughout the 6 h of ventilation (H1 to H6). Results from physiologically variable ventilation (PVV, filled circles) and pressure-controlled ventilation (PCV, empty circles) are expressed as mean ± standard deviation. Relative changes compared to H0 after the application of 6-h PCV (white box) or PVV (gray box) are reported on the right panels. PaO_2, arterial partial pressure of oxygen; FiO_2, fraction of inspired oxygen. *p < 0.05 versus H0; #p < 0.05 versus PCV.

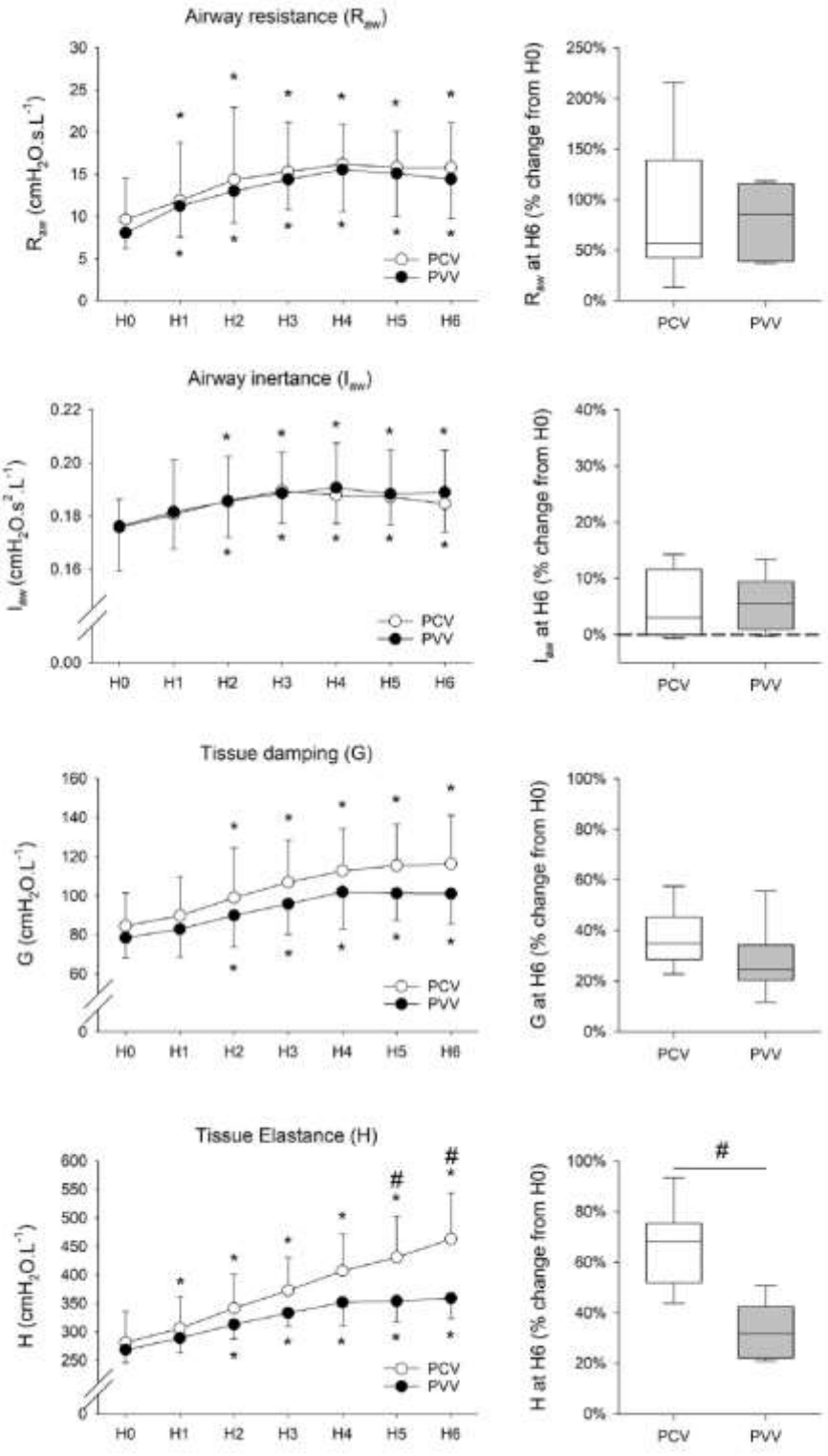

Figure 21. Respiratory mechanical parameters (left panels) in rabbits at the onset (H0) and during 6 h of ventilation (H1 to H6) receiving physiologically variable ventilation (PVV, filled circles) or pressure-controlled ventilation (PCV, empty circles). Values are expressed as mean ± standard deviation. Relative changes compared to H0 after the application of 6-h PCV (white box) or PVV (grey box) are reported in the panels on the right. R_{aw}, airway resistance; I_{aw}, airway inertance; G, respiratory tissue damping; H, respiratory tissue elastance. *p < 0.05 versus H0; #p < 0.05 versus PCV.

SECONDARY OUTCOMES:

- **Ventilation parameters**

Mean inspiratory pressure, PEEP, driving pressure, and FiO2 were maintained unchanged during the 6-h ventilation with no difference in these parameters between the study groups. The ventilatory parameters are summarized in Figure 22.

At the onset of the study (H0), PCV and PVV animals were ventilated with identical mean VT [6.90 ± 0.78 (mean ± SD) versus 6.67 ± 0.44 mL/kg in PCV and PVV, respectively]. Despite a constant $P_{driving}$ throughout the 6-h ventilation, there was a significant and progressive reduction in mean VT in both experimental groups, starting from H1 in the PCV group ($p < 0.001$) and from H2 in the PVV group ($p < 0.001$). Notably, after 6 h of ventilation, mean VT was significantly lower in animals ventilated with PCV ($p < 0.05$). To target normocapnia, a significant increase in RR was necessary in both experimental groups, in comparison to H0, with no evidence for a statistical difference in RR between the experimental groups.

- **Lung aeration and shunt fraction**

The relative change in lung aeration area during the 6-h ventilation period is represented in Figure 23. While lung aeration remained unchanged during the study period in the PVV group, a significant and progressive deterioration in lung aeration appeared from the first hour of ventilation in the PCV group ($p=0.02$). This decrease in lung aeration area became significant between the two groups at H6 ($p=0.007$). No difference in the intrapulmonary shunt fraction (Qs/Qt) was observed at H0 between the protocol groups; however, Qs/Qt was significantly elevated at H6 only in animals ventilated with PCV ($p<0.001$). This increase resulted in significantly higher Qs/Qt in PCV compared to PVV at H6 ($p=0.002$).

- **Pathological findings**

Histological, cellular, and protein assessment results are summarized in Figure 24. Although no difference in the WBC count at H0 was observed between groups, a significant increase was detected at H6 in the PCV group ($p=0.04$). In addition, there was a tendency for a significant increase in the total cell count in the BALF in the PCV group in comparison to PVV ($p=0.051$). Moreover, the differential cell counts in the BALF revealed a significantly higher number of lymphocytes in the PCV group ($p=0.02$). Proteins and cytokine concentrations in the BALF and in the lung tissue revealed no significant difference

between the two groups (see Appendix). After application of PCV and PVV, there was no evidence for a difference in the overall histological injury score (Figure 24-D).

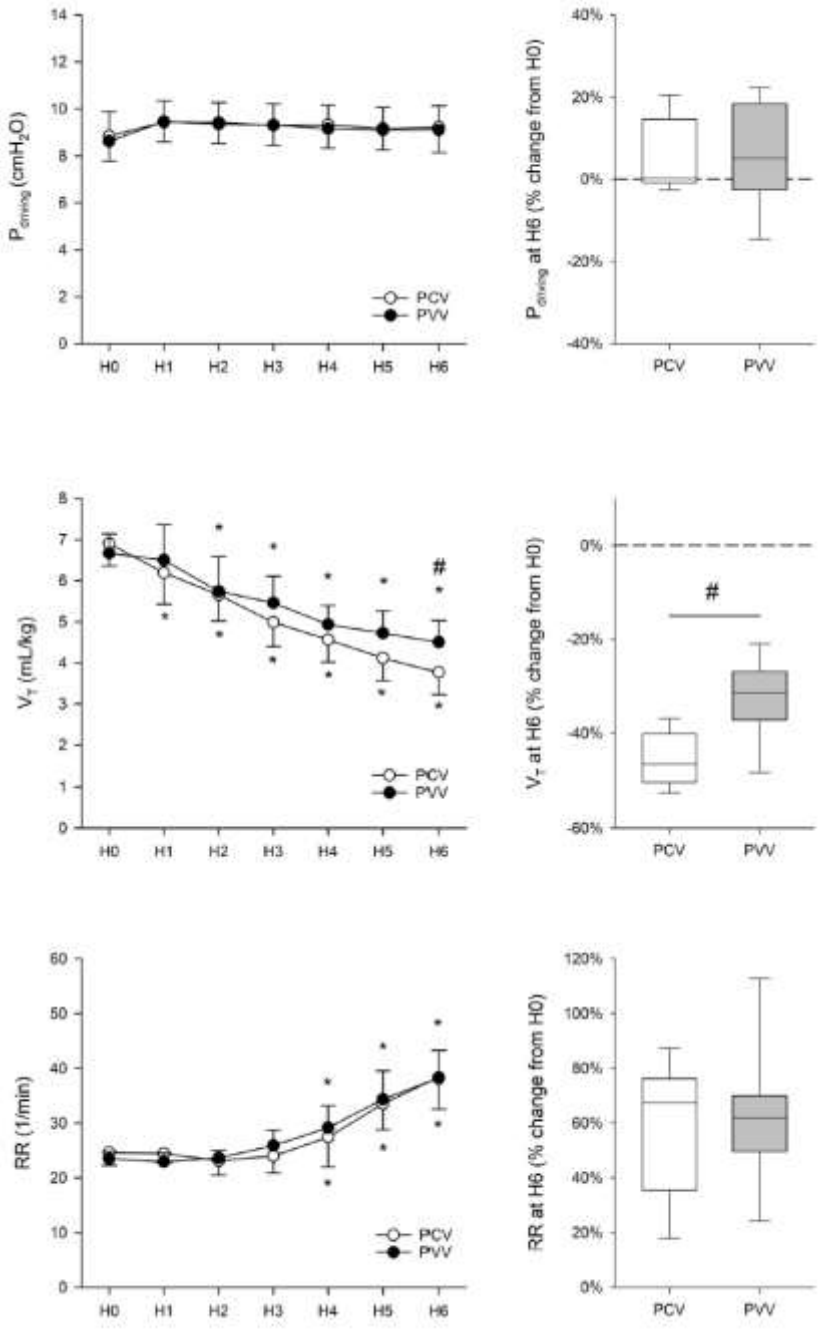

Figure 22. Driving pressure ($P_{driving}$), tidal volume (V_T), and respiratory rate (RR), measured at the onset (H0) and throughout the 6 h of ventilation (H1 to H6). Results from physiologically variable ventilation (PVV, filled circles) and pressure-controlled ventilation (PCV, empty circles) are expressed as mean ± standard deviation. Relative changes compared to H0 after the application of 6-h PCV (white box) or PVV (grey box) are reported on the right panels. *p < 0.05 versus H0; #p < 0.05 versus PCV.

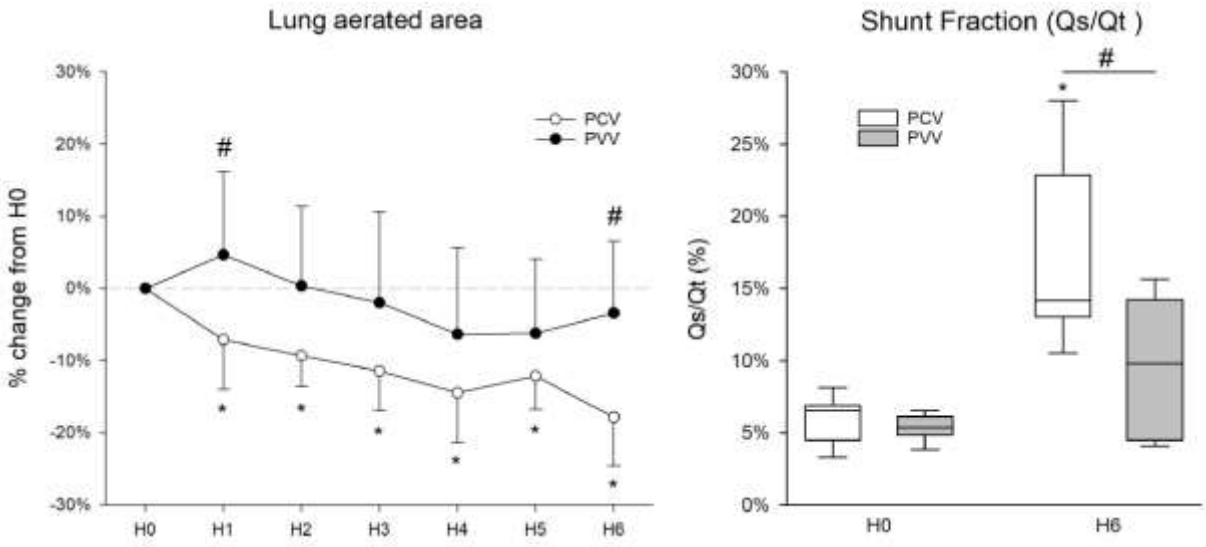

Figure 23. Percentage change of lung aerated area (left graph) obtained from chest X-ray imaging during the 6 h of ventilation (H1 to H6) relative to the lung aerated area at the ventilation onset (H0). Values are expressed as mean ± standard deviation. On the right, shunt fraction (Qs/Qt, in percentage) before and after 6 h of mechanical ventilation (H0 and H6, respectively). Results from physiologically variable ventilation (PVV) and pressure-controlled ventilation (PCV) are represented with filled and empty circles/boxes, respectively. *p < 0.05 versus H0; #p < 0.05 versus PCV.

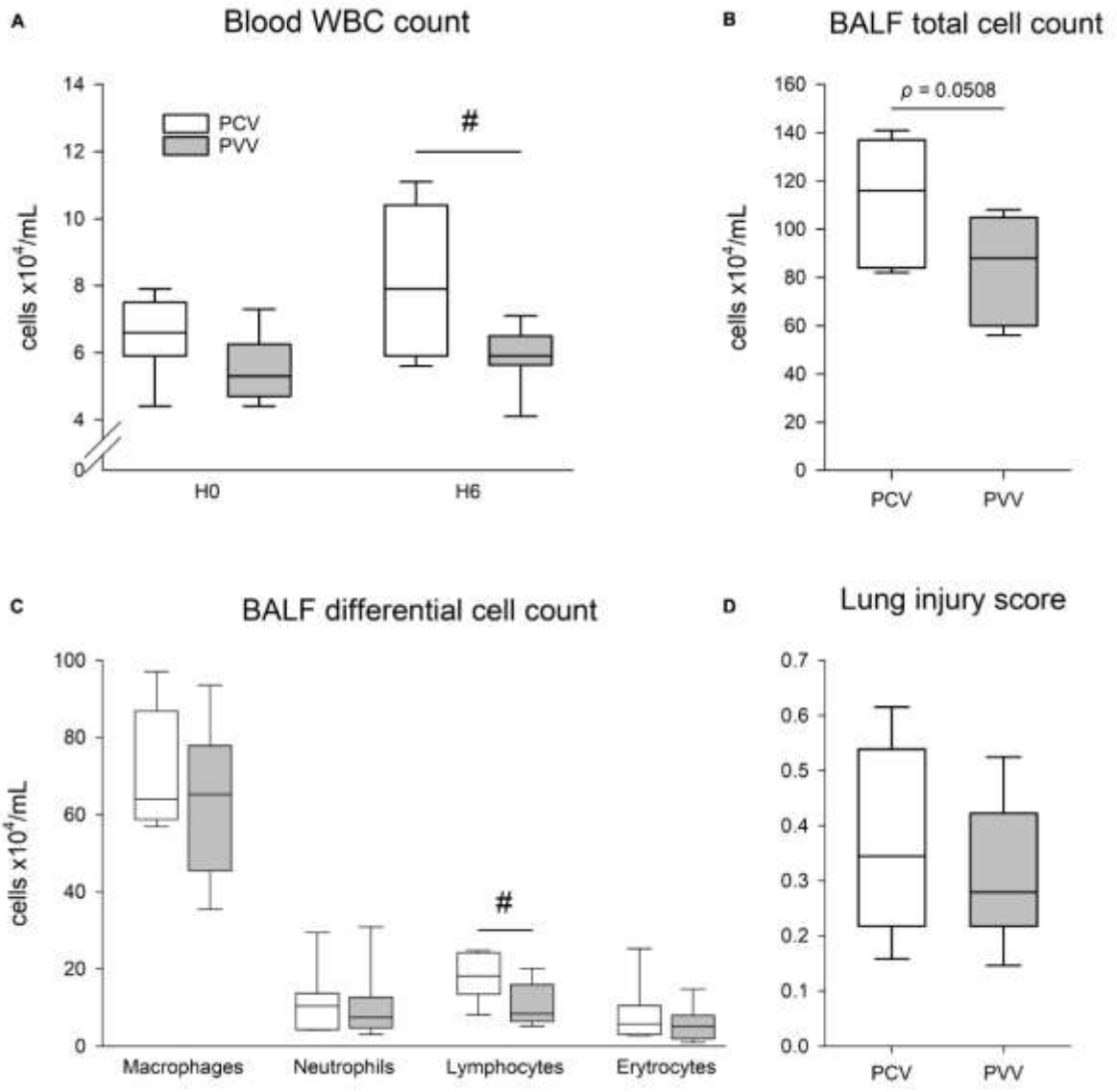

Figure 24. (**A**) Total white blood cell (WBC) count in the blood at the onset (H0) and at the end of the 6-h period of mechanical ventilation (H6) with pressure-controlled (PCV, white) or physiologically variable modes (PVV, grey). (**B, C**) Total and differential cell counts in the bronchoalveolar lavage fluid (BALF) after 6 h of mechanical ventilation with PCV and PVV. (**D**) Lung injury score (range: 0–1) assessed in the lung histological sections after application of 6 h of PVV or PCV. Data are represented as median and quartiles #p < 0.05 versus PCV.

GENERAL DISCUSSION

The potential effects of variable ventilation have been reported in the scientific literature since the late 90's. Experiments in acute lung injury have provided experimental evidence on the benefits of this variable mode in several aspects of respiratory function. Moreover, in 2012, an editorial was published in the *Critical Care Medicine* journal which strongly advocated for the use of variable ventilation. To quote: "Adding noise to mechanical ventilation: so obvious!" (137). Nevertheless, as of today, this 'so obvious' beneficial modality is still not available for clinical practice. More than 20 years passed since the first experiments on variable ventilation (82), however, the evidence supporting the mechanisms and outcomes of this ventilatory mode are still conflicting to some extent. Therefore, the aim of this research work was to thoroughly characterise the benefits of variable ventilation in both healthy and diseased lungs and, ultimately, facilitate the translation of this mode of ventilation from the bench to the bedside.

The current research work compared the use of conventional (monotonous) mechanical ventilation and physiologically variable ventilation (PVV, emulating the variability of spontaneous breathing). In comparison to pressure-controlled ventilation, the application of physiologically variable ventilation improved respiratory function, reflected in better gas exchange, respiratory mechanics, lung aeration and tissue injury/inflammation. These benefits of variable over conventional ventilation were observed in experimental models of healthy, acutely- and chronically-injured lungs alike. Our data suggests that the application of variability during mandatory mechanical ventilation is capable of preventing derecruitment and lung function deterioration during prolonged ventilation.

Benefits of PVV in gas exchange

One of the main respiratory outcomes that was observed with PVV was a benefit in terms of gas exchange, namely a better oxygenation ratio (PaO_2/FiO_2). This benefit was very significant in the experimental setting of asthma and COPD but not apparent in the context of ARDS and healthy lungs.

In healthy-lung animals, PaO_2/FiO_2 was similar between groups PCV and PVV and was within the physiological range (i.e. about 400mmHg). In the setting of ARDS, benefits in oxygenation have been described in previous reports (82, 92, 95, 97, 99, 114, 117, 120, 123). In our ARDS model, a severe degree of hypoxemia and V/Q mismatch required high FiO_2 (>60%) and provoked persistent hyperlactataemia (~4 mmol/L) and acidemia (~pH 7.30), suggesting inadequate tissue oxygen delivery. We may hypothesise that this setting

of profound heterogeneity of V/Q distribution may have precluded the PVV pattern to overcome PCV in terms of oxygenation. Differently, in the models of asthma and COPD, the oxygenation was significantly improved by the PVV pattern in comparison to conventional ventilation. In such models, the benefits of the PVV pattern in gas exchange became clearly manifest, likely because the tissue injury and V/Q distribution is less heterogeneous than in ARDS.

Most available data from previous studies demonstrate an improvement in gas exchange during variable ventilation (improved oxygenation, improved CO_2 clearance or both) in comparison to conventional ventilation (69, 82-85, 88, 89, 92, 95, 96, 99, 101, 103, 105-109, 114, 117-120, 123, 124). Additionally, a great number of studies found a reduced venous admixture (intrapulmonary shunt fraction) and a better redistribution of the pulmonary blood flow when comparing the variable ventilation with a conventional mode (86, 88, 92, 96, 97, 105-107, 109, 113, 117, 118, 120). However, a certain number of studies did not verify improved gas exchange with variable ventilation in both healthy and diseased lungs (87, 90, 91, 93, 97, 98, 100, 102, 110, 116, 122, 125, 128). Importantly, no study has observed detrimental effects of variable ventilation on both oxygenation and CO_2 clearance in comparison to conventional ventilation.

Benefits of PVV in respiratory mechanics and ventilatory pressures

The respiratory mechanics were another primary endpoint of the present series of research works. In all the diseases that were studied, we observed a significant improvement of lung elastance after prolonged application of PVV in comparison to PCV. The deterioration of ventilatory parameters was also significantly reduced by PVV: 1) in healthy lungs and asthma, PVV permitted a lower $P_{driving}$, in comparison to PCV; 2) in COPD, for the same $P_{driving,}$ a higher V_T and lung aeration was observed. Globally, all the aforementioned benefits can be explained by a protective effect of PVV on the lung volume loss and derecruitment that is associated with prolonged mechanical ventilation.

From the available literature on variable ventilation, the vast majority of the experimental works reported an improvement in ventilatory and/or mechanical parameters with the application of the variable mode. For equivalent values of MV and mean V_T, the variable pattern promoted lower mean/peak airway pressures and/or better elastance / compliance (69, 82, 83, 85-90, 92, 95-97, 99-103, 105-109, 111, 113, 114, 116-118, 123-125, 127, 134). There were also reports of reduced dead space (89, 103, 105), better regional aeration (97, 113) and less alveolar collapse and tissue heterogeneity (86, 100, 116). However, some studies did not observe significant differences in mechanical outcomes

(84, 91, 93, 110, 122, 128). Once again, it is noteworthy that no study has described detrimental mechanical effects in comparison to conventional modes.

Benefits of PVV in lung pathology and inflammation

In both healthy and ARDS lungs, we observed that PVV significantly reduced inflammation (as assessed by lung normalized ^{18}F-fluorodeoxyglucose uptake). In an asthma model, we observed a significantly lower histological injury score, lower cell count and protein concentration in the BALF after the application of PVV. In a model of COPD, the plasma leucocytes were increased after the application of PCV but not with PVV. There was no evidence for a difference in the histological analysis in animals with healthy lungs, ARDS or COPD. Likewise, in all the diseases that were studied, there was no significant difference between the ventilation modes for any cytokine or surfactant protein (TNF-α, IL-6, IL- 8, SP-B, SP-D, E-cadherin).

In agreement with the previous literature, the pathological and molecular findings are not consensual. Lung injury was differently assessed by multiple research groups using histological scores, oedema indices, concentration of different proteins and cells in the plasma, lung tissue and BALF. Imaging techniques were also used to assess lung tissue pathology. While some studies revealed pathology results favouring the use of variable ventilation over conventional ventilation (69, 82, 84, 85, 87, 88, 91, 102, 114, 117, 119, 125), others found no significant difference (90, 93, 95, 97, 99, 105, 111, 120, 123, 128). Of note, no work has reported higher degrees of tissue injury with variable ventilation in comparison to conventional ventilation.

The production and release of surfactant triggered by the variable ventilation pattern was also addressed by several studies. In particular, different precursors and components of alveolar surfactant were found to be increased (84, 85, 100, 116, 123), decreased (85) or unchanged (69, 85, 95, 123) in comparison to conventional ventilation.

A plethora of methods has been also applied to quantify lung inflammation after delivery of variable ventilation: from cell and cytokine quantifications to *in vivo* imaging and microscopy techniques. While some studies observed lower indices of inflammation after variable ventilation (69, 84-86, 88, 90, 91, 102, 112, 114, 116, 124, 126), others did not observe significant differences (87, 93-95, 100, 108, 111, 117, 119, 120, 122, 125, 128). Only a single study found a higher level of interleukin 6 (IL-6), a pro-inflammatory marker, in comparison to conventional ventilation (127). Hence, the available literature is inconclusive on the relationship between inflammation outcomes and variable ventilation (129).

The variable ventilation patterns

At least to some extent, the inconsistent findings in previous reports are due to very distinct experimental settings and variability patterns. As a matter of fact, the concept of '*variability'* in the breathing pattern applied in previous research is not homogeneous. While some studies used spontaneous (physiological) breathing recordings as driving signals to the ventilator (82, 88, 89, 93, 95, 97, 101, 103, 106-109, 113, 125, 134), others have used a wide range of computer-generated signals, with mathematically random patterns (69, 83-87, 90, 92, 96, 99, 100, 102, 110, 111, 114, 116-124, 126-128, 138).

The respiratory signal used in most published research on variable ventilation uses 'artificial' signals to modulate the ventilator. Otherwise said, the pattern used for variable ventilation did not respect the physiological characteristics of awake, spontaneous breathing. One research group has used *'biologically variable ventilation'* (88, 95, 98, 105-109), by setting a variable RR with a constant inspiratory flow (thus, the VT was directly proportional to the cycle frequency). Other researchers have used mathematically random VT and/or RR with different distributions (gaussian, power-law, etc.).

The originality of our ventilation approach stems from the use of a physiological breathing pattern, recorded in awake rabbits prior to the period of mechanical ventilation, and therefore the term 'physiologically variable ventilation'. The driving signal of PVV reproduced the tidal variations of RR and V_T with the exact characteristics of awake breathing. Furthermore, while a "healthy-breathing" pattern was applied to animals with healthy lungs, ARDS and asthma, for the experiment in COPD animals, the breathing pattern was recorded after the induction of the disease. Thus, the coefficients of variation of the ventilation signal were slightly different between experiments and likely more tailored to the specific respiratory condition.

In this regard, it is important to note that the degree of variability is a major difference between the published reports on variable ventilation. The pioneer works that brought attention to the benefits of variable ventilation used a V_T that varied between 75% and 135% of the mean, corresponding to a coefficient of variation of 11.5% (82, 134). Subsequently, it was demonstrated that excessive amounts of variability were less beneficial and triggered negative outcomes (83, 110, 127), likely through alveolar overdistention, reduction of venous blood return and V/Q mismatch. These heterogenous approaches on variability led to conflicting results on the respiratory outcomes in the different studies. Regarding the results presented in this thesis, the CV of V_T and RR recorded during the rabbit's spontaneous breathing were roughly between 10 and 15%.

This values are in line with the very first report of variable ventilation, recorded in the awake dog (82).

The rabbit as model of respiratory diseases

Another aspect that deserves consideration is the choice of the rabbit as an animal model for respiratory diseases. This species has several advantages for preclinical studies in respiratory research that have been recognized in the literature (139, 140). Using the rabbit as a model also allowed the reduction and refinement of animal use (3R policy) for research purposes (135), in comparison to rodents. Rodents have less extensive airway branching than larger mammals and do not have respiratory bronchioles. In humans, bronchioles are major sites of injury for both emphysema and airway diseases. Therefore, small animals are "a very poor model for diseases such as chronic bronchitis (141). Furthermore, the adult rabbit has an ideal "baby-size" which permits preclinical studies using human paediatric devices, catheters, monitors, etc., facilitating the clinical translation of the experimental results. In addition, repeated measurements of lung function and repeated blood sampling can be readily made in the rabbit, thus, each animal may be used as its own control (142), which greatly reduced the number of animals used in the research protocols of this thesis (3R policy).

Limitations

The methodological aspects constituting limitations in each of the three research protocols are considered individually in each publication. However, some aspects warrant further discussion in a global manner.

One aspect that is common to all experimental trials is the small sample size. Each experimental group is constituted by a dozen research subjects and thus, the precision of estimates and the measure error/deviation could easily be optimised by increasing *n*. However, in all respect of the 3R policy, and considering the fact that the results were strongly significant in most essays, we did not request further authorisations from the Ethics Committee to include supplementary animals in the experiments.

Another aspect that limits the conclusions of the present studies is the length of mechanical ventilation. We applied five to six hours of ventilation, which is a relatively prolonged ventilation time in comparison to most published experiments; however, this does not allow to draw conclusions on the potential effects of several days of mechanical ventilation, as per routine use in the ICU. In our series of research works, we mostly verified that the benefits of PVV became more evident over time, and that the differences between PCV

and PVV became more significant after 6 hours of ventilation. Nevertheless, we cannot conclude whether a longer experimental time would have increased or decreased the benefits of PVV. Limited data is available about prolonged application of variable ventilation, however, one reported demonstrated that 24 h of mechanical ventilation with variable V_T did not attenuate pulmonary inflammation in comparison to conventional ventilation (143).

Another aspect that limits the generalisation of our findings is that we only studied settings of mandatory ventilation (in opposition to assisted ventilation). Our research subjects were under neuro-muscular blockade, thus avoiding the breathing effort. On one hand, this experimental setting permitted the application of the PVV pattern with the exact characteristics of the pre-recorded signal of spontaneous breathing; on the other hand, this protocol design does not allow patient-ventilator interactions and desynchrony events might become then an issue. Therefore, care must be taken when generalising our observations to the context of assisted ventilation. In spite of that, assisted variable ventilation ("noisy pressure-support ventilation) has been shown to be beneficial in terms of ventilatory pressures, gas exchange and mechanics when compared to "monotonous" pressure support (92, 96, 119). We also did not investigate the effects of variability during the expiratory phase of the breathing cycle; our studies were limited to the benefits of variability in the inspiratory pressure and frequency, thus, we are not able to conclude if further variability during the expiratory phase would have had an added value.

The advantages of PVV in respiratory mechanics were observed in all the diseases studied in this thesis work. However, it is important to note that the improvements in respiratory tissue elastance is likely to be even more beneficial than reported. The parameters reflecting the dissipative and elastic properties were obtained for the total respiratory system and, notably, the chest wall contributes to about half of the total respiratory damping and elastance in this species (144). Since this significant chest wall component is expected to be constant throughout the ventilation period, changes in pulmonary tissue parameters are likely to be even more pronounced. The partition of the lung tissue properties could have been performed if we had measured the chest wall component using an oesophageal pressure catheter.

Finally, it is important to note that all 3 diseases that were experimentally induced in animal models to study aspects of mechanical ventilation are complex clinical syndromes, with multiple pathophysiological mechanisms that are only partially reproduced in the laboratory setting. It is well recognized that the animal models of acute and chronic lung injury have biological differences in comparison to the human diseases, with distinct expression of inflammatory cells, histological injury, and phenotype (139, 141, 145).

Despite the aforementioned limitations, there is a strong aspect which is worth noting in regards of the results: independently of the disease model or the particular ventilation setting that was applied in each research protocol, PVV has globally improved the respiratory function by means of better gas exchange, respiratory mechanics, lung aeration and tissue inflammation. These reproducible benefits, which are further supported by previous literature reporting advantages of variable ventilation in other experimental settings (and using other patterns of variability / ´noise´ during mechanical ventilation), likely anticipate similar effects of this mode of ventilation in human lungs. Therefore, clinical trials should be done in order to assess the clinical benefits of PVV.

Final considerations

As of today, there are 2 small clinical trials reported in the literature which addressed the application of variable ventilation in humans. One clinical study in patients submitted to open-abdominal surgery for aortic aneurisms (n=41) demonstrated significant benefits of variable ventilation, with lower ventilatory pressures and dead space and higher lung compliance, oxygenation, and CO_2 clearance (89). Inversely, another trial in open abdominal surgery (n=50) did not find significant differences in gas exchange, respiratory mechanics, nor cytokine expression (122).

In summary, the present work provides a considerable amount of evidence supporting the benefits of physiologically variable ventilation in both healthy and diseased lungs. The evident advantages of variable ventilation over conventional modes warrant further investigation to assess its potential benefits for human use.

CONCLUSION

The application of physiologically variable ventilation to experimental models of healthy and diseased lungs (ARDS, asthma and COPD) prevented deterioration in respiratory function, in comparison to conventional pressure-controlled ventilation. Although the particular advantages of physiologically variable ventilation were different in each of the investigated diseases, improvements were globally observed in terms of oxygenation, respiratory mechanics, ventilatory pressures, lung aeration and tissue inflammation.

A reduction in alveolar derecruitment and lung tissue stress leading to better aeration and gas exchange may explain the benefits of physiologically variable ventilation. This modality could become clinically useful to mitigate the impact of ventilator-induced lung injury and the pulmonary complications after prolonged mechanical ventilation.

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APPENDIX

PUBLICATIONS

▪ **Dos Santos Rocha A**, Fodor GH, Kassai M, Degrugilliers L, Bayat S, Petak F, Habre W. Physiologically variable ventilation reduces regional lung inflammation in a pediatric model of acute respiratory distress syndrome. Respir Res. 2020 Oct 31;21(1):288. doi: 10.1186/s12931-020-01559-x.

▪ **Dos Santos Rocha A**, Südy R, Peták F, Habre W. Physiologically variable ventilation in a rabbit model of asthma exacerbation. Br J Anaesth. 2020 Dec;125(6):1107-1116. doi: 10.1016/j.bja.2020.08.059.

▪ **Dos Santos Rocha A**, Südy R, Bizzotto D, Kassai M, Carvalho T, Dellacà RL, Peták F, Habre W. Benefit of Physiologically Variable Over Pressure-Controlled Ventilation in a Model of Chronic Obstructive Pulmonary Disease: A Randomized Study. Front Physiol. 2021 Jan 13; 11:625777. doi: 10.3389/fphys.2020.625777.

Dos Santos Rocha et al., Respir Res. 2020 Oct 31;21(1):288

Respiratory Research

RESEARCH Open Access

Physiologically variable ventilation reduces regional lung inflammation in a pediatric model of acute respiratory distress syndrome

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Abstract

Background: Benefits of variable mechanical ventilation based on the physiological breathing pattern have been observed both in healthy and injured lungs. These benefits have not been characterized in pediatric models and the effect of this ventilation mode on regional distribution of lung inflammation also remains controversial. Here, we compare structural, molecular and functional outcomes reflecting regional inflammation between PVV and conventional pressure-controlled ventilation (PCV) in a pediatric model of healthy lungs and acute respiratory distress syndrome (ARDS).

Methods: New-Zealand White rabbit pups (n = 36, 670 ± 20 g [half-width 95% confidence interval]), with healthy lungs or after induction of ARDS, were randomized to five hours of mechanical ventilation with PCV or PVV. Regional lung aeration, inflammation and perfusion were assessed using x-ray computed tomography, positron-emission tomography and single-photon emission computed tomography, respectively. Ventilation parameters, blood gases and respiratory tissue elastance were recorded hourly.

Results: Mechanical ventilation worsened respiratory elastance in healthy and ARDS animals ventilated with PCV (11 ± 8%, 6 ± 3%, $p < 0.04$), however, this trend was improved by PVV (1 ± 4%, −6 ± 2%). Animals receiving PVV presented reduced inflammation as assessed by lung normalized [^{18}F]fluorodeoxyglucose uptake in healthy (1.49 ± 0.62 standardized uptake value, SUV) and ARDS animals (1.86 ± 0.47 SUV) compared to PCV (2.33 ± 0.775 and 2.28 ± 0.3 SUV, respectively, $p < 0.05$), particularly in the well and poorly aerated lung zones. No benefit of PVV could be detected on regional blood perfusion or blood gas parameters.

Conclusions: Variable ventilation based on a physiological respiratory pattern, compared to conventional pressure-controlled ventilation, reduced global and regional inflammation in both healthy and injured lungs of juvenile rabbits.

Keywords: Mechanical ventilation, ARDS, Variable ventilation, Positron emission tomography, Regional ventilation

Introduction

Acute respiratory distress syndrome (ARDS), characterized by the acute onset of severe hypoxic respiratory failure, remains a prevalent and often lethal condition in intensive care [1]. Although mechanical ventilation is a crucial life-saving treatment for ARDS, there is a considerable body of evidence indicating that prolonged positive-pressure ventilation can initiate, perpetuate or aggravate injury to lung tissue [2, 3]. The resulting

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exaggerated mechanical stress, along with the monotonous alveolar opening and closing, exerts shear stress and increased strain in the lung tissue [4], conditions that contribute to ventilator-induced lung injury (VILI).

While various modalities of mechanical ventilation have been proposed to reduce VILI [5–8], protective ventilation with monotonous tidal volume (VT) may not be the only rational strategy. In recent years, it has been advocated that mechanical ventilation reproducing the natural variability of breathing is better than conventional modes [9, 10]. Variable ventilation has been shown to be beneficial for gas exchange and respiratory mechanics in various animal models with healthy [11–13] or injured lungs, including ARDS [14–17]. We have previously established a variable ventilation modality using pre-recorded breathing patterns of healthy animals [18]. This physiologically variable ventilation (PVV) is characterized by breath-to-breath variability of VT and respiratory rate, in contrast to the monotonous conventional ventilation modes.

Recent interest in variable ventilation stems from the need to reduce cyclic alveolar reopening during mechanical ventilation, especially in injured lungs, to avoid development or propagation of lung inflammation, atelectasis and subsequent hypoxemia [19]. Whereas some studies demonstrated the beneficial effect of introducing variability into lung recruitment [20, 21], and others reported improvement in global respiratory mechanical and functional parameters [11–18], there is still a lack of detailed knowledge about the pathophysiological background related to the functional and regional behavior of the lung during variable ventilation. Moreover, the potential of PVV in the context of pediatric ARDS has not been characterized. To investigate the effect of PVV, lung functional and structural changes were compared to those obtained with conventional monotonous ventilation in normal lungs and ARDS, in a pediatric model. Global respiratory parameters were measured to characterize the overall lung condition. Regional lung aeration, pulmonary perfusion and inflammation were assessed by functional imaging using positron-emission tomography (PET) and single-photon emission computed tomography (SPECT) combined with X-ray computed tomography (CT).

Methods

A more detailed description of the methods can be found in Additional file 1.

Experimental animals

New Zealand White rabbit pups of both sexes, aged 4 to 5 weeks, were included in the present study (mean weight: 630 g, 370–860 g). This age can be approximated to an equivalent human age of 6 to 8 months [22]. Rabbits underwent tracheostomy and continuous intravenous (iv) anesthesia using propofol (10 mg/kg/h), fentanyl (5 µg/kg/h), midazolam (0.2 mg/kg/h) and atracurium (0.6 mg/kg/h).

Study protocol

The protocol of the study is depicted in Fig. 1. Under baseline (BL) conditions, pressure-controlled ventilation was applied, using a positive end-expiratory pressure (PEEP) of 6 cmH_2O, a fraction of inspired oxygen (FiO_2) of 0.4, a VT of 8 ml/kg and a respiratory rate to achieve normocapnia (end-tidal CO_2 of 5.5–6%). Arterial and central venous blood gas analyses and respiratory mechanical measurements were performed at BL. Subsequently, animals were randomized for the absence (CTRL) or presence (ARDS) of lung injury. Mild ARDS, according to the Berlin definition [23], was induced by combination of intravenous lipopolysaccharide (20 µg/kg) and injurious ventilation (VT = 40 ml/kg, 0 cmH_2O PEEP, $FiO_2 = 1.0$) with a target range of partial pressure of arterial oxygen (PaO_2)/FiO_2 ratio of 250–300 mmHg. When the target range of PaO_2/FiO_2 was reached, animals were further randomized for the ventilation mode: five-hour mechanical ventilation (VT = 8 ml/kg, PEEP = 6 cmH_2O) was applied using either pressure-controlled ventilation (PCV) or PVV. FiO_2 was adjusted according to PaO_2/FiO_2: using $FiO_2 = 0.4$ above 250 mmHg; $FiO_2 = 0.6$ between 200–250 mmHg; $FiO_2 = 0.8$ between 100–200 mmHg, and $FiO_2 = 0.9$ in the case that PaO_2 decreased below 100 mmHg. Arterial blood gas and respiratory mechanics were measured hourly (T1–T5). After 5 h (T5), in vivo lung imaging was performed under continuous application of the ventilation mode. Subsequently, animals were euthanized with iv sodium thiopental (100 mg/kg). Bronchoalveolar lavage was performed ex vivo in the right lung, and the left lung was extracted for histological analysis.

Experimental procedures

Measurement of respiratory mechanical parameters

Respiratory mechanical parameters were assessed by the wave-tube method of the forced oscillation technique, as detailed previously [14]. The constant-phase model [24] was fitted to the spectra to separate airway and tissue compartments of the respiratory system. Airway resistance (Raw), tissue damping (G) and tissue elastance (H) were estimated from the fits.

Application of physiologically variable ventilation

A commercially available pediatric ventilator (Servo-i, Maquet Critical Care, Solna, Sweden) was used with special firmware. The applied variable

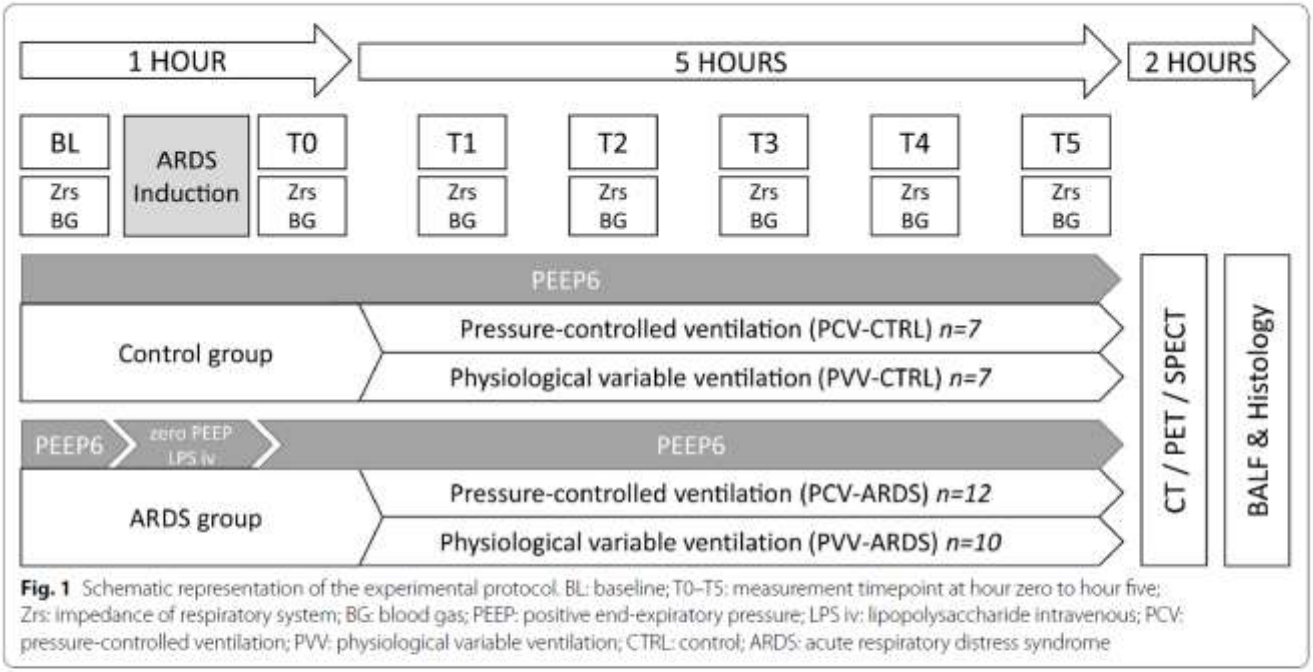

Fig. 1 Schematic representation of the experimental protocol. BL: baseline; T0–T5: measurement timepoint at hour zero to hour five; Zrs: impedance of respiratory system; BG: blood gas; PEEP: positive end-expiratory pressure; LPS iv: lipopolysaccharide intravenous; PCV: pressure-controlled ventilation; PVV: physiological variable ventilation; CTRL: control; ARDS: acute respiratory distress syndrome

pattern was the reproduction of physiological breathing in rabbit pups, obtained using unconstrained whole-body plethysmography.

Lung imaging

Structural imaging of the respiratory system was acquired using CT. Regional lung perfusion was assessed though SPECT imaging using ^{99m}Tc-labeled iv albumin macroaggregates. Regional distribution of inflammatory activity was assessed using PET imaging of fluorodeoxyglucose (^{18}F-FDG) [25]. Lung radiodensity was expressed in mean pixel value (MPV), while PET and SPECT activity were expressed as standardized uptake value (SUV) normalized for voxelwise fraction of lung tissue [26].

CT images were segmented to well aerated, poorly aerated and non-aerated zones, based on radiodensity, as well as to ventral and dorsal halves. These segmented zones were considered when analyzing PET and SPECT images.

Measurements of secondary outcomes

Cell and cytokine content of the bronchoalveolar lavage fluid (BALF) was analyzed as detailed previously [18]. A histological lung injury score was determined according to the American Thoracic Society guidelines [27]. Tracheal pressure, airflow, arterial pressure, central venous pressure (CVP) and electrocardiogram were digitized and continuously recorded. Mean arterial pressure (MAP) and heart rate (HR) were assessed from these curves.

Experimental outcomes

The primary outcomes of the present study were defined as respiratory mechanical parameters (Raw, tissue damping and elastance), arterial blood gas parameters (lactate, pH, PaO_2/FiO_2 and $PaCO_2$) and imaging parameters. Secondary outcomes were hemodynamic and ventilation parameters, cytokine levels and lung injury histological indices.

Statistical methods

Data are presented as mean ± half-width of 95% confidence interval. Normality of the data was assessed for each variable with the Shapiro–Wilk test. In case of a failed normality test, the variable was log-transformed. Repeated measures analyses of variance (ANOVA) using linear mixed-effect model fits by a restricted maximum likelihood (REML) method were applied to calculate statistical significances followed by Dunnett or Holm-Sidak post-hoc tests, using a significance level of $p < 0.05$, and all p values two-sided.

Results

Study population

Forty-four rabbits were randomized into one of four experimental groups. Eight rabbits were excluded from the analysis due to vital issues precluding the 5 h of ventilation (pneumothorax, n = 7; hemorrhage, n = 1). Therefore, 36 rabbits were included in the final analyses, with

the following distribution: 12 rabbits were included in the PCV-ARDS group, 10 rabbits in PVV-ARDS, 7 rabbits in PCV-CTRL and 7 rabbits in PVV-CTRL.

Respiratory mechanics

Parameters characterizing respiratory mechanics obtained prior to initiating the 5-h ventilation are displayed in Additional file 1: Table S1. Changes in respiratory mechanical parameters relative to those obtained immediately after the induction of lung injury are displayed in Fig. 2. Applying PCV for 5 h led to significant increases in tissue elastance (T1–T5, $p < 0.01$) in the control animals and in Raw in the ARDS model (T1–T5, $p < 0.03$). Conversely, ventilating the lungs with PVV resulted in a significant decrease in tissue damping in control animals (T1–T5, $p < 0.01$), whereas no change in respiratory mechanics was detected in the ARDS model. Comparison of the two ventilation modes revealed significantly lower relative changes with PVV in tissue damping for the control animals (T4-T5, $p < 0.03$) and tissue elastance for the ARDS model (T1-T5, $p < 0.01$).

Gas exchange

Figure 3 depicts the blood gas parameters during the 5-h ventilation. Inducing lung injury led to significant impairment of the blood oxygenation index (PaO_2/FiO_2), confirming the presence of mild to moderate ARDS, according to the Berlin definition [23]. Further drift in PaO_2/FiO_2 was observed in the PVV-ARDS group that resulted in statistically significant decreases after the

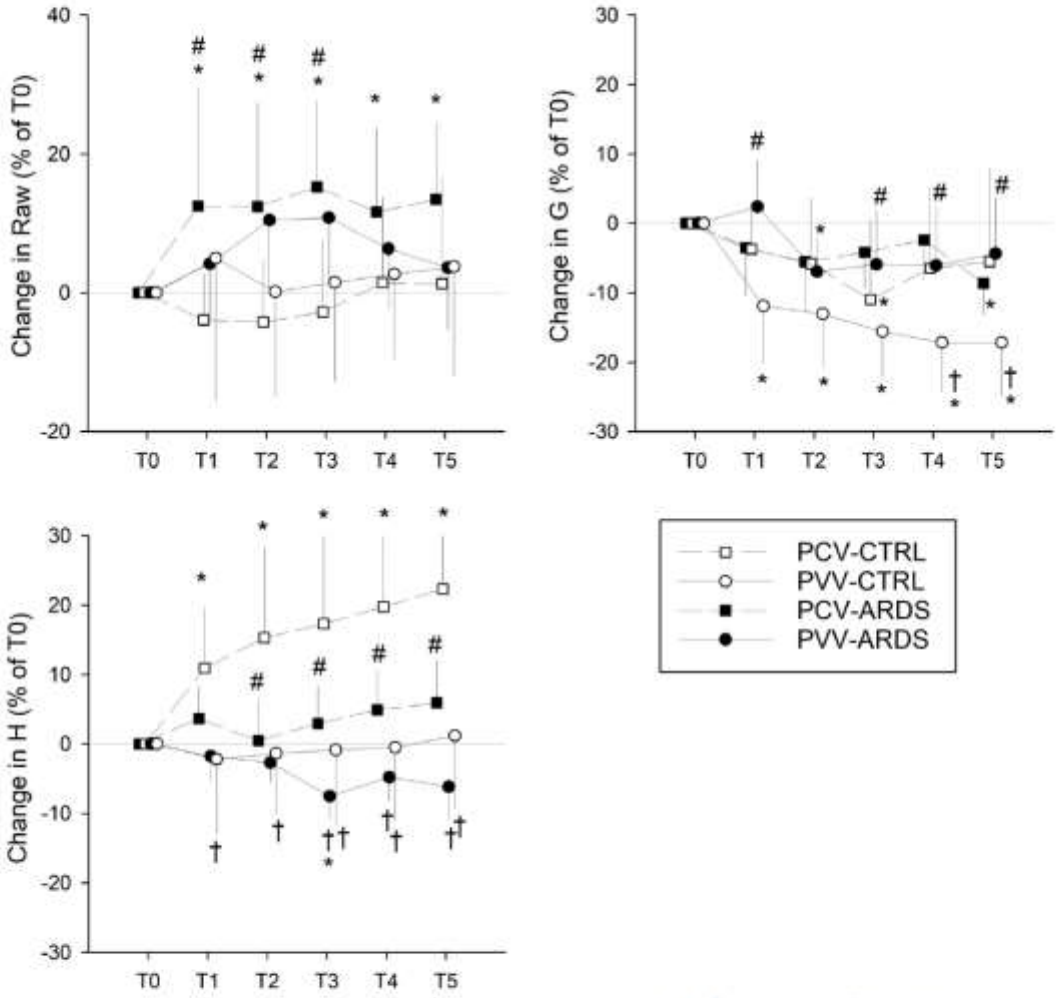

Fig. 2 Changes in respiratory mechanical parameters relative to those obtained immediately after induction of lung injury (T0). Values expressed as mean ± half-width of 95% confidence interval. Raw: airway resistance; G: respiratory tissue damping; H: respiratory tissue elastance, T0: immediately after induction of lung injury; T1–T5: time points at the end of the corresponding hour of the 5-h long ventilation period; PCV: pressure-controlled ventilation; PVV: physiological variable ventilation; ARDS: presence of lung injury; CTRL: absence of lung injury. *$p < 0.05$ vs. T0, #$p < 0.05$ vs. CTRL, †$p < 0.05$ vs. PCV

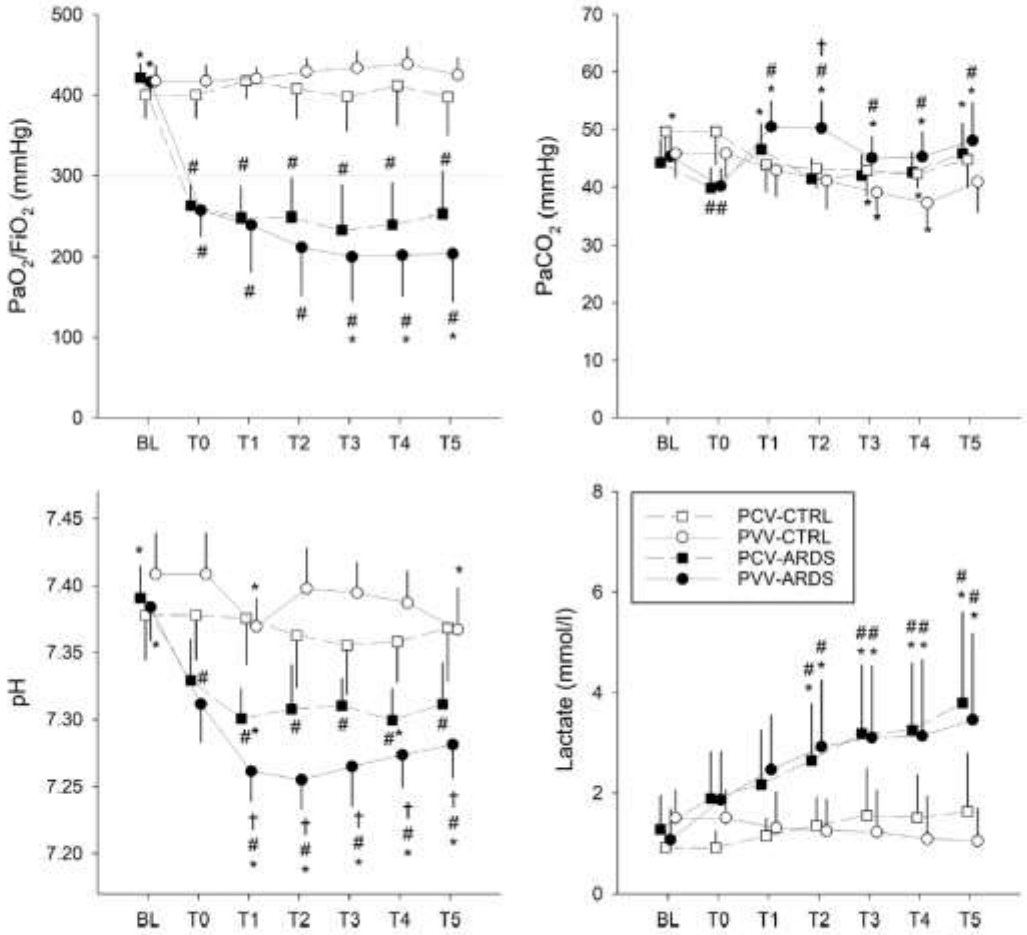

Fig. 3 Blood gas parameters obtained before and during the 5-h ventilation period. Values expressed as mean ± half-width of 95% confidence interval. PaO_2: partial pressure of arterial oxygen concentration; FiO_2: fraction of inspired oxygen; $PaCO_2$: partial pressure of arterial carbon dioxide concentration; BL: baseline; T0: immediately after induction of lung injury; T1–T5: time points at the end of the corresponding hour of the 5-h long ventilation period; PCV: pressure-controlled ventilation; PVV: physiological variable ventilation; ARDS: presence of lung injury; CTRL: absence of lung injury. *$p < 0.05$ vs. T0, #$p < 0.05$ vs. CTRL, †$p < 0.05$ vs. PCV

third hour of mechanical ventilation (T3–T5, $p < 0.045$). Monotonous ventilation with PCV had no effect on the blood gas parameters in the control animals, whereas a systematic decrease in pH and plasma lactate concentration was observed in the ARDS groups (T1–T5, $p < 0.001$). Applying variable ventilation for 5 h in the control group had no systematic effect on gas exchange, whereas higher $PaCO_2$ levels (T1–T5, $p < 0.05$) were associated with significantly diminished pH and elevated lactate in animals with ARDS (T1–T5, $p < 0.01$).

Lung imaging

Representative CT, PET and SPECT images with the corresponding regional aeration maps in control and ARDS conditions are shown in Fig. 4. More heterogeneous lung structure, as indicated by heterogeneous regional distribution of ^{18}F-FDG uptake and ^{99m}Tc-labeled albumin macroaggregates, was observed in the presence of ARDS. The PET uptake values calculated for the total lung and at regional levels are summarized for the study groups in the left panels of Fig. 5. When averaging the entire lung, significantly lower mean ^{18}F-FDG uptake was evidenced for the lungs in the animals ventilated with PVV, regardless of the presence of lung injury. This difference was also detected at the regional level in rabbits with healthy lungs ventilated with PVV ($p < 0.04$). Characterizing the differences in ^{18}F-FDG uptake among the various aeration zones, defined by CT density, revealed the highest activity in the well aerated zones, with 2 to threefold differences compared to the non-aerated zones ($p < 0.01$, *well aerated vs. poorly aerated or non-aerated*). Likewise, ventral (non-dependent) regions presented significantly higher ^{18}F-FDG uptake

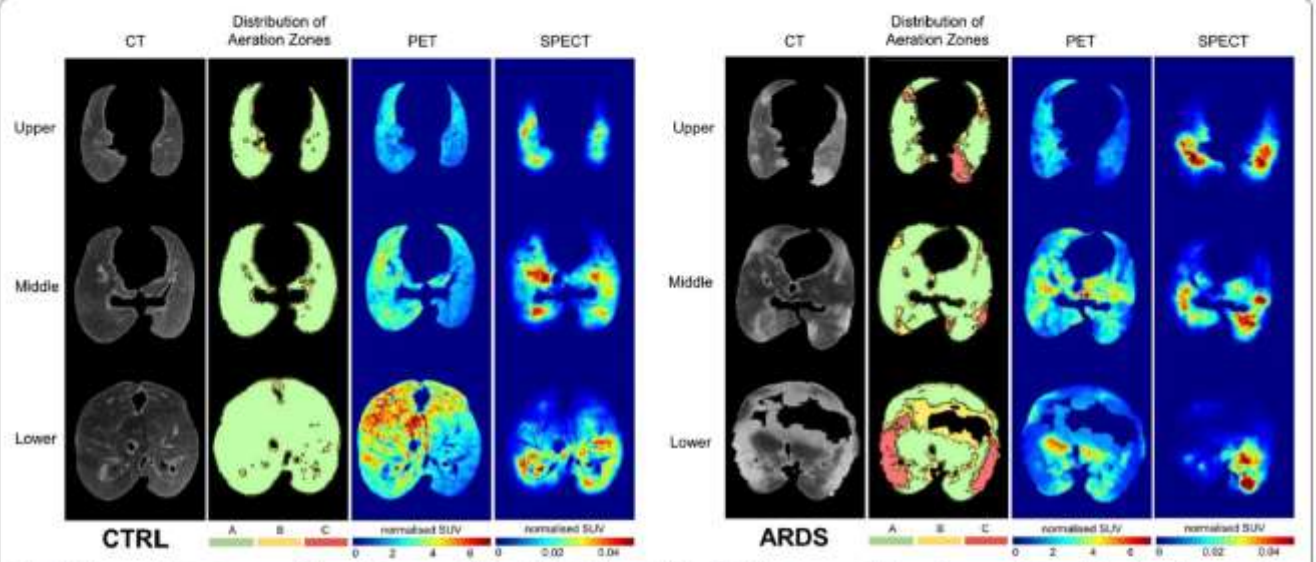

Fig. 4 Representative images of CT, aeration maps, PET and SPECT (from left to right) in upper, middle and lower sections of the lung (from top to bottom) in the different experimental groups (CTRL and ARDS). Aeration zones A, B and C represent the well, poorly and non-aerated lung zones, respectively. PET and SPECT heating maps are represented in SUV normalized for tissue fraction for fludeoxyglucose and ^{99m}Tc-labeled albumin macroaggregates, respectively

compared to dorsal (dependent) regions in both ventilation modes. Furthermore, significantly decreased mean ^{18}F-FDG uptake was observed in the control animals ventilated with PVV compared to those with PCV ($p < 0.01$).

No evidence for a difference in SPECT activity was detected between the protocol groups (Fig. 5, right panels). However, regional perfusion was significantly and consistently higher in the well aerated zones and the dorsal zones of the lung, without differences between the experimental groups.

Secondary outcomes

The detailed results on secondary outcomes (hemodynamic and ventilation parameters, cytokine levels and lung injury histological indices) can be found in Additional file 1. In the presence of ARDS, significantly higher driving pressure was required to maintain the same minute ventilation than in healthy animals ($p < 0.01$, ARDS vs. CTRL, Additional file 1: Figure S2). In the CTRL group, a progressive reduction in driving pressure was observed with PVV ($p < 0.01$ vs. T0, Additional file 1: Figure S2), which was not observed in animals ventilated with PCV.

No differences were detected between the two ventilation modes in regards of the hemodynamic parameters (Additional file 1: Figure S4), lung injury score (Additional file 1: Table S2), cytokine and cell content of BALF (Additional file 1: Table S3).

Discussion

In the present study, a combined approach consisting of lung functional and structural assessment was used to investigate differences in the global and regional effects of PVV and the conventional monotonous pressure-controlled mode in a pediatric model of normal lungs and ARDS. The use of PVV decreased pulmonary inflammation, as assessed by ^{18}F-FDG uptake, independent of lung condition. The decreased lung inflammation observed with PVV was also detected as an improvement in respiratory tissue elastance. Neither the use of PCV nor PVV affected blood gas and lung morphology indices.

Respiratory system mechanical parameters obtained in BL conditions or following induction of lung injury exhibited excellent agreement with previous data from the same species with similar weight range [14–16, 28]. Furthermore, the time course of the respiratory mechanical parameters over 5 h of ventilation in the control groups is in accordance with that observed previously in an experimental model using adult rabbits [18].

Since increases in tissue damping and elastance reflect lung volume loss and stiffening of the lung tissue [29, 30], the lack of an increase of elastance in the PVV-CTRL group suggests that lung derecruitment did not occur, and this conclusion is also supported by the lower inspiratory driving pressure achieved in this group. Moreover, the significant differences in elastance between the PCV-ARDS and PVV-ARDS groups observed after the 5-h ventilation suggest a protective effect of the variability on the conservation

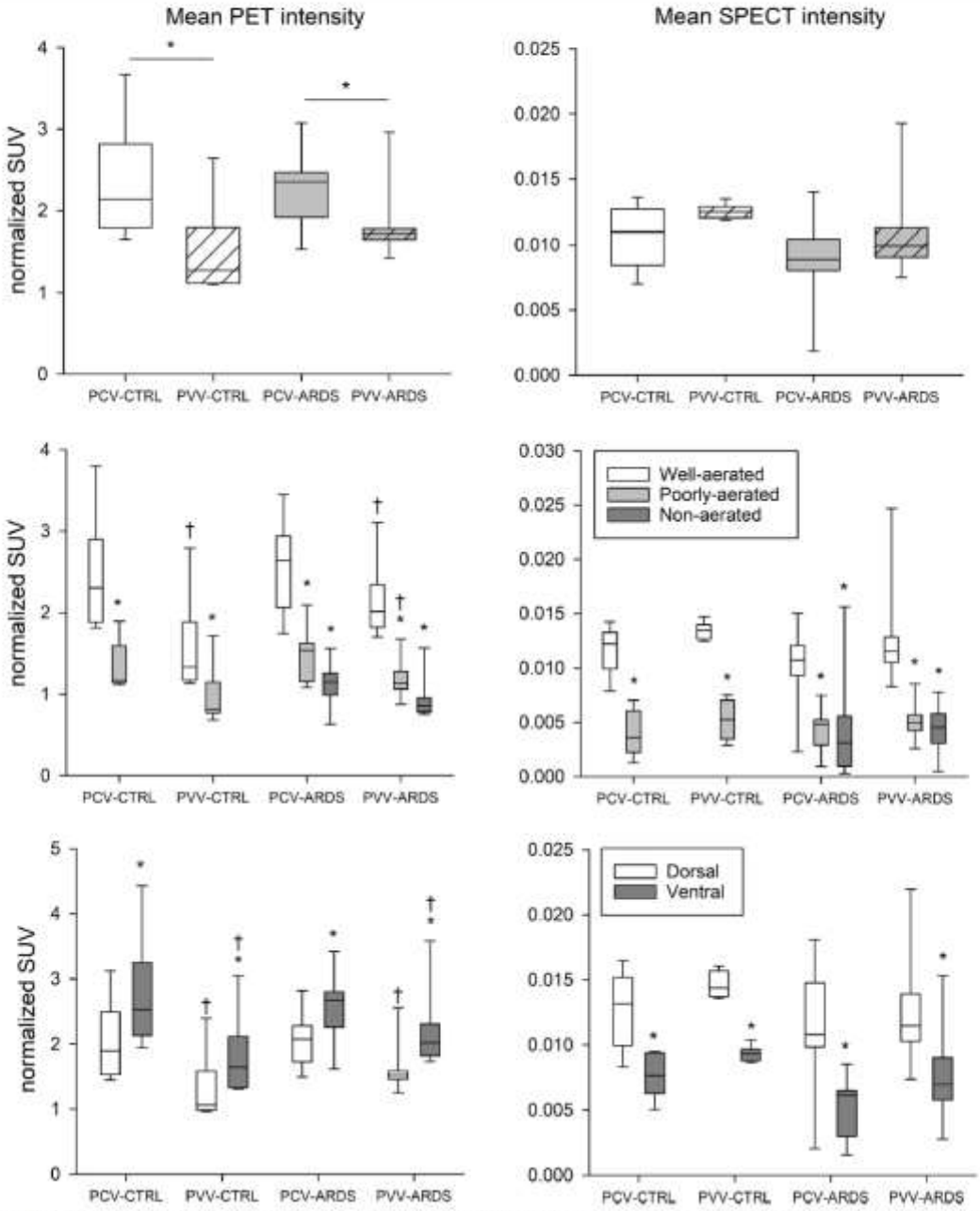

Fig. 5 Left panels depict pulmonary inflammation characterized by PET imaging normalized to the tissue fraction. Right panels show pulmonary circulation characterized by SPECT imaging, normalized to tissue fraction. Upper panels represent mean PET and SPECT intensities averaged for the entire lung. Middle panels demonstrate the regional distribution based on aeration zones. Bottom panels represent the regional distribution based on the dependent (dorsal) and non-dependent (ventral) zones. SUV: standardized uptake value; PCV: pressure-controlled ventilation; PVV: physiological variable ventilation; ARDS: presence of lung injury; CTRL: absence of lung injury. *$p < 0.05$ vs. well-aerated or vs. dorsal, †$p < 0.05$ vs. PCV

of lung volume in the presence of ARDS. Studies using models of mild-to-moderate lung injury have found similar beneficial effects on respiratory mechanics for variable ventilation [15, 31], and this protective effect was not observed in the presence of more severe ARDS [14].

Global and regional lung metabolic activity were measured by ^{18}F-FDG uptake, a reliable biomarker of inflammation in the lung [32]. This marker is indicative of neutrophil activation in acute lung injury and ARDS [33–35]. Previous studies have shown that voxelwise ratio of lung parenchyma and air content influences ^{18}F-FDG

uptake quantification, requiring normalization for the tissue fraction [26, 36], which was performed in the current study. After 5 h of ventilation, we observed significantly lower indices of global and regional lung inflammation in the animals ventilated with PVV. Specifically, a significantly higher inflammatory activity characterized the well aerated and non-dependent lung zones, both in control and injured groups. This finding is consistent with results from previous experiments studying injured lungs, in which lung inflammation assessed by ^{18}F-FDG uptake was correlated with regional strain [37, 38]. The significantly lower inflammation associated with PVV may be explained by the fact that the variability of the delivered VT contributes to tidal recruitment [12, 15], therefore reducing strain in the open, aerated zones. It is worth noting that PVV exerts the most beneficial effect in the well and poorly aerated zones under both control and ARDS conditions (Fig. 5). Conversely, the collapsed non-aerated zones were obviously unaffected by ventilation modes since these units were not subjected to strain. These findings further confirm the importance of focusing on regional ventilation when assessing the benefit of ventilation strategies. SPECT imaging confirms differences in regional distribution of lung perfusion when it is related to aeration zones. However, the lower blood perfusion in the ventral lung regions as compared to the dorsal zones can be attributed to the gravity effect and/or to the blood shift to the dorsal zones as a consequence of positive pressure and lung overdistension.

The beneficial effects of PVV on respiratory mechanics and lung inflammation were not reflected in changes in blood gas parameters. The lack of improvement in oxygenation may be related in part to the more severe hypoxemia in this group, which required a higher FiO_2 (65% vs 55% in groups PVV-ARDS and PCV-ARDS, respectively). Moreover, the increase in lactate levels suggest the development of metabolic acidosis in both groups of ARDS animals, which may be the consequence of inadequate tissue oxygen delivery. Moreover, the timespan of the experiment (5 h) may be too short to detect effects on gas exchange. We may hypothesize that the more prominent inflammation observed in the PCV groups would build up and potentially cause gas exchange problems over the course of days.

The presence of ARDS was evident in the elevated lung injury score compared to control groups. In agreement with previous studies, lung injury score did not differ between the ventilation modes [14, 39]. The discrepancy between the functional and structural findings may be explained by the faster onset of functional changes, compared to the relatively longer time needed for morphological changes to become apparent. Lung inflammation quantified using BALF cell counts and pro-inflammatory cytokines, unlike in vivo imaging, did not reveal differences between the ventilation modes. In vivo imaging gives a more comprehensive measure of pulmonary inflammation at the early phase of ARDS, as it demonstrates the alveolar as well as the interstitial compartments of the lung. Additionally, ^{18}F-FDG uptake reflects the acute metabolic activation of neutrophils and captures lung inflammation without barrier disruption, opposite to BALF neutrophils and cytokines, providing a more rapid assessment of inflammatory processes. In this context, it is worth noting that the control groups also showed increased inflammation and lung injury indices (BALF cytokines and histological injury score). These findings suggest that, despite the use of protective ventilation in the control groups, prolonged mechanical ventilation triggered the development of lung inflammation. This could potentially explain the lack of significant difference in normalized ^{18}F-FDG uptake between control and ARDS lungs.

The similarity in the values of systemic hemodynamic parameters observed for the experimental groups is expected from the similarity in the overall lung perfusion as assessed by SPECT imaging. However, the significantly higher regional perfusion measured in the dependent zones can be attributed to the physiological distribution of lung perfusion that occurs in supine position [40] and is enhanced under positive pressure ventilation [41]. Considering the regional aeration of lung tissue, the significantly lower perfusion observed in the poorly and non-aerated zones can be explained by the hypoxic pulmonary vasoconstriction mechanism [42].

There are some methodological aspects of the present study that warrant consideration. In this study we used a Cone Beam CT [43]. This device uses less radiation and creates higher resolution images than the regular fan beam CT; however, it produces more scatter artefacts, which can alter the measured values [44, 45]. Due to technical limitations, breath gating was not performed in any of the acquisitions; therefore, basal lung areas had artefacts due to motion of the abdominal organs during breathing. The lung volume containing these artefacts was similar however, among rabbits.

The animal model to induce ARDS calls for some considerations as well. The components of the model were chosen to mimic the various pathophysiological aspects of ARDS observed in humans. Namely, intravenous LPS contributes to the inflammatory component of the disease and it has also been described to induce surfactant dysfunction [46]. Injurious ventilation using high VT combined with no PEEP contributes to development of volume- and barotrauma due to the supraphysiologic tidal volumes and respiratory pressures, whereas the absence of PEEP promotes tidal closures and exerts shear

stress on the lung tissues [47]. The use of an FiO_2 of 1.0 during this injurious ventilation period facilitates lung volume loss and development of ventilation heterogeneities [48]. While the surfactant dysfunction can restore to some extent during the 5-h timeframe of the experimental protocol, the functional and morphological damage is still present in the lungs, supported by the marked and highly significant changes observed between the control and ARDS groups regardless of the ventilation mode applied.

Measurements of respiratory mechanical parameters also warrant some considerations. While Raw is mainly specific to the flow resistance of the conducting airways [49], the tissue parameters damping and elastance include not only pulmonary components but are also influenced by other structures of the total respiratory system, mainly the chest wall [49]. Previous literature attributed a chest wall contribution of approximately 30–50% to these parameters [50] and since the chest wall contribution is not expected to change after lung injury and mechanical ventilation [51], the observed changes are interpreted as being mainly of pulmonary origin. Therefore, the corresponding changes registered in tissue damping and elastance are predictably underestimating the real pulmonary changes.

Conclusions

Our data demonstrate the beneficial effect of variable ventilation based on a physiological breathing pattern in healthy lungs and in mild to moderate ARDS, in an experimental pediatric model. This positive effect was detected in the absence of deterioration in respiratory tissue elastance and in decreased regional lung inflammation measured by PET imaging. Ventilation for five hours with physiologically variable ventilation provided better protection on aerated lung zones than with monotonous pressure-controlled ventilation. While further studies in humans might be needed, our results suggest that the application of a physiological breathing pattern as the driving signal of mechanical ventilation may have a better lung protective ability than conventional modes in scenarios where prolonged mechanical ventilation is required.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12931-020-01559-x.

Additional file 1. Supplementary information on methods and ancillary results (Figures S1–S4 and Tables S1–S3).

Abbreviations

ANOVA: Analyses of variance; ARDS: Acute respiratory distress syndrome; BALF: Bronchoalveolar lavage fluid; BL: Baseline; CT: X-ray computed tomography; CTRL: Control; CVP: Central venous pressure; FDG: Fluorodeoxyglucose; FiO_2: Fraction of inspired oxygen; G: Tissue damping; HR: Heart rate; H: Tissue elastance; MAP: Mean arterial pressure; MPV: Mean pixel value; PaO_2: Partial pressure of arterial oxygen concentration; PaO_2/FiO_2: Blood oxygenation index; PCV: Pressure-controlled ventilation; PEEP: Positive end-expiratory pressure; PET: Positron-emission tomography; PVV: Physiologically variable ventilation; Raw: Airway resistance; SPECT: Single-photon emission computed tomography; SUV: Standardized uptake value; T0–T5: Timepoint (hour) zero to timepoint (hour) five; VILI: Ventilator-induced lung injury; VT: Tidal volume.

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Authors' contributions

ADSR: study design, experimental work, data collection, data analyses and article drafting. GHF: study design, experimental work, data collection, data analyses and article drafting. MK: data collection and data analyses. LD: data collection and data analyses. SB: study design, interpretation of results and article drafting. FP: study design, data analyses, interpretation of results and article drafting. WH: study design, data analyses, interpretation of the results and article drafting. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The experimental protocol was approved by the Experimental Ethics Committee of the University of Geneva and the Animal Welfare Committee of the Canton of Geneva, Switzerland (no. GE 64/17). All procedures were performed according to the current animal protection laws of Switzerland (LPA, RS455).

Consent for publication

Not applicable.

Competing interests

Engineering support was provided by Getinge AB (Solna, Sweden), who provided special firmware for the ventilator that allowed the application of variable ventilation patterns. The authors have no further conflicts of interest.

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Publisher's Note

- Online data supplement –

Physiologically variable ventilation reduces regional lung inflammation in a pediatric model of acute respiratory distress syndrome

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METHODS

Ethical statement

The experimental protocol was approved by the Experimental Ethics Committee of the University of Geneva and the Animal Welfare Committee of the Canton of Geneva, Switzerland (no. GE 64/17, modified by GE/144/17 and GE 30/18, 02 May 2017, 21 August 2017 and 19 February 2018). All procedures were performed according to the current animal protection laws of Switzerland (LPA, RS455) and reported in compliance with ARRIVE guidelines.

Experimental animals

New Zealand White rabbit pups of both sexes, aged 4 to 5 weeks, were included in the present study (mean weight: 630 g, 370–860 g). Animals were purchased from the farm of the University of Geneva (Arare, Geneva, Switzerland) and were delivered at least two days before the experiments to allow acclimatization. The rabbits had access to food and water *ad libitum* before the experiments and were kept in cages together with their mothers.

Study design

The protocol of the study is depicted in Figure 1 of the main manuscript. Following animal preparation, anaesthesia and surgery, ventilation was initiated in supine position using a commercially available neonatal ventilator (Servo-i, Maquet Critical Care, Solna, Sweden) to apply a pressure-controlled mode, a positive end-expiratory pressure (PEEP) of 6 cmH_2O, a fraction of inspired oxygen (FiO_2) of 0.4, a VT of 8 ml/kg and a respiratory rate to achieve normocapnia (end-tidal CO_2 of 5.5–6%). Before the beginning of the experimental protocol, two deep inflations (30 cmH_2O peak pressure maintained for 5 seconds each) were applied to normalize lung volume history. Subsequently, following a 5-minute stabilization period, baseline (BL) measurements were carried out: arterial and central venous blood samples were

obtained for blood gas analysis and respiratory mechanical properties were measured. Animals were randomized for the absence (CTRL) or presence (ARDS) of lung injury. Animals randomized to lung injury were exposed to a combination of intravenous injection of 20 µg/kg lipopolysaccharide (LPS, from *Escherichia coli* O111:B4, Sigma, Saint Louis, Missouri, USA) and injurious ventilation for 30 minutes (VT=40 ml/kg, PEEP=0 cmH_2O, FiO_2=1.0 with a respiratory rate to achieve the same minute ventilation as in BL) to provoke mild ARDS, according to the Berlin definition (146). Arterial blood gas analyses were performed to measure partial pressure of arterial oxygen (PaO_2), and the results were used to assess the degree of injury. If the target range of PaO_2/FiO_2 ratio (250–300 mmHg) had not been achieved, injurious ventilation was repeated for further 10-minute periods. When the target range of PaO_2/FiO_2 was reached, collapsed alveoli were opened with 2 deep inflations (30 cmH_2O peak pressure maintained for 5 seconds each), and, after a 5-minute stabilization period, another set of blood gas and respiratory mechanical data was collected (T0).

Animals were further randomized for the ventilation mode: five-hour mechanical ventilation was applied using either pressure-controlled ventilation (PCV) or PVV, at a PEEP of 6 cmH_2O, an average VT of 8 ml/kg, and a minute ventilation to match that of BL. FiO_2 was adjusted according to PaO_2/FiO_2: using FiO_2=0.4 above 250 mmHg; FiO_2=0.6 between 200–250 mmHg; FiO_2=0.8 between 100–200 mmHg, and FiO_2=0.9 in the case that PaO_2 decreased below 100 mmHg. Arterial blood gas and respiratory mechanics were checked hourly (T1–T4) and ventilation was adjusted if necessary. After 5 hours (T5), a final set of arterial and venous blood gas samples and respiratory mechanical data was obtained. The animals were then transported to the imaging facility while undergoing sedation and mechanical ventilation. Lung imaging was performed under continuous application of the ventilation mode. When the imaging acquisition finished, animals were euthanized with a single injection of intravenous

sodium thiopental (100 mg/kg). Bronchoalveolar lavage was performed *ex vivo* in the right lung, and the left lung was extracted for histological analysis.

Experimental procedures

All experiments were carried out at the laboratory of the Unit for Anaesthesiological Investigations, University Hospitals of Geneva and University of Geneva. Imaging procedures were carried out at the microPET/SPECT/CT Imaging Laboratory, at the Geneva site of the Centre d'Imagerie Biomédicale. Animal transport between the two research facilities was performed under sedation and mechanical ventilation. Because of time limitations of isotope production, animal preparation and mechanical ventilation were performed overnight to allow imaging acquisition during office hours.

Anesthesia and surgical preparation

Anesthesia was induced using intramuscular injection of xylazine (3 mg/kg) and ketamine (25 mg/kg) and maintained using a continuous intravenous infusion of propofol (10 mg/kg/h), fentanyl (5 µg/kg/h), and midazolam (0.2 mg/kg/h) through a 24 G catheter (Abbocath, Abbot Medical, Baar/Zug, Switzerland) in a marginal ear vein. Animals were placed on a thermostatic heating pad (Harvard Apparatus, South Natick, MA, USA) and a rectal thermometer probe was used to keep internal body temperature within the range of 38–39°C. Fluid balance of the animals was maintained by administering a 4 ml/kg/h continuous infusion of Ringer's Acetate. Local anesthesia with lidocaine 1% was applied to the anterior cervical region, prior to a surgical tracheostomy using a 2.5-mm uncuffed tube (2.5 mm Portex, Smiths Medical, Kent, UK). The experimental procedures described below were performed under mechanical ventilation, using the pressure-controlled mode with a VT of 8 ml/kg, a respiratory rate to achieve normocapnia (5.5–6.0% end tidal CO_2), a PEEP of 3 cmH_2O and FiO2 of 0.4. A

continuous infusion of atracurium (0.6 mg/kg/h) was initiated to induce neuromuscular blockade once proper depth of anesthesia was ensured.

The left femoral artery and right jugular vein were cannulated with a 20 G catheter (Abbocath, Abbot Medical, Baar/Zug, Switzerland) for invasive blood pressure measurements as well as, arterial and central venous blood gas sampling. Electrocardiogram was recorded using subcutaneous needle electrodes. Tracheal pressure, airflow, arterial pressure, central venous pressure (CVP) and ECG were digitized (sampling rate 1 kHz) and continuously recorded (ADInstruments, Powerlab model 8/35 and LabChart 7, Dunedin, New Zealand). Mean arterial pressure (MAP) and heart rate (HR) were calculated from the recorded traces.

Measurement of respiratory mechanical parameters

Respiratory mechanical parameters were assessed by the wave-tube method of the forced oscillation technique, as detailed previously (93). Briefly, a loudspeaker-in-a-box system was connected to the tracheal tube using a polyethylene wave-tube with known geometrical properties (length: 100 cm, internal diameter: 0.193 cm). A small amplitude (±1 cmH2O peak-to-peak pressure) forcing signal was generated by the loudspeaker and introduced to the respiratory system by short, 8-s long periods of apnea. The wave-tube and the chamber of the loudspeaker were pressurized to the level of PEEP to maintain the same mean airway pressure during the recordings. Two identical pressure transducers (Honeywell Differential Pressure Sensor model 24PCEFA6D, Charlotte, North Carolina, USA) were connected to the two ends of the wave-tube and lateral pressures were recorded using low-pass filtering at 25 Hz. Pressure signals were digitized at 256 Hz using an analog–digital data acquisition board (NI USB-6211, National Instruments, Austin, TX, USA). Input impedance of the respiratory system (Zrs) was calculated from a fast Fourier transformation of the transfer function of the lateral pressures

(147). Three to four comparable recordings were performed at each experimental step and the spectra obtained from these recordings were ensemble-averaged for further analysis.

Airway and tissue compartments of the respiratory system were separated by fitting the constant-phase model (148) to the spectra using a global optimization method. The model consists of an airway compartment with resistive (Raw, airway resistance) and inertive components (Iaw, airway inertance) in series with a constant-phase tissue compartment, including tissue damping (G) and tissue elastance (H). As established previously (149), Raw reflects the flow resistance of the central conductive airways, Iaw is related to the acceleration and deceleration of the air column in the central airways, tissue damping characterizes the energy loss within the respiratory tissues, and tissue elastance describes the energy storage properties of the respiratory tissues (elastance). The impedance of the instrumental dead space (including the measurement circuit and the tracheal tube) was measured and subtracted from the Zrs spectra before model fitting.

Blood gas analyses

Arterial and venous blood samples were analyzed by a point-of-care blood gas analyzer (i-Stat, Abbott Laboratories, Chicago, IL, USA), and PaO_2, $PaCO_2$, pH and lactate concentrations were determined. Oxygenation index was calculated as PaO_2/FiO_2.

Application of physiologically variable ventilation

A commercially available pediatric ventilator (Servo-i, Maquet Critical Care, Solna, Sweden) was used with special firmware. The ventilation pattern was applied via custom-made computer software in a looped manner. The applied variable pattern was the reproduction of physiological breathing in rabbit pups, obtained from a randomly selected rabbit pup using unconstrained whole-body plethysmograph. The rabbit was placed in a sealed transparent box that had an intentional leak to allow continuous supply of fresh gas. A pressure transducer was

connected to the box (Honeywell Differential Pressure Sensor model 24PCEFA6D, Charlotte, North Carolina, USA) and the signal was digitized at 1 kHz (ADInstruments, Powerlab model 8/35 and LabChart 7, Dunedin, New Zealand) along with the feed of a digital camera pointed at the box. The internal temperature and humidity of the box were recorded. The rabbit was placed twice daily in the box for 40 min on five consecutive days to allow it to become accustomed to the box environment. Data recorded during the last 40-min period was used. Post-processing of the data included deletion data segments in which movement artefacts (verified by the recording of the camera feed) and sniffing behavior occurred. The resulting 10-min recording of spontaneous breathing was used as a driving pattern of the variable ventilation. Replicates with different average respiratory rates were created of a single pattern, by retaining the original ratios of breath-to-breath pressure and frequency. An inspiratory to expiratory (I:E) time ratio of 1:2 was used. The ventilation was adjusted to achieve an overall average VT of 8 ml/kg and an overall rate was selected to achieve normocapnia (5.5–6.0% end tidal CO_2). Ventilation was checked and adjusted hourly, if necessary. The applied variable ventilatory pattern is presented in Figure S1, along with its characteristics throughout the study in Figures S2 and S3.

Lung imaging

Structural, perfusion and metabolic status of the respiratory system were assessed by *in vivo* imaging. A small animal tri-modal imaging device (Triumph, TriFoil Imaging, Chatsworth, CA, USA) was used for all imaging acquisition. After 5 h of mechanical ventilation, animals were transferred to the imaging device in supine position to acquire CT imaging of the lung. To assess the regional distribution of lung perfusion, ^{99m}Tc-labeled albumin macroaggregates (mean activity 33.3±3.9 MBq/kg), were injected in the jugular vein, and SPECT imaging of the lung was performed 15 min later. Finally to assess the regional distribution of inflammatory

activity (150), fluorodeoxyglucose (^{18}F-FDG, mean activity 35.2±3.0 MBq/kg), was injected into the ear vein. Following a 60-min uptake period, PET imaging of the lung was acquired.

Image analysis

During post-processing, images obtained with CT, PET and SPECT were spatially registered and the lung was semi-automatically segmented using ITK-Snap (*itksnap.org*). Binary masks were created based on the density histogram of the CT, where the thresholds between three zones were manually marked (well aerated, poorly aerated and non-aerated). These masks were later applied to the PET and SPECT images. Lung radiodensity as well as PET and SPECT activity were calculated and compared in these three regions and also in the dorsal and ventral half of the lung using a custom-made script written in MATLAB (version R2018a, Mathworks Inc, Natick, MA, USA). PET and SPECT activity were expressed as standardized uptake value (SUV), after corrections for radioactive decay occurring after injection and before detection and for animal body weight. SUV values were normalized for the voxelwise fraction of lung tissue, as described previously (151) to more appropriately represent the metabolic activity of the lung tissue regardless of the degree of aeration (151, 152).

Bronchoalveolar lavage

The cell content of the bronchoalveolar lavage fluid (BALF) was analyzed as detailed previously (125). Following euthanasia and clamping of the left main bronchus, a small catheter was introduced into the right bronchus through the tracheal tube. Pre-heated (38°C) phosphate-buffered saline (PBS) with 1% bovine serum albumin (BSA) was used to wash the left lung. The injected BALF was recovered gently using gravity as fully as possible and was centrifuged at 412 G for 5 min at 5°C and the supernatant stored at –20°C until analysis. The cell pellet was re-suspended in PBS/BSA and Cytospin preparations were obtained by centrifugation at 58 *G* for 7 min. The slides were fixed, and May-Grünwald-Giemsa staining

was applied for differential cell counting. Cells were counted using image acquisition software (Panoramic viewer, 3DHISTECH Ltd, Budapest, Hungary). As the distribution of the cells was not homogeneous, the cells were counted within rectangles with an edge length equivalent to the radius of the circular Cytospin. The number of cells was normalized to the surface area of the rectangles. Enzyme-linked immunosorbent assay (ELISA) analysis was performed on undiluted BALF supernatant to assess the presence of the inflammatory cytokines TNF-α (MyBiosource MBS2021700, San Diego, CA, USA), IL-1β, IL-6 and IL-8 (Raybiotech Norcross, GA, USA). Measurements were performed according to the manufacturer's instructions.

Lung histology

Formaldehyde, 4%, at a hydrostatic pressure of 20 cmH_2O was filled into the left lung. Apical, middle and basal lobe regions of the left lung were excised and fixed before embedding them in paraffin. Lung tissue sections (5 µm) were stained with hematoxylin and eosin. An expert technician who was blinded to group allocations performed the analysis in accordance with American Thoracic Society guidelines (145). Histological status of the lung was quantified using scores for the presence of neutrophils in the alveolar and interstitial spaces, the presence of hyaline membranes, proteinaceous debris filling the airspaces and alveolar septal thickening. A histology score was determined for each lung region separately (apical, middle and basal), and averaged to obtain an overall lung injury score.

Sample size

Since the primary outcomes included changes in respiratory tissue elastance (H), the sample size was estimated based on our previous data on this parameter (125) to detect 20% between-group differences; assuming a coefficient of variation of 15% in the injured lung and 8% in the healthy lungs, a statistical power of 0.8 and 2-sided alpha error of 0.05. The estimation resulted

in a required sample size of 9 rabbits for the injured groups. Considering the smaller variability in the healthy rabbits, a sample size of 6 was calculated for these groups. To take into account potential drop-outs, we included 7 animals in each healthy group and 12 animals in each injured group.

Allocating animals to experimental groups

A block randomization procedure was used to assign the animals to one of the four experimental groups. The website randomizer.org was used to generate the blocks.

Statistical methods

Data are presented as mean ± half-width of 95% confidence interval. Normality of the data was assessed for each variable with the Shapiro-Wilk test. In case of a failed normality test, the variable was log-transformed. Three-way repeated measures analyses of variance (ANOVA) using linear mixed-effect model fits by a restricted maximum likelihood (REML) method were applied to analyze respiratory mechanical, blood gas, and cytokine data with factors ventilation mode (PCV or PVV), injury (healthy or ARDS) and time. In case of a significant test, Dunnett's post-hoc tests were used for time (using T0 as a reference level), ventilation mode (using PCV as reference) and injury (using control as reference). Imaging parameters were analyzed using three-way repeated measures ANOVA with factors ventilation mode, injury and aeration zone or position (dorsal or ventral). Histology lung injury score, global CT density, cytokine levels and BALF cell counts were analyzed using two-way ANOVA with Holm-Sidak post-hoc tests using injury and ventilation mode as between-group variables. The statistical tests were performed within the R environment with the *lme4, lsmeans* and *stats* packages and SigmaPlot (version 13, Systat Software, Inc. Chicago, IL, USA). The statistical tests were performed with a significance level of $p < 0.05$, and all p values were two-sided.

RESULTS

Respiratory mechanics

Parameters characterizing respiratory mechanics obtained prior to initiating the 5-hour ventilation are displayed in Table S1. No significant differences were observed in any parameter between the study groups under the BL conditions. Induction of lung injury led to significant increases in tissue damping and elastance in groups PCV-ARDS and PVV-ARDS ($p<0.001$) with no difference between the two ARDS groups.

Morphological findings

Lung injury scores, summarized in Table S2, were significantly elevated in animals with ARDS (0.79±0.01, 0.78±0.01 for the PCV-ARDS and PVV-ARDS groups, respectively) compared to those with healthy lungs (0.43±0.02 for both control groups, $p<0.05$). Differences in lung injury scores were also observed for the global CT density, which reflects the amount of well aerated zones, with values being significantly higher in the ARDS groups (375±32 mean pixel value (MPV) and 382±31 MPV for PCV-ARDS and PVV-ARDS groups, respectively) than those obtained in healthy controls (306±51 MPV and 298±23MPV for PCV-CTRL and PVV-CTRL groups, respectively, $p<0.05$).

Molecular and cellular content of bronchoalveolar lavage fluid

The results of the cellular and molecular evaluation of lung injury are summarized in Table S3. Differences in the cytokine and cell content of BALF were observed between ARDS and CTRL groups. No differences based on ventilation mode were detected.

Ancillary analysis

Ventilation parameters characterizing the 5-hour experimental period (VT, RR) are depicted in Figures S1-S3. Significantly lower values of inspiratory driving pressure (IDP) were required

to maintain the same minute ventilation in healthy animals ($p < 0.01$, ARDS vs. CTRL), with a possibility of progressive reduction in IDP in the PVV-CTRL group ($p < 0.01$ vs. T0). Such reduction was not observed in the PCV-CTRL group. No differences were detected in the hemodynamic parameters (MAP, CVP, HR) between the ventilation modes during the 5-hour ventilation period (Figure S4).

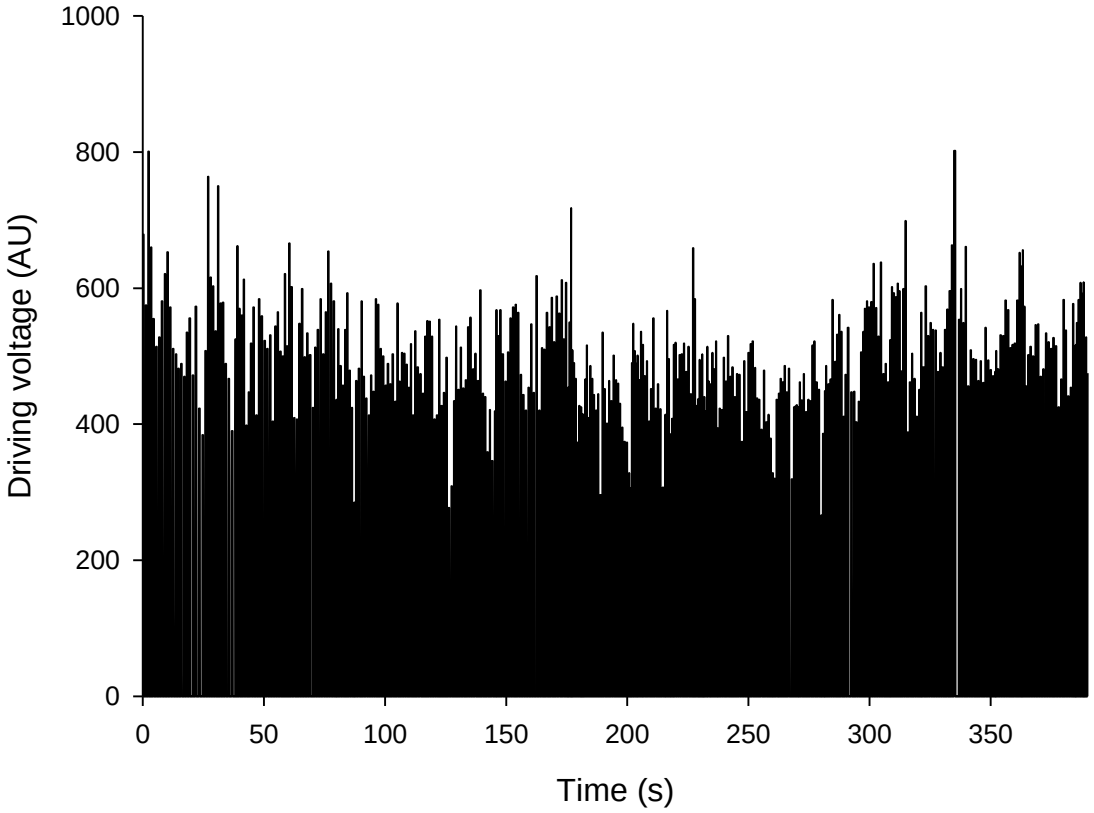

Figure S1

Trace of the physiological breathing pattern applied in groups PVV-ARDS and PVV-CTRL during the 5-hour ventilation period. AU: arbitrary unit.

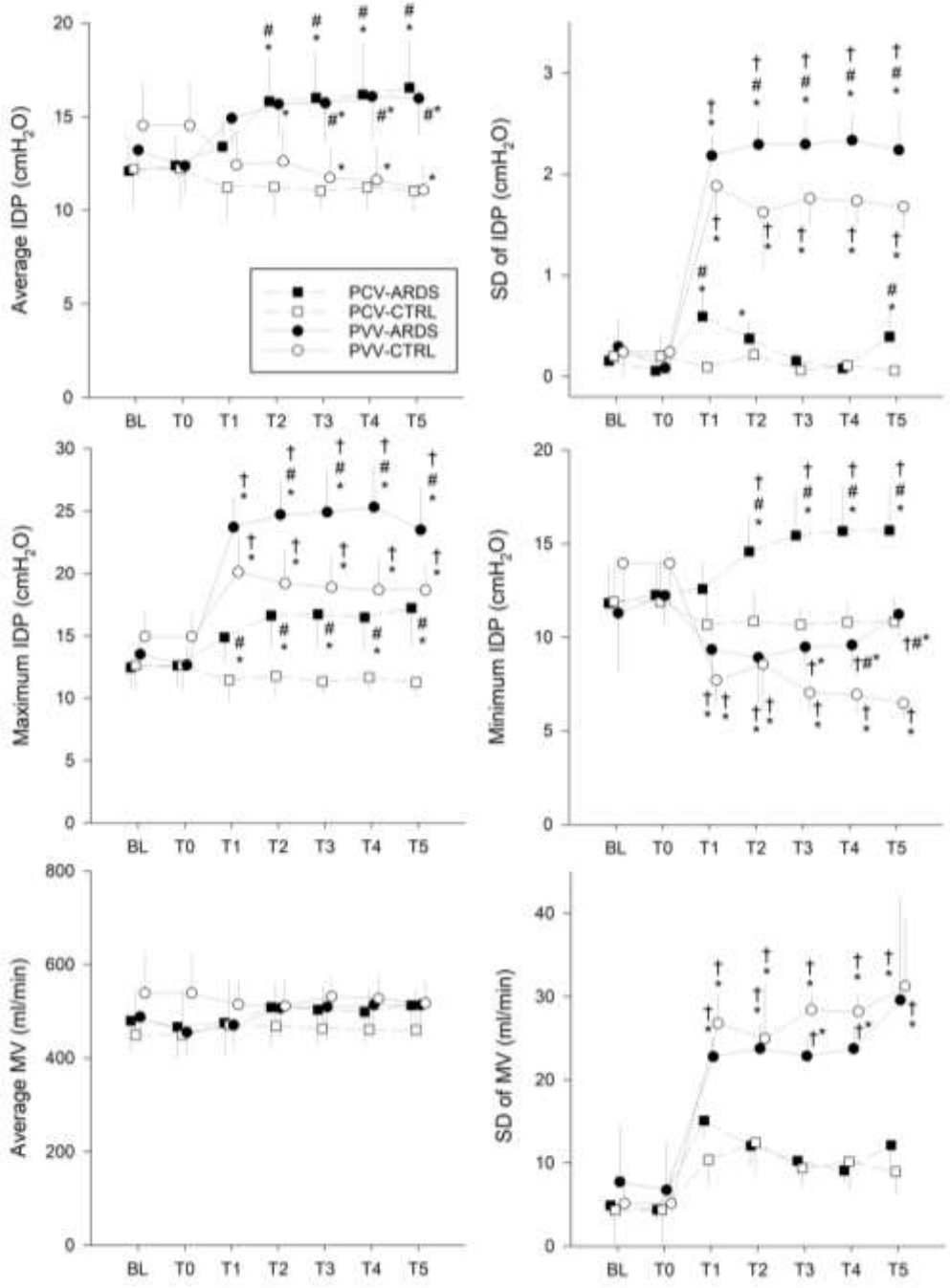

Figure S2

Ventilation parameters obtained before and during the 5-hour long ventilation period. Values expressed as mean ± half-width of 95% confidence interval.

IDP: inspiratory driving pressure, MV: minute ventilation, SD: standard deviation. BL: baseline, T0: immediately after induction of lung injury, T1-T5: average value during the corresponding hour of the 5-hour long ventilation period. PCV: pressure-controlled ventilation, PVV: physiological variable ventilation, ARDS: presence of lung injury, CTRL: absence of lung injury.

*: $p < 0.05$ vs. T0, #: $p < 0.05$ vs. CTRL, †: $p < 0.05$ vs. PCV.

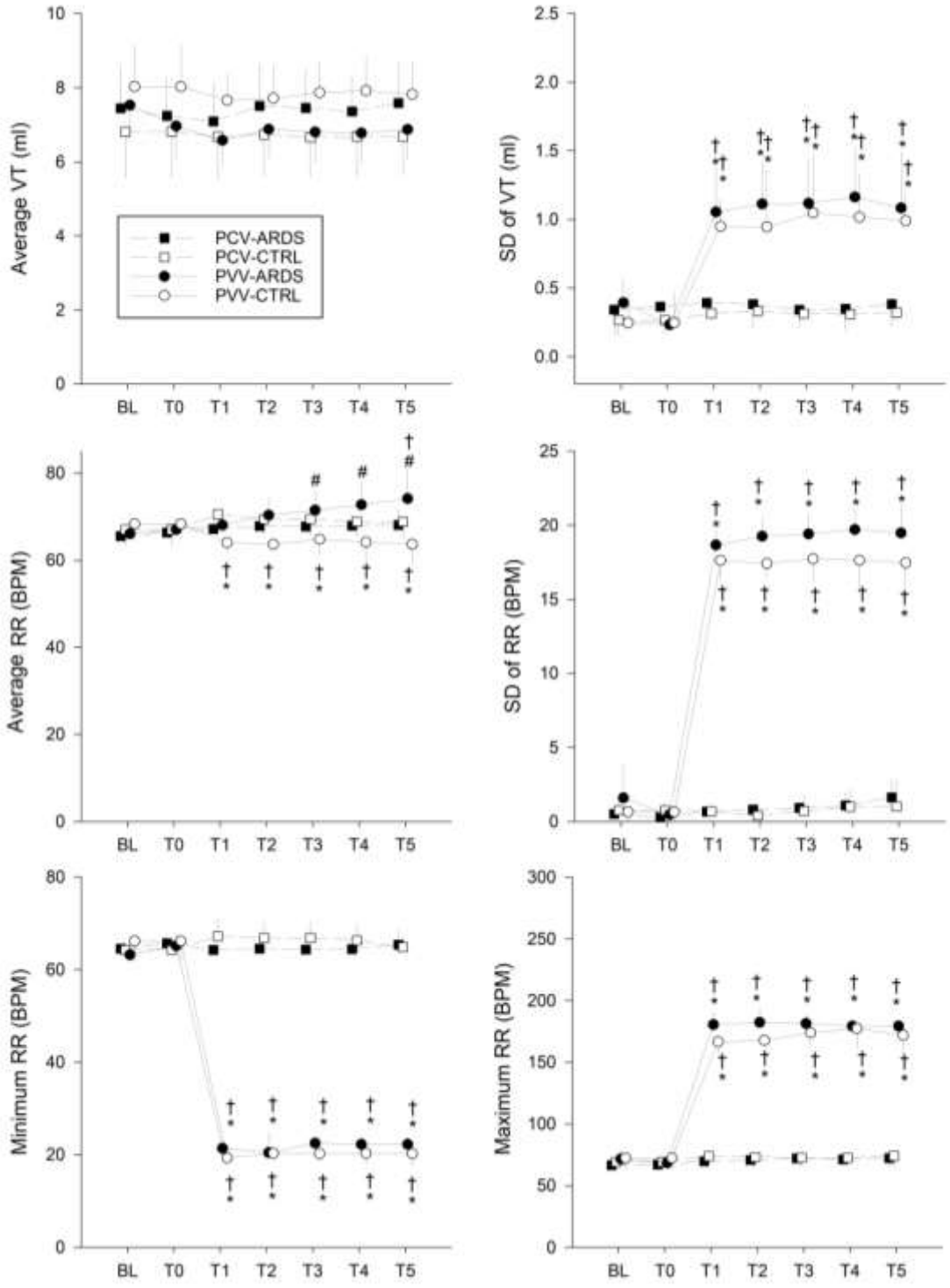

Figure S3

Ventilation parameters obtained before and during the 5-hour long ventilation period. Values expressed as mean ± half-width of 95% confidence interval.

VT: tidal volume, RR: respiratory rate, BPM: breath per minute, SD: standard deviation.

BL: baseline, T0: immediately after induction of lung injury, 1H-5H: average value during the corresponding hour of the 5-hour long ventilation period. PCV: pressure-controlled ventilation,

PVV: physiological variable ventilation, ARDS: presence of lung injury, CTRL: absence of lung injury.

*: $p < 0.05$ vs. T0, #: $p < 0.05$ vs. CTRL, †: $p < 0.05$ vs. PCV, n.s.: not significant.

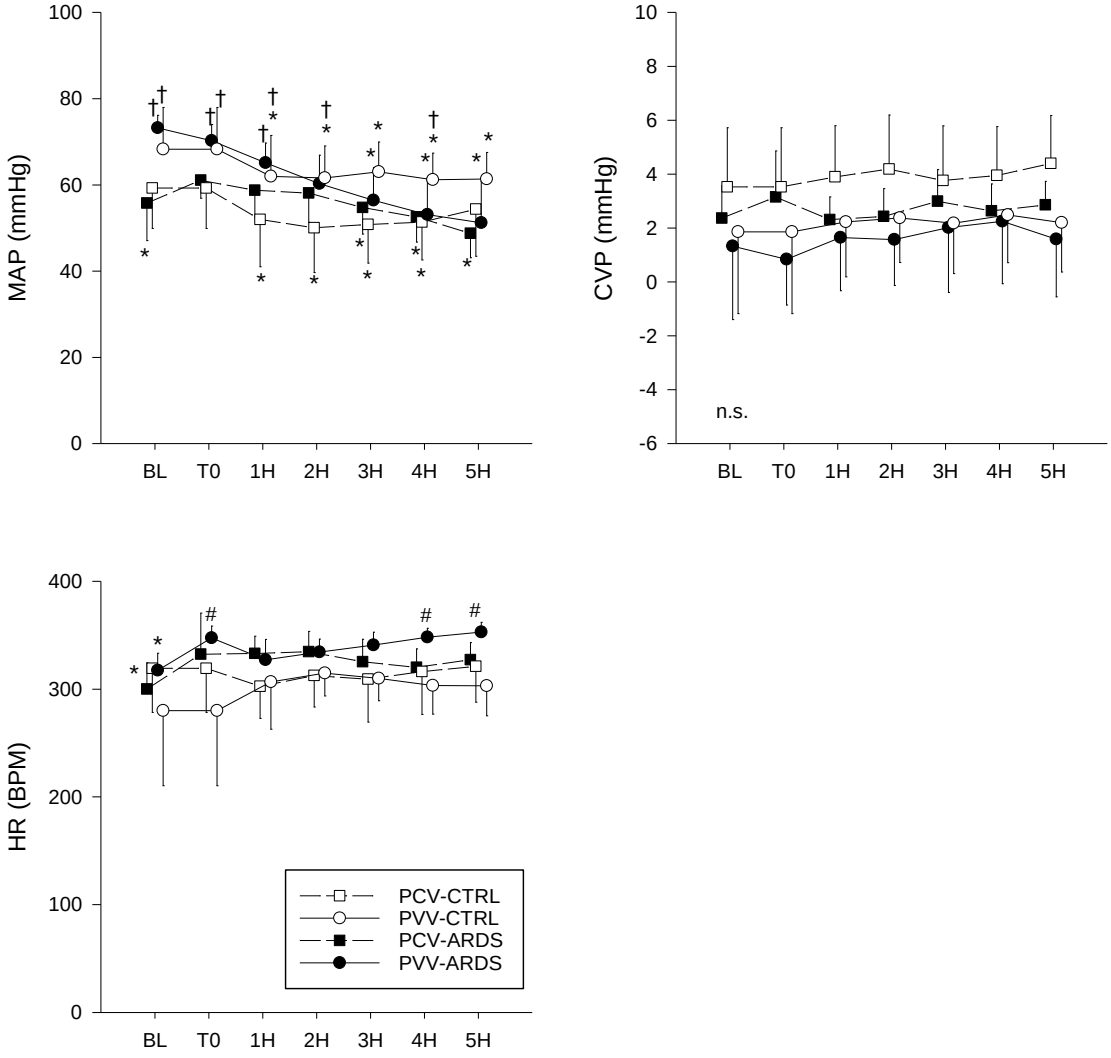

Figure S4

Haemodynamic parameters obtained before and during the 5-hour long ventilation period. Values expressed as mean ± half-width of 95% confidence interval.

MAP: mean arterial pressure, CVP: central venous pressure, HR: heart rate, BPM: beat per minute. BL: baseline, T0: immediately after induction of lung injury, 1H-5H: average value during the corresponding hour of the 5-hour long ventilation period. PCV: pressure-controlled ventilation, PVV: physiological variable ventilation, ARDS: presence of lung injury, CTRL: absence of lung injury.

*: $p < 0.05$ vs. T0, #: $p < 0.05$ vs. CTRL, †: $p < 0.05$ vs. PCV, n.s.: not significant.

	PCV-CTRL		PVV-CTRL		PCV-ARDS		PVV-ARDS	
	BL	T0	BL	T0	BL	T0	BL	T0
Raw ($cmH_2O.s/l$)	36.5±9.7	36.5±9.7	34.2±6.4	34.2±6.4	37.2±4.4	34.1±5.4	38.5±3.5	36.8±3.7
G (cmH_2O/l)	315±81	315±81	245±35	245±35	293±49	336±52 *	291±33	338±33 *#
H (cmH_2O/l)	1028±174	1028±174	894±151	894±151	955±104	1291±181*#	958±122	1353±75 *#

Table S1

Values of respiratory mechanical parameters at baseline (BL) and immediately after induction of lung injury (T0). Values expressed as mean ± half-width of 95% confidence interval.

Raw: airway resistance, G: respiratory tissue damping, H: respiratory tissue elastance.

PCV: pressure-controlled ventilation, PVV: physiological variable ventilation, ARDS: presence of lung injury, CTRL: absence of lung injury.

*: $p < 0.05$ vs. BL, #: $p < 0.05$ vs. CTRL

	PCV-CTRL	PVV-CTRL	PCV-ARDS	PVV-ARDS
Global CT density (AU)	306±51	298±23	375±32 #	382±31 #
Lung injury score	0.43±0.02	0.43±0.02	0.79±0.01 #	0.78±0.01 #

Table S2

Morphological indices of lung injury. Values expressed as mean ± half-width of 95% confidence interval. AU: arbitrary unit.

PCV: pressure-controlled ventilation, PVV: physiological variable ventilation, ARDS: presence of lung injury, CTRL: absence of lung injury.

#: $p < 0.05$ vs. CTRL

	PCV-CTRL	PVV-CTRL	PCV-ARDS	PVV-ARDS
IL-1β (pg/ml)	116.5±32.1	126.2±37.8	168.5±7.6 #	170.7±17.5 #
IL-6 (pg/ml)	3.17±1.89	4.93±5.77	8.67±2.89 #	9.93±2.77 #
IL-8 (pg/ml)	113.0±49.3	164.8±79.8	245.5±11.6 #	242.8±19.3 #
TNF-a (pg/ml)	15.01±3.29	23.18±24.86	18.38±1.35	22.99±10.51
Protein (mg/ml)	0.13±0.17	0.49±1.09	0.78±0.25 #	1.00±0.44 #
Macrophages (%)	79.1±11.4	67.5±7.1	40.5±12.5 #	34.0±10.4 #
Neutrophils (%)	12.4±7.4	19.5±9.1	51.9±14.3 #	60.1±11.0 #
Lymphocytes (%)	8.3±5.3	12.4±4.0	7.3±3.2	5.5±1.3 #

Table S3

Cytokine and cell content of the bronchoalveolar lavage fluid. Values expressed as mean ± half-width of 95% confidence interval.

PCV: pressure-controlled ventilation, PVV: physiological variable ventilation, ARDS: presence of lung injury, CTRL: absence of lung injury.

#: $p < 0.05$ vs. CTRL.

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Respiration and the Airway

RESPIRATION AND THE AIRWAY

Physiologically variable ventilation in a rabbit model of asthma exacerbation

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Abstract

Background: Mechanical ventilation during status asthmaticus is challenging and increases the risk of severe complications. We recently reported the value of physiologically variable ventilation (PVV) in healthy and acutely injured lungs. We investigated whether PVV provides benefits compared with pressure-controlled ventilation (PCV) in an experimental model of severe acute asthma.

Methods: Allergen-sensitised rabbits were anaesthetised and randomised to either PCV (n=10) or PVV (n=12) during sustained bronchoconstriction induced by allergen and cholinergic stimuli for 6 h. The PVV pattern was generated from pre-recorded spontaneous breathing. Ventilation parameters, oxygenation index (PaO_2/FiO_2), and respiratory mechanics were measured hourly. Histological injury and inflammation were quantified after 6 h of ventilation.

Results: PVV resulted in lower driving pressures (13.7 cm H_2O [12.5–14.9], mean [95% confidence interval]), compared with pressure-controlled ventilation (17.6 cm H_2O [15.4–19.8]; P=0.002). PVV improved PaO_2/FiO_2 (PVV: 55.1 kPa [52–58.2]; PCV: 45.6 kPa [39.3–51.9]; P=0.018) and maintained tissue elastance (PVV: +8.7% [−0.6 to 18]; PCV: −11.2% [−17.3 to −5.1]; P=0.03). PVV resulted in less lung injury as assessed by lower histological injury score (PVV: 0.65 [0.62–0.65]; PCV: 0.71 [0.69–0.73]; P=0.003), cell count (PVV: 247 10^4 ml^{-1} [189–305]; PCV: 447 10^4 ml^{-1} [324–570]; P=0.005), and protein concentration in bronchoalveolar lavage fluid (PVV: 0.14 μg ml^{-1} [0.10–0.18]; PCV: 0.21 μg ml^{-1} [0.15–0.27]; P=0.035).

Conclusions: Applying physiological variable ventilation in a model of asthma exacerbation led to improvements in gas exchange, ventilatory pressures, and respiratory tissue mechanics, and reduced lung injury. A global reduction in lung shear stress and recruitment effects may explain the benefits of PVV in status asthmaticus.

Keywords: gas exchange; lung injury; mechanical ventilation; respiratory mechanics; status asthmaticus

Editor's key points

- Acute asthma attacks requiring mechanical ventilation are frequently complicated by barotrauma and further lung injury.
- Physiologically variable ventilation may confer lung protection in severe acute asthma.
- In an experimental laboratory model of severe asthma, allergen-sensitised, anaesthetised rabbits were randomised to either pressure-controlled ventilation or physiologically variable ventilation (generated from pre-recorded spontaneous breathing).
- Physiologically variable ventilation resulted in lower driving pressures, improved PaO_2/FiO_2, and reduced histological and biochemical markers of lung injury.
- Physiologically variable ventilation in a model of asthma exacerbation led to improvements in gas exchange, ventilatory pressures, and respiratory tissue mechanics, and reduced lung injury.

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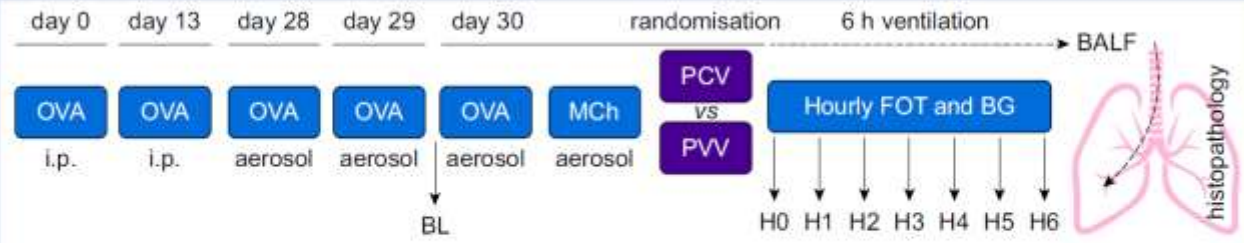


Fig 1. Schematic representation of the experimental protocol. BALF, bronchoalveolar lavage fluid; BG, blood gas; BL, baseline; FOT, forced oscillation technique; H0–H6, measurement time points at hour 0 to hour 6; MCh, methacholine; OVA, ovalbumin; PCV, pressure-controlled ventilation; PVV, physiologically variable ventilation.

Asthma is a major public health concern worldwide, with significant morbidity and mortality.[1,2] Severe exacerbations require mechanical ventilation in up to 4% of the patients with asthma.[3,4] Optimisation of the ventilator settings in acute severe asthma is a major challenge because of a high risk of inducing barotrauma and subsequent lung injury caused by dynamic hyperinflation and high inspiratory pressures.[5,6] Therefore, optimising ventilation modalities may reduce morbidity during asthma exacerbations.

Conventional volume- or pressure-controlled ventilation modes did not improve outcomes in the clinical setting of asthma exacerbation.[7] However, preclinical data suggest variable ventilation provides benefit that conventional modes do not for both healthy[8–10] and injured lungs.[11–16] In contrast to conventional ventilation, variable ventilatory modes were shown to have beneficial effects on respiratory mechanical and functional parameters,[8–14,17] lung recruitment,[18,19] and histological damage.[20] We have demonstrated the advantages of physiologically variable ventilation (PVV) based on pre-recorded spontaneous breath-by-breath variation of tidal volume and ventilatory frequency over mathematically derived variability in injured lungs.[11] However, the benefits of PVV in the context of acute asthma exacerbations have not yet been characterised.

In this study, we hypothesised that prolonged application of PVV has advantages over pressure-controlled ventilation (PCV) with respect to gas exchange, respiratory mechanics, and ventilation pressures during acute exacerbation of asthma. We compared PVV with PCV in a well-established experimental model of acute severe asthma.

Methods

Study design

The experimental protocol was approved by the Animal Welfare Committee of the Canton of Geneva and the Experimental Ethics Committee of the University of Geneva, Switzerland (no. GE 99/18, 19 July 2018). All procedures were performed according to the current animal protection laws of Switzerland (LPA, RS455). The current report follows the ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines. New Zealand White rabbits of both sexes (seven males, 20 females; weight 2.5kg [0.24]; mean [95% confidence interval]), aged 8–10 weeks, were purchased from the farm of University of Geneva (Arare, Geneva, Switzerland) and were delivered at least 2 days before the experiments to allow acclimatisation. The rabbits had access to food and water *ad libitum* before the experiments.

Model of developing susceptibility to acute severe asthma

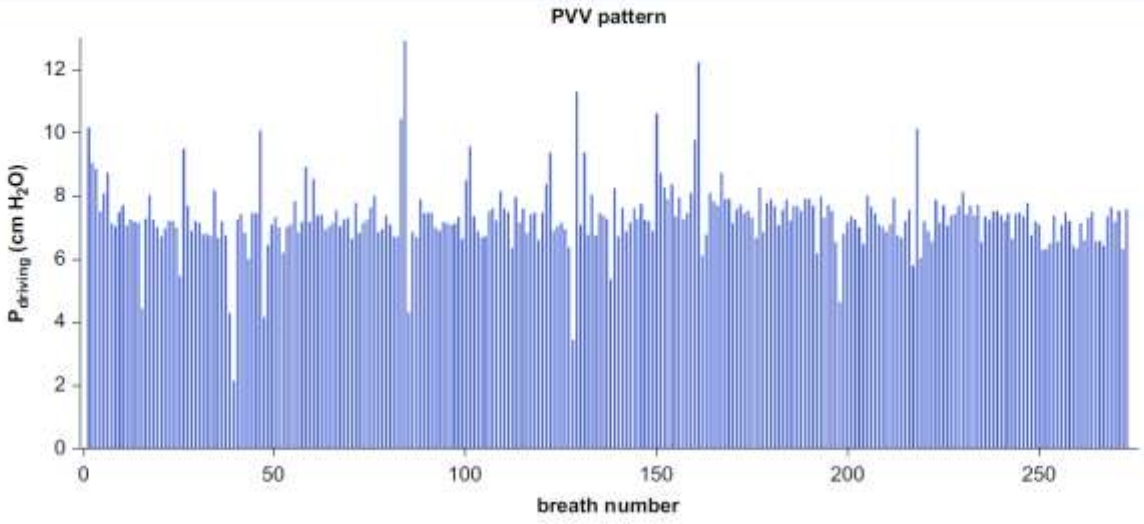
Rabbits were sensitised via intraperitoneal injections of 0.1 mg ovalbumin (OVA; Sigma Aldrich, Buchs, Switzerland) and 10 mg aluminium hydroxide (Sigma Aldrich) on days 0 and 13 (Fig. 1). Rabbits received nebulised OVA 50 mg (2.5 mg ml^{-1}) through a face mask under spontaneous breathing, from days 28–30, using an ultrasonic Nebuliser (SYST'AM LS2000, Ledat, France). On day 30, animals were anaesthetised, tracheotomised, and ventilated initially with volume-controlled mode (7 ml kg^{-1}) using a neonatal ventilator (Servo-i; Maquet Critical Care, Solna, Sweden) and were then randomised to receive 6 h of either PVV or PCV.

Anaesthesia and surgical preparation

On day 30 (Fig. 1), anaesthesia was induced with intramuscular ketamine (25 mg kg^{-1}) and xylazine (3 mg kg^{-1}). Anaesthesia was maintained with continuous intravenous (i.v.) propofol (10 mg kg^{-1} h^{-1}), fentanyl (5 μg kg^{-1} h^{-1}), and midazolam (0.2 mg kg^{-1} h^{-1}) through a 24 G catheter (Abbocath; Abbot Medical, Baar/Zug, Switzerland) inserted in the ear vein. Adequacy of anaesthesia was ensured by absence of spontaneous movement to painful stimulation and cardiovascular parameters of sympathetic activity (stable heart rate and arterial blood pressure). Body temperature was monitored through a rectal thermometer and maintained at 38–39°C using a thermostatic heating pad (Harvard Apparatus, South Natick, MA, USA). A surgical tracheostomy using a 3.5 mm uncuffed tube (3.5 mm Portex; Smiths Medical, Kent, UK) was performed after infiltration of the anterior cervical region with lidocaine 1% (Sintetica, Mendrisio, Switzerland). After ensuring adequate anaesthesia and analgesia, continuous i.v. atracurium (0.6 mg kg^{-1} h^{-1}) was administered for neuromuscular block. The left femoral artery was cannulated with a 20 G catheter (Abbocath; Abbot Medical) for arterial blood sampling and invasive pressure measurements.

Measurement of respiratory mechanical parameters

Respiratory mechanical parameters were assessed using the wave-tube method for the forced oscillation technique, as described previously.[21] The airway and tissue compartments



	V_T (ml kg^{-1})		RR (min^{-1})	
	PCV	PVV	PCV	PVV
mean	6.8 (0.3)	6.6 (0.1)	38.6 (4.2)	34.7 (3.1)
CV (%)	1.6 (0.2)	8.3 (0.2)	0.8 (0.3)	15.1 (1.5)
minimum	6.5 (0.3)	1.9 (0.2)	37.6 (3.9)	16.1 (1.2)
maximum	7.1 (0.4)	8.8 (0.1)	39.5 (4.2)	61.1 (4.3)

Fig 2. Changes in driving pressure ($P_{driving}$) during the application of a physiologically variable ventilation (PVV) pattern based on pre-recorded spontaneous breathing in a representative rabbit (top). Characteristics of the ventilation variables for both modes of ventilation (CV, coefficient of variation; PCV, pressure-controlled ventilation; RR, respiratory rate; V_T, tidal volume).

of the respiratory system were separated by fitting a well-validated model[22] to the obtained spectra. The model consists of an airway compartment with a resistive (Raw, airway resistance) and an inertive component (Iaw, airway inertance) in series with a constant-phase tissue compartment including tissue damping (G) and tissue elastance (H). As previously shown,[23] Raw reflects the flow resistance of the central airways, Iaw relates to the acceleration and deceleration of air in the central airways, G characterises the energy loss in the respiratory tissues, whereas H describes the energy storage properties of the tissues (elastance). The impedance of the instrumental apparatus was measured and subtracted from the total impedance spectra of the respiratory sythem.

Assessment of gas exchange

Arterial blood was analysed by a point-of-care blood gas analyser (i-Stat; Abbott Laboratories, Chicago, IL, USA). Partial pressure of oxygen (PaO_2), carbon dioxide ($PaCO_2$), and pH were measured. Oxygenation index was calculated as PaO_2 divided by the fraction of inspired oxygen (FiO_2).

Physiologically variable ventilation pattern

The PVV pattern was applied via a neonatal ventilator (Servo-i, Maquet Critical Care) with special firmware, using custom-made computer software. The PVV pattern was based on a recording of spontaneous breathing from healthy rabbits that were awake using whole-body plethysmography. A pressure transducer (Honeywell Differential Pressure Sensor model 24PCEFA6D; Honeywell, Charlotte, NC, USA) was connected to a custom-made sealed plexiglass box (external dimensions of 300×300×500 mm; internal volume 37.63 L) and the signal was digitised at 1 kHz (Powerlab model 8/35 and LabChart 7; ADInstruments, Dunedin, New Zealand) along with the feed of a digital camera. The box temperature and humidity were recorded. Rabbits were placed in the plethysmograph box for 30 min periods for 5 consecutive days so they can get accustomed to the environment. A 15 min recording of spontaneous breathing was obtained on the last day, and parts of the recording that corresponded to movement artifacts, as verified by the camera recording, were deleted before the recording was used. Replicates of the same pattern were created with different average ventilatory frequencies, maintaining the exact ratios of breath-to-breath pressure and frequency for each replicate. An inspiratory to expiratory (I/E) time ratio of 1:3 was used. The characteristics of the PVV pattern are summarised in Fig. 2.

Acute severe asthma protocol

On day 30, tracheal pressure, blood pressure, and electrocardiogram were continuously recorded (sampling rate 1 kHz, ADInstruments, Powerlab model 8/35, and LabChart 7) during

mechanical ventilation. Driving pressure ($P_{driving}$) was calculated as the difference between PEEP and peak inspiratory pressure. After establishing stable haemodynamic and respiratory conditions, two deep inflations (25 cm H_2O peak pressure maintained for 10 s each) were applied to normalise lung volume history. Respiratory mechanics were measured, and arterial blood samples were obtained for a white blood cell (WBC) differential count and for gas analysis at baseline (BL; Fig. 1).

OVA 5 mg (2.5 mg ml^{-1}) was nebulised through the tracheal tube using vibrating mesh technology (Aerogen® Solo Nebulizer System; Hamilton Medical, Bonaduz, Switzerland). Methacholine (250 μg ml^{-1}) was subsequently nebulised until a doubling of the airway resistance was achieved. At this stage (H0), animals were randomised to receive 6 h of either PCV or PVV. The ventilation parameters in both groups were set to a FiO_2 of 40%, a PEEP of 3 cm H_2O, an average tidal volume of 7 ml kg^{-1}, and a ventilatory frequency to achieve end-tidal CO_2 of 5.5–6%. Arterial blood gas and respiratory mechanics were assessed hourly (H1–H6) and peak inspiratory pressure was adjusted to maintain end-tidal CO_2 in the physiological range. After 6 h (H6), a blood sample was obtained for differential WBC count. Animals were euthanised by injecting a single intravenous dose of sodium thiopental (100 mg kg^{-1}). Bronchoalveolar lavage was performed *ex vivo* in the right lung, and the left lung was extracted for histological analyses.

Bronchoalveolar lavage

The cell content of the bronchoalveolar lavage fluid (BALF) was analysed as described elsewhere.[17] Enzyme-linked immunosorbent assays were performed according to the manufacturer's instructions on undiluted BALF supernatant and on frozen lung tissue homogenates to assess the presence of inflammatory cytokines TNF-α (MyBiosource MBS2021700; MyBioSource, Inc., San Diego, CA, USA), interleukin-6 (IL-6) and IL-8, (Raybiotech Norcross, GA, USA), surfactant protein B (SP–B; LSBio LS-F47557, LifeSpan BioSciences Inc., Muttenz, Switzerland), surfactant protein D (SP-D; Blue Gene Biotech E04S0170, Paris, France), and E-cadherin (LSBio LS-F43438; LifeSpan BioSciences Inc.).

Peripheral whole blood cell count

Arterial blood was sampled at baseline and H6 for total and differential cell count using an automated quantification with a pocH-100iV DIFF haematology analyser (Sysmex Digitana AG, Horgen, Switzerland).

Lung histology

Formaldehyde (4%) was filled into the left main bronchi at a hydrostatic pressure of 20 cm H_2O. Apical, middle, and basal lobe regions of the left lung were excised, fixed, and embedded in paraffin. Lung tissue sections (5 μm) were stained with haematoxylin–eosin. An expert technician blinded to group allocations quantified the lung injury score in accordance with American Thoracic Society guidelines,[24] considering the presence of neutrophils in the alveolar and interstitial spaces, hyaline membranes, proteinaceous debris filling the airspaces, and alveolar septal thickening. The overall histological injury score was calculated by averaging the score of each lung region (apical, middle, and basal).

Co-primary outcomes

The co-primary outcomes of the present study were the PaO_2/FiO_2 and tissue elastance (H).

Secondary outcomes

We assessed the following secondary outcomes:

1. Airway pressure ($P_{driving}$)
2. Respiratory mechanics (G and Raw)
3. Lung injury (histological injury scores; bronchoalveolar lavage cell counts; cytokine and protein levels; peripheral WBC count)

Statistical analysis

Data are presented as mean [95% confidence interval]. Normality of the data was assessed for each variable using the Shapiro–Wilk test, and logarithmic transformation was applied if necessary. Two-way repeated-measures analyses of variances were applied to analyse the absolute values of respiratory mechanics and blood gas parameters, using ventilation mode (PCV or PVV) and time (H0 to H6) as factors. In case of significance, Dunnett's *post hoc* test was used to assess significances for ventilation mode (using PCV as reference) and time (using H0 as a reference). Relative changes in all parameters between H0 and H6, blood cell counts, histological lung injury score, BALF cell counts, and cytokine levels were analysed using an unpaired *t*-test or a Mann–Whitney *U*-test, depending on normality. The statistical tests were performed using SigmaPlot (version 13; Systat Software, Inc., Chicago, IL, USA). Results were considered significant for a level of $P<0.05$, and all *P*-values are two-sided.

Sample size estimation

We estimated the sample size based on similar experimental conditions in rabbits for tissue elastance (H).[17] We aimed at detecting 20% between-group differences, assuming an inter-individual variation of 15%, a statistical power of 0.8 and a two-sided alpha error of 0.05. The calculation resulted in a sample size of 12 rabbits per group. Considering a potential 10% drop-out rate, we performed allergen sensitisation on 27 rabbits.

Results

Study population

Allergen sensitisation was performed on 27 animals. Five rabbits were excluded from the experimental protocol because of lethal bronchospasm at day 30. Consequently, 22 rabbits were randomised to receive 6 h of PCV ($n=10$) or PVV ($n=12$) after bronchoconstriction (Fig. 1). Haemodynamic parameters were similar at each time point during ventilation between the experimental groups (full details are available in Supplementary Fig. S1). Tidal volume, ventilatory frequency, FiO_2, and PEEP were kept constant during the 6 h ventilation in both study groups (Fig. 2).

Co-primary outcomes

PaO_2/FiO_2 levels

At baseline, PaO_2/FiO_2 levels were in the physiological range (Fig. 3). Inducing bronchoconstriction led to comparable

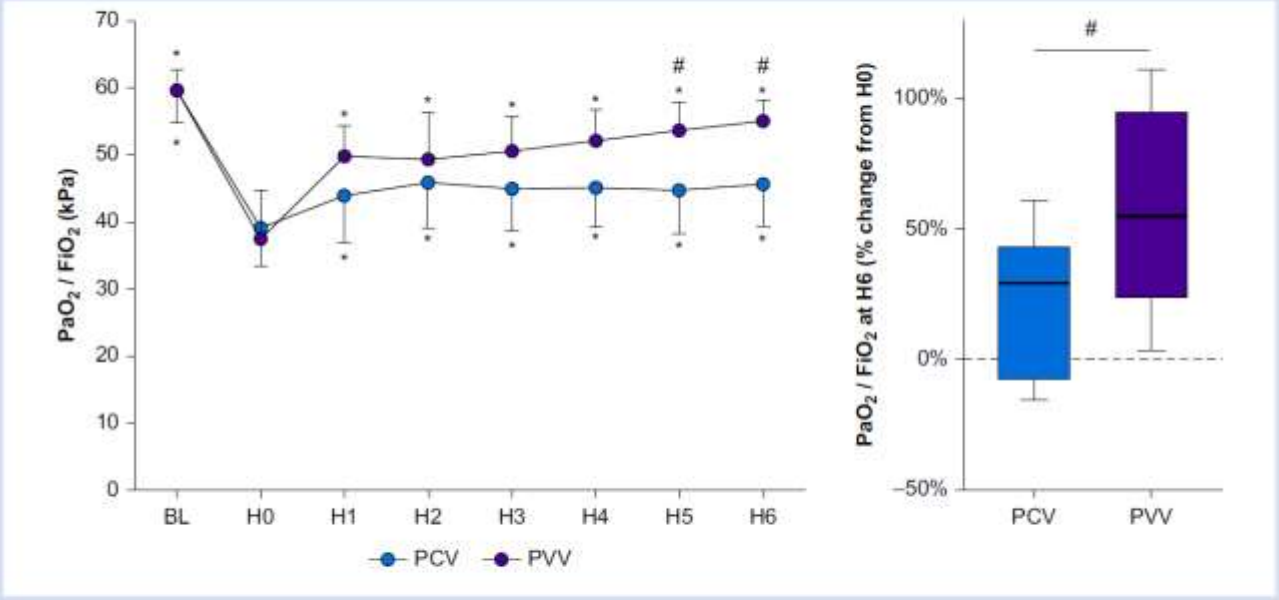


Fig 3. Absolute values of oxygenation index (left, PaO_2/FiO_2) measured at baseline (BL), immediately after induction of bronchoconstriction by ovalbumin and methacholine (H0) and during 6 h of ventilation (H1–H6). Results from physiologically variable ventilation (PVV; n=12) and pressure-controlled ventilation (PCV; n=10) are represented with filled and empty symbols, respectively. Values are expressed as mean (half-width of 95% confidence interval). Relative changes compared with H0 after the application of 6 h PCV or PVV are reported on the right graph. FiO_2, fraction of inspired oxygen; PaO_2, arterial partial pressure of oxygen. *$P<0.05$ *vs* H0; #$P<0.05$ *vs* PCV.

decreases in PaO_2/FiO_2 (H0). The ventilation mode significantly affected oxygenation, with higher PaO_2/FiO_2 at H5 and H6 in the PVV group ($P=0.018$; Fig. 3). No differences between PCV and PVV groups were observed after 6 h of ventilation in arterial pH (7.42 [0.02] *vs* 7.43 [0.03], respectively) or $PaCO_2$ (5.7 [0.3] kPa *vs* 5.6 [0.4] kPa), respectively).

Tissue elastance (H)

PVV decreased H ($P<0.001$) throughout the 6 h of ventilation, whereas PCV further increased H ($P=0.011$; Fig. 4, lower panel).

Secondary outcomes

Airway driving pressure

Nebulisation of OVA and methacholine increased $P_{driving}$ similarly in each group (Fig. 5). From H2 to H6, animals ventilated with PVV received lower $P_{driving}$ than those ventilated with PCV ($P=0.045$ and $P=0.002$ for H2 and H6, respectively; Fig. 4). The continuous monitoring of expiratory pressure and flow revealed no evidence for intrinsic PEEP or air trapping during the study period.

Respiratory mechanics

OVA and methacholine nebulisations approximately doubled the Raw and induced increases in G (Fig. 4). Raw remained elevated in both experimental groups throughout the 6 h ventilation, independent of the ventilatory mode. In comparison with H0, PCV did not alter G whereas PVV reduced G ($P=0.002$) throughout the 6 h of ventilation.

Lung injury

Rabbits receiving PVV had less lung injury, as adjudged by histological score compared with rabbits receiving PCV (Fig. 6; $P=0.003$). The total protein ($P=0.035$) and cell count in BALF ($P=0.005$) were also lower in animals ventilated with PVV (Fig. 6). Total and differential WBC counts in the blood were similar between the ventilation modes throughout the experimental protocol (Supplementary Fig. S2). Levels of inflammatory cytokines, surfactant proteins and E-cadherin were similar in each experimental group (full details are available in Supplementary Table S1).

Discussion

We found that prolonged ventilation with a physiologically variable mode was beneficial in the presence of sustained bronchoconstriction in a model of allergic asthma, as evidenced by improved gas exchange, reduced respiratory tissue stiffness and decreased ventilatory driving pressure. These functional benefits of PVV were also reflected in reduced histological injury score and lung inflammation.

We used an established rabbit model of allergy sensitisation, as it mimics the key molecular, cellular, and functional features of asthma.[25–32] Combining allergic and cholinergic stimulation led to sustained bronchoconstriction, with persistent elevation of airway resistance. In agreement with the literature, it is likely that the sustained bronchoconstriction resulted from the early and late phases of an allergic lung response.[30,31,33] This study was designed to induce sustained bronchoconstriction mimicking *status asthmaticus*. Although the sensitisation regimen using OVA might not

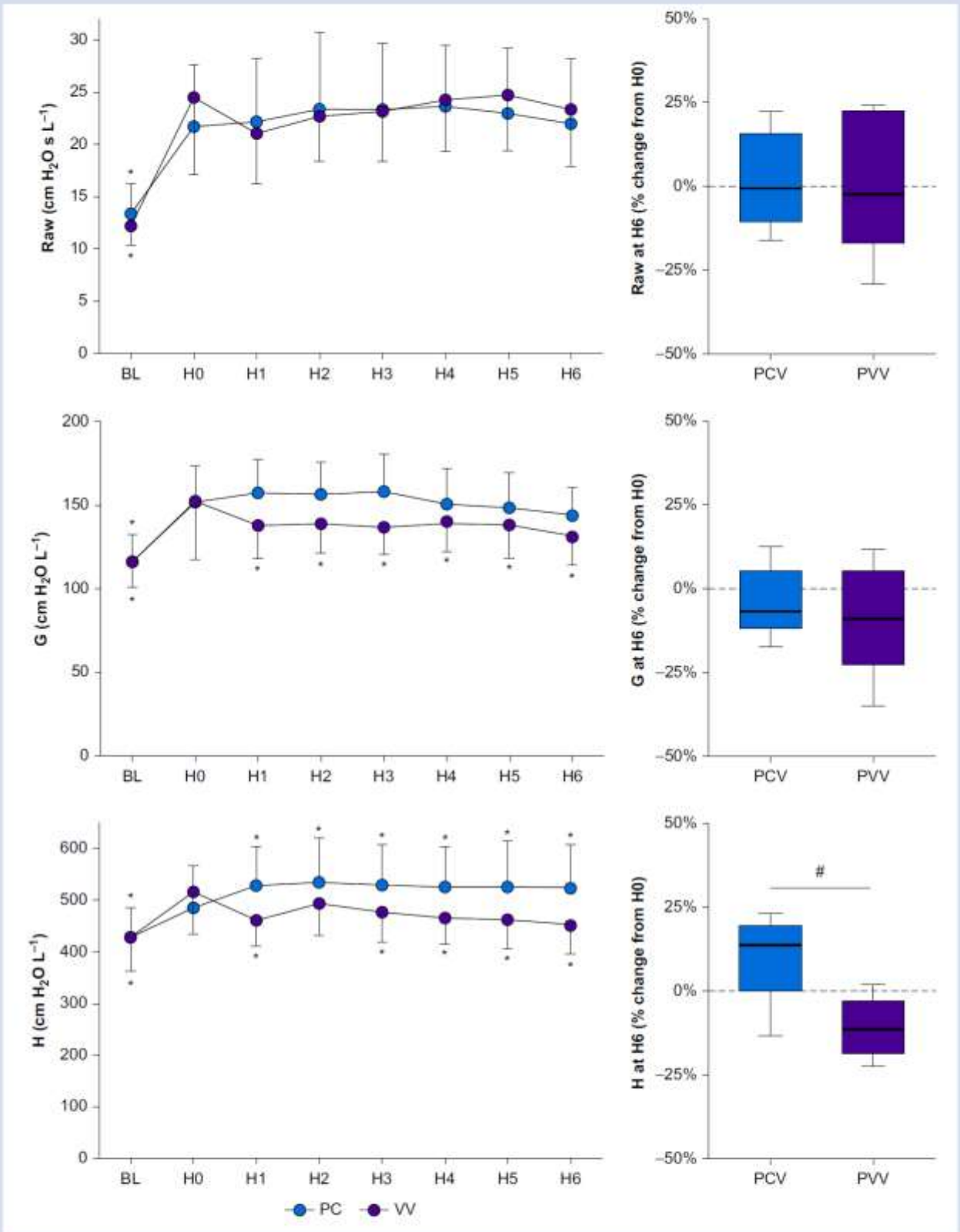


Fig 4. Absolute values of respiratory mechanical parameters (left) at baseline (BL) in rabbits, after bronchoconstriction with ovalbumin and methacholine (H0) and during 6 h of ventilation (H1–H6) with physiologically variable ventilation (PVV; n=12) or pressure-controlled ventilation (PCV; n=10). In the right panels, relative changes after 6 h of ventilation compared with those immediately after induction of bronchoconstriction (H0). Values are expressed as mean (half-width of 95% confidence interval). Raw, airway resistance; G, respiratory tissue damping; H, respiratory tissue elastance. *P<0.05 *vs* H0; #P<0.05 *vs* PCV.

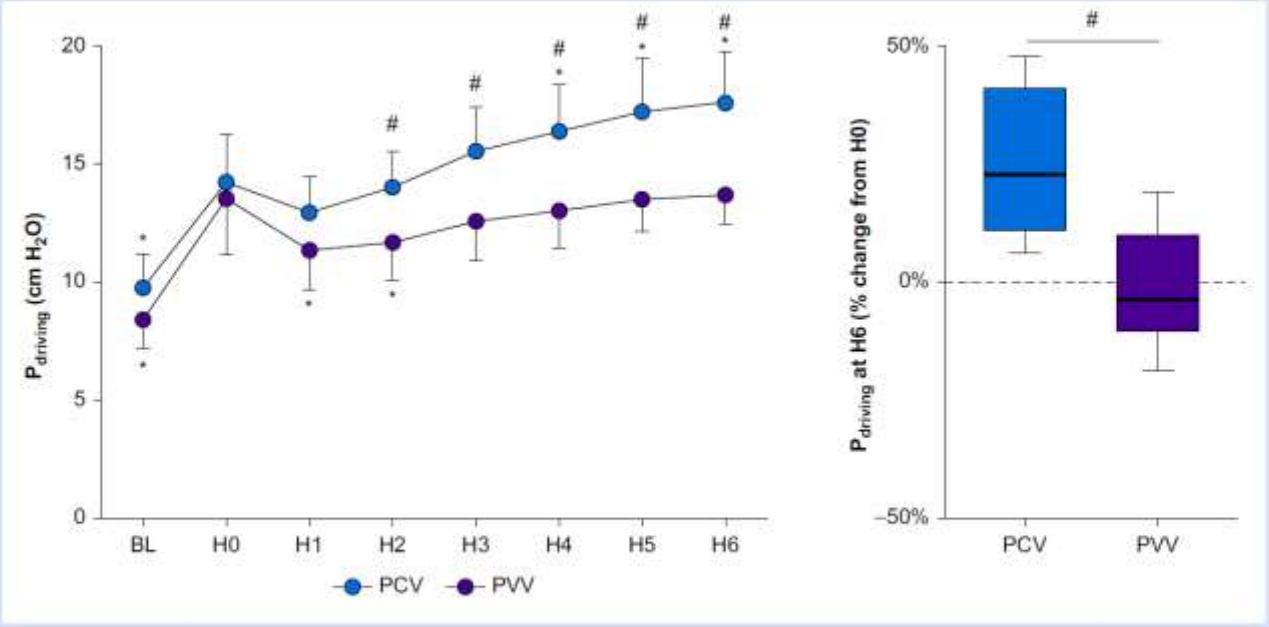


Fig 5. Absolute values of driving pressure (left, $P_{driving}$) measured at baseline (BL), immediately after induction of bronchoconstriction by ovalbumin and methacholine (H0) and during 6 h of ventilation (H1–H6). Results from physiologically variable ventilation (PVV; n=12) and pressure-controlled ventilation (PCV; n=10) are represented with filled and empty symbols, respectively. Values are expressed as mean (half-width of 95% confidence interval). Relative changes of $P_{driving}$ compared with H0 after the application of 6 h PCV or PVV (right). *P<0.05 vs H0; #P<0.05 vs PCV.

reproduce all the features of allergic asthma, a sustained increase in airway smooth muscle tone was achieved during the 6 h study period.

It is noteworthy that findings in lung histology and blood and BALF cellular profiles reflect a combination of the injurious effects of sensitisation, allergen- and cholinergic-induced sustained bronchoconstriction, and prolonged ventilation. Therefore, we are confident that parameters for which improvements were observed after 6 h of PVV are related to the applied ventilation rather than to the course of the allergic response. The PVV pattern generated from the current population was recorded in awake, unrestrained rabbits. The coefficient of variation in ventilatory frequency and driving pressure was about half that reported and applied previously in healthy and injured lungs.[11,17] This difference may be related to the conditions of spontaneous breathing recording in the current study, which used whole-body plethysmography in rabbits acclimated for several days. Despite the lower variation, the benefits with the PVV pattern were apparent. Furthermore, considering the excellent agreement between Raw measured in the present study from sensitised animals and the Raw obtained previously from healthy rabbits,[17] the basal airway smooth muscle tone was not elevated. Therefore, it was a reasonable choice to apply PVV based on the spontaneous breathing recorded before sensitisation.

Lower driving pressures and higher oxygenation index were observed with PVV. This improvement was apparent despite identical ventilatory frequency, tidal volume, and CO_2 levels between PCV and PVV. Similar benefits were reported after cholinergic-induced bronchospasm in healthy animals with better outcomes in respiratory mechanics, oxygenation, and ventilation pressures.[15] Furthermore, the benefit of PVV was also demonstrated in allergen-sensitised rats for preventing bronchial hyperresponsiveness.[16] We also observed the improvement in respiratory tissue viscoelastic parameters (G and H) when PVV was applied in the presence of sustained bronchoconstriction. As increased H reflects lung volume loss caused by peripheral airway closure,[34,35] our results suggest that PVV reversed the derecruitment and lung volume loss resulting from the allergic lung response and, most importantly, resulting from the prolonged ventilation in supine position. This finding is further supported by the lower peak inspiratory pressures required in the PVV group compared with the PCV group and by the improvement in PaO_2/FiO_2. The beneficial effect of prolonged application of PVV on respiratory mechanics is in accordance with previous results obtained in healthy and injured lungs.[11,17]

The benefits of PVV were also evident in the lower lung injury score and the reduced cellular and protein content in the BALF. These parameters are reported to be markers of alveolar damage, inflammation, and capillary leakage.[8,24,36–38] Accordingly, our findings suggest that PVV reduced the degree of lung injury after 6 h of ventilation. The improvement in respiratory tissue viscoelastic parameters and reduced driving pressure with PVV reflects reduced derecruitment. Thus, the

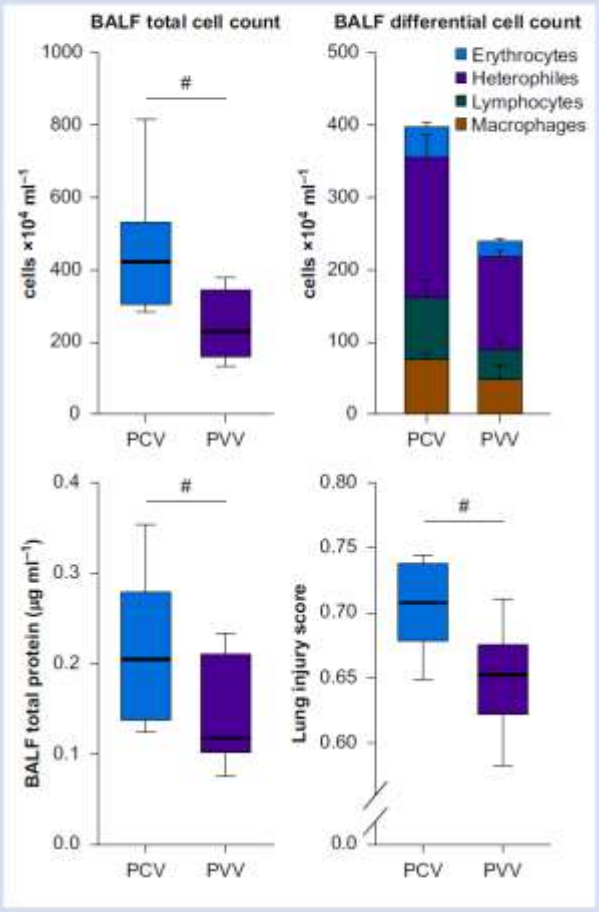


Fig 6. Total and differential cell counts (upper left and right, respectively) and total protein concentration (bottom left) in the bronchoalveolar lavage fluid (BALF) after 6 h of mechanical ventilation with physiologically variable ventilation (PVV; n=12) or pressure-controlled ventilation (PCV; n=10). On the bottom right, lung injury score assessed in the lung histological sections after 6 h application of PVV or PCV. #P<0.05.

lower lung injury score and the reduced BALF cell and protein content may be related to a decrease in alveolar shear stress in the animals ventilated with PVV. In addition to the reduced derecruitment with PVV, the diminished tissue injury may also contribute to the improvement in oxygenation, through a better diffusing capacity. Therefore, improved oxygenation can be explained by a dual advantage of macro- and micro-structural effects of PVV.

A strength of our study was the use of a well-validated technique to assess airway and tissue viscoelastic mechanical properties.[17,21,25] However, parameters reflecting the dissipative and elastic properties were obtained for the total respiratory system, and, therefore, the chest wall contributes to about half of the total respiratory G and H in this species.[39] As this significant chest wall component is expected to be constant throughout the ventilation period, changes in pulmonary tissue parameters are likely to be even more pronounced.

In summary, PVV in an experimental model of acute severe asthma led to improved oxygenation and respiratory tissue mechanics while reducing driving pressure and lung injury, compared with conventional PCV. A recruitment effect in combination with a global reduction in lung tissue shear stress may explain the evident benefits of PVV over conventional ventilation. The results of this experimental work suggest that a physiologically variable pattern may be a beneficial strategy for severe asthma requiring mechanical ventilation.

Authors' contributions

Study design: ADSR, RS, WH
Experimental work: ADSR, RS
Data collection: ADSR, RS
Data analyses: all authors
Interpretation of results: FP, WH
Drafting of the manuscript: ADSR, FP, WH
All authors have read, critically revised, and accepted the final manuscript.

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Declarations of interest

Engineering support was provided by Getinge AB (Solna, Sweden) by providing a special firmware for the ventilator that allowed the application of variable ventilation patterns. The authors declare that they have no further conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bja.2020.08.059.

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- online supplementary data -

Benefits of physiologically variable ventilation in a model of asthma exacerbation: an experimental randomised trial

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Keywords (5): *status asthmaticus*, mechanical ventilation, respiratory mechanics, lung injury, gas exchange

Short running title: Variable ventilation and *status asthmaticus*

Figure S1

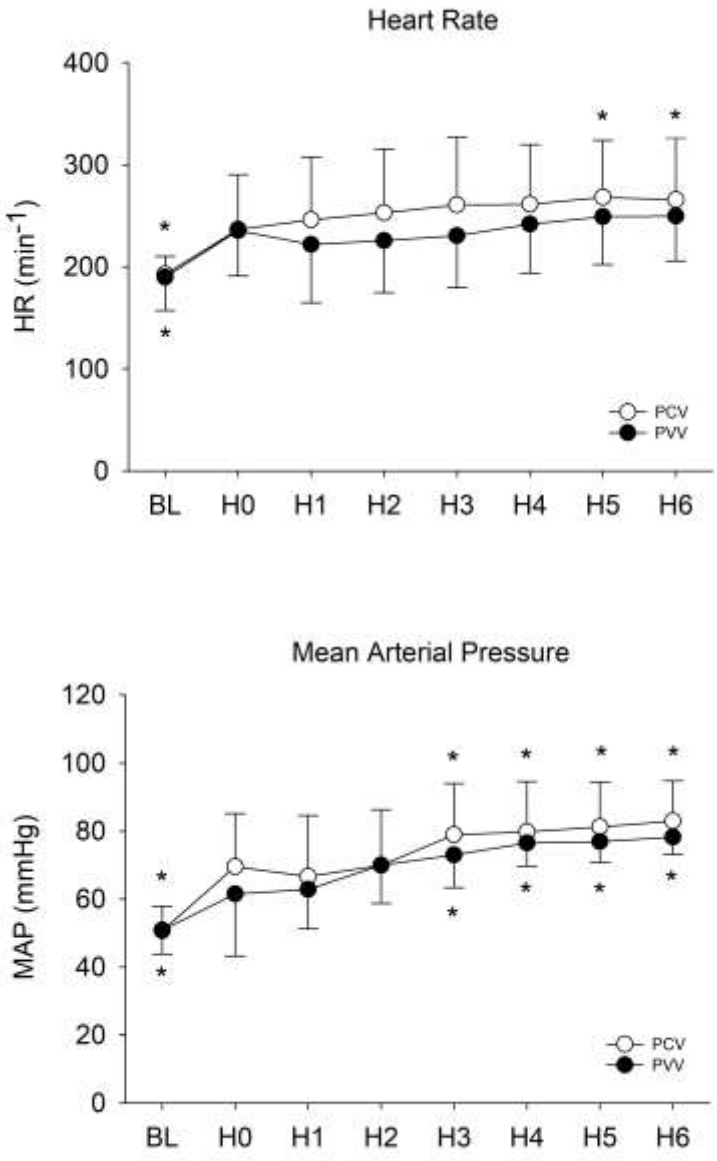

Figure S1. Hemodynamic parameters obtained during the 6-hour ventilation period. Values are expressed as mean ± half-width of 95% confidence interval. MAP: mean arterial pressure; HR: heart rate; BL: baseline; H0 to H6: average value during the corresponding hour of the 6-hour long ventilation period; PCV: pressure-controlled ventilation; PVV: physiological variable ventilation. *: $p < 0.05$ vs. H0

Figure S2

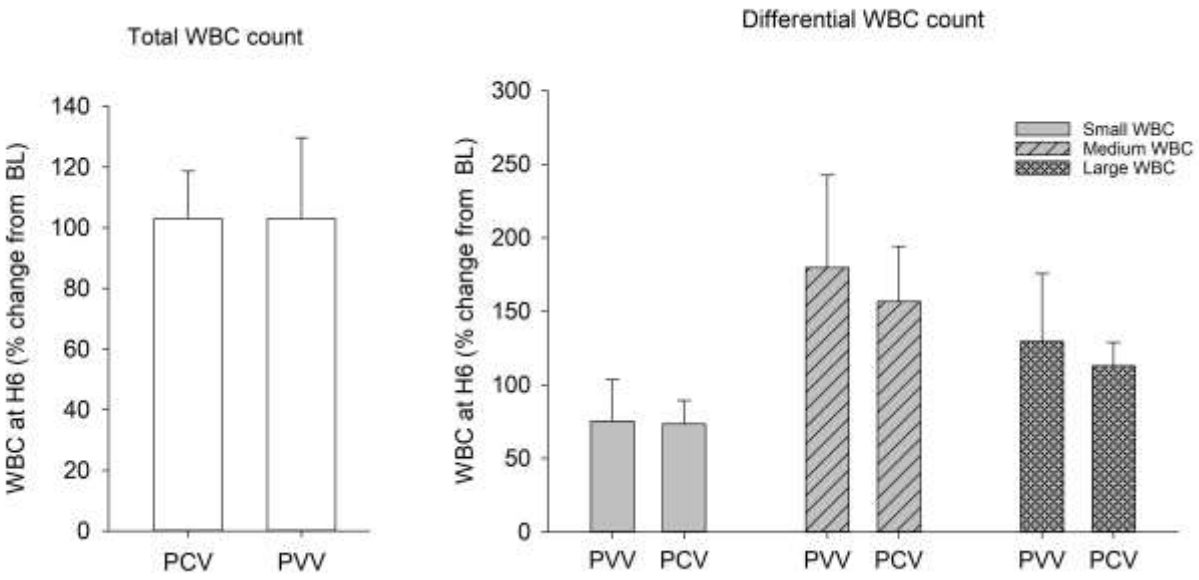

Figure S2. Total White Blood Cell (WBC) counts (left graph) and differential WBC counts (right graph) after 6 hours of ventilation are presented as relative change compared to baseline (BL) values. Values are expressed as mean ± half-width of 95% confidence interval.

Table S1

BALF	PCV	PVV
TNF-α (pg ml^{-1})	33.0 (18.6)	31.4 (10.4)
IL-6 (pg ml^{-1})	1.51 (0.53)	1.49 (0.26)
IL- 8 (pg ml^{-1})	364 (5)	363 (5)
SP-B (ng ml^{-1})	0.027 (0.02)	0.017 (0.01)
SP-D (ng ml^{-1})	0.51 (0.25)	0.35 (0.08)
E-cadherin (ng ml^{-1})	0.08 (0.06)	0.05 (0.04)
Lung tissue	PCV	PVV
TNF-α (pg mg^{-1} of protein)	*out of range*[*]	*out of range*[*]
IL-6 (pg mg^{-1} of protein)	6.57 (0.42)	6.40 (1.36)
IL- 8 (pg mg^{-1} of protein)	421.8 (46.3)	414.3 (29.2)
SP-B (ng mg^{-1} of protein)	0.82 (0.10)	0.79 (0.13)
SP-D (ng mg^{-1} of protein)	3.72 (0.80)	4.41 (0.43)
E-cadherin (ng mg^{-1} of protein)	3.63 (0.25)	3.42 (0.52)

Table S1. Protein concentration in the supernatant of the bronchoalveolar lavage fluid (BALF), and frozen lung tissue homogenate, expressed as mean (half-width of 95% confidence interval), for PCV (pressure-controlled ventilation) and PVV (physiological variable ventilation). TNF, *tumour necrosis factor;* IL*, interleukin*; SP, *surfactant protein*. [*] optical density values were out of the absorbance detection limits, equally in both experimental groups.

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Benefit of Physiologically Variable Over Pressure-Controlled Ventilation in a Model of Chronic Obstructive Pulmonary Disease: A Randomized Study

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Introduction: The advantages of physiologically variable ventilation (PVV) based on a spontaneous breathing pattern have been demonstrated in several respiratory conditions. However, its potential benefits in chronic obstructive pulmonary disease (COPD) have not yet been characterized. We used an experimental model of COPD to compare respiratory function outcomes after 6 h of PVV versus conventional pressure-controlled ventilation (PCV).

Materials and Methods: Rabbits received nebulized elastase and lipopolysaccharide throughout 4 weeks. After 30 days, animals were anesthetized, tracheotomized, and randomized to receive 6 h of physiologically variable ($n = 8$) or conventional PCV ($n = 7$). Blood gases, respiratory mechanics, and chest fluoroscopy were assessed hourly.

Results: After 6 h of ventilation, animals receiving variable ventilation demonstrated significantly higher oxygenation index (PaO_2/FiO_2 441 ± 37 (mean ± standard deviation) *versus* 354 ± 61 mmHg, $p < 0.001$) and lower respiratory elastance (359 ± 36 *versus* 463 ± 81 cmH_2O/L, $p < 0.01$) than animals receiving PCV. Animals ventilated with the variable mode also presented less lung derecruitment (decrease in lung aerated area, −3.4 ± 9.9 *versus* −17.9 ± 6.7%, $p < 0.01$) and intrapulmonary shunt fraction (9.6 ± 4.1 *versus* 17.0 ± 5.8%, $p < 0.01$).

Conclusion: PVV applied to a model of COPD improved oxygenation, respiratory mechanics, lung aeration, and intrapulmonary shunt fraction compared to conventional ventilation. A reduction in alveolar derecruitment and lung tissue stress leading to better aeration and gas exchange may explain the benefits of PVV.

Keywords: COPD, variable ventilation, animal model, gas exchange, lung mechanics

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is the most prevalent respiratory disease, with a reported prevalence of 251 million cases worldwide (Chronic obstructive pulmonary disease (COPD), 2017). In addition to the high burden and morbidity of the disease (Gershon et al., 2013; Lopez-Campos et al., 2016), COPD is the third leading cause of death worldwide (Lozano et al., 2012). COPD is characterized by chronic lung inflammation with airway remodeling and airflow limitation, which is associated with irreversible emphysematous destruction of the alveoli. These permanent alterations of the pulmonary structure progressively impair gas exchange and respiratory mechanics, leading to different degrees of respiratory insufficiency.

Chronic obstructive pulmonary disease is a progressive disease with exacerbations leading potentially to acute respiratory failure necessitating mechanical ventilation (Gacouin et al., 2015). Furthermore, even under stable conditions, COPD patients may require mechanical ventilation for respiratory life-support while undergoing general anesthesia. Considering that COPD patients have a higher incidence of respiratory complications induced by mechanical ventilation (Edrich and Sadovnikoff, 2010; HausmanJr., Jewell and Engoren, 2015; Numata et al., 2018; Szylinska et al., 2020), it is therefore of utmost importance to optimize ventilation modalities in this population.

Variable ventilation is a recently developed modality that mimics physiological breathing by incorporating breath-by-breath variations in tidal volume and respiratory rate. A variable breathing pattern has been advocated to be superior to monotonous ventilation by means of optimizing gas exchange and recruitment of alveoli, given the non-linearity of the respiratory system (Suki et al., 1998; Brewster et al., 2005). Several studies have demonstrated the benefits of variable ventilation on gas exchange (Carvalho et al., 2009, 2011) and lung mechanics as well as in preventing ventilator-induced lung injury in animal models with normal lungs (Mutch et al., 2000a; Arold et al., 2003; Walesa et al., 2018), acute respiratory distress syndrome (ARDS) (Lefevre et al., 1996; Boker et al., 2002; Funk et al., 2004; Spieth et al., 2009), emphysema (Henriques et al., 2016), and prematurity (Pillow et al., 2011; Berry et al., 2012) and in the presence of atelectasis (Mutch et al., 2000b; McMullen et al., 2006) and asthma (Dos Santos Rocha et al., 2020). However, the potential beneficial effects of variable ventilation in a model of COPD have not yet been characterized. We hypothesize that the deterioration of lung mechanics and oxygenation during mechanical ventilation of lungs with main features of COPD will be prevented by a ventilation mode reproducing the variability of spontaneous breathing. To test this hypothesis, we compared physiologically variable ventilation (PVV) to conventional pressure-controlled ventilation (PCV) in an experimental model of COPD.

MATERIALS AND METHODS

Ethics Statement

The current study was approved by the Animal Welfare Committee of the Canton of Geneva and the Experimental Ethics Committee of the University of Geneva, Switzerland (GE 184/18, 2 January 2019). All procedures were performed in accordance to current Swiss animal protection laws (LPA, RS455). The ARRIVE guidelines were followed to report this study.

Study Design

The study protocol is represented in **Figure 1**. Adult New Zealand White rabbits [male n = 8, female n = 7, aged 20 weeks, weighing 3.4 kg (range 2.88–3.76 kg)] were purchased from the University of Geneva's farm (Arare, Geneva, Switzerland). Pathological aspects of COPD were experimentally induced over 4 weeks. On day 30, rabbits were anesthetized, tracheotomized, and randomized to receive 6 h of either PCV or PVV. After 6 h, animals were euthanized with sodium thiopental (100 mg/kg), and lung post-mortem analyses were performed.

Experimental Procedures

COPD Model Preparation

All rabbits received a scheme of aerosol treatments over 4 weeks, as described in **Figure 1**, to induce a persistent lung injury that reproduced pathological aspects of COPD, as described previously (Sajjan et al., 2009; Ganesan et al., 2010). On day 0, porcine elastase 15 U/kg (Elastase suspension 3 U/mg Worthington, BioConcept, Allschwil, Switzerland) was aerosolized using a vibrating mesh nebulizer (Aerogen® Solo Nebulizer System, Hamilton Medical, Switzerland). Subsequently, once a week from day 3 to day 24, rabbits received nebulized lipopolysaccharide 20 µg/kg (*Escherichia coli* O111:B4, Sigma, St. Louis, MO, United States). All nebulized substances were delivered to the lower airways through a supraglottic airway device (v-gel®, Docsinnovent Ltd., London, United Kingdom), to reduce the mucosal damage of repeated intubation (Engbers et al., 2017) and aerosol dispersion in the upper airway. Under sedation with 2% sevoflurane for approximately 10 min (the duration of the nebulization), animals received pressure-support ventilation with 10 cmH_2O of inspiratory pressure and 3 cmH_2O of positive end-expiratory pressure (PEEP) using a clinical ventilator (Primus®, Dräger, Lübeck, Germany). After elimination of sevoflurane, animals were weaned from the ventilator and the supraglottic airway device was removed. Periprocedural care included artificial tears, external heating, and supplemental oxygen. Furthermore, an animal welfare score was quantified after each nebulization and twice per week, to assess the general and respiratory condition of the rabbits (**Supplementary Table S1**). In case of respiratory distress, supplemental oxygen was administered until symptoms resolved. The rabbits had access to food and water *ad libitum* before and after the experiments.

Anesthesia and Surgical Preparation

On day 30, anesthesia was induced by intramuscular injection of ketamine 25 mg/kg and xylazine 3 mg/kg. Cannulation of the ear vein with a 24 G catheter (Abbocath, Abbott Medical, Baar/Zug, Switzerland) was performed. After infiltration of the anterior cervical region with lidocaine 1% (Sintetica, Mendrisio, Switzerland), a surgical tracheostomy with a 3.5-mm uncuffed tube (3.5 mm Portex, Smiths Medical, Kent,

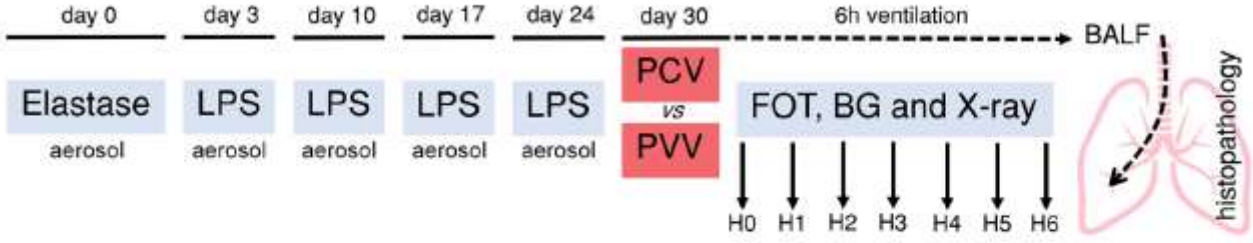

FIGURE 1 | Schematic representation of the experimental protocol. LPS, lipopolysaccharide; PCV, pressure-controlled ventilation; PVV, physiologically variable ventilation; FOT, forced oscillation technique; BG, blood gas; BALF, bronchoalveolar lavage fluid; H0 to H6, measurement time points at hour 0 to hour 6.

United Kingdom) was performed. Intravenous anesthesia with propofol 10 mg/kg/h, fentanyl 5 µg/kg/h, and midazolam 0.2 mg/kg/h was administered via the ear vein. The left femoral artery and right internal jugular vein were cannulated with a 20 G catheter for arterial and venous blood sampling and invasive blood pressure measurements.

After confirming adequate anesthesia and analgesia through the absence of movement in response to painful stimuli and cardiovascular monitoring (stable heart rate and arterial blood pressure), neuromuscular blockade was performed with atracurium besylate 0.6 mg/kg/h. Body temperature was monitored with a rectal thermometer and kept between 38 and 39°C with a thermostatic heating pad (Harvard Apparatus, South Natick, MA, United States). Intravenous fluid replacement was administered with Ringer's acetate 2 mL/kg/h.

Mechanical Ventilation Settings

Mechanical ventilation was applied using a computer-controlled custom-made turbine ventilator connected to a heated pediatric pneumotachograph (PNT 3700 Hans Rudolph Inc., Shawnee, KS, United States) and pressure transducers (Honeywell Differential Pressure Sensor model 24PCEFA6D, Charlotte, NC, United States). Custom-made software designed in Labview® was used to control the ventilator and to continuously record tracheal airflow (V′), airway pressure (P_{aw}), and tidal volume (V_T). After tracheostomy and surgical preparation, a sustained inspiratory pressure of 25 cmH_2O was applied twice for 10 s to normalize lung volume history in all animals. Subsequently, animals were randomized to receive 6 h of either PCV or PVV. While the ventilatory pattern was essentially different between PCV and PVV, all the ventilation parameters were set equally between groups, as follows: an inspiratory pressure was set to deliver an average V_T of 7 mL/kg, a PEEP of 3 cmH_2O, a fraction of inspired oxygen (FiO_2) of 0.4, and an inspiratory to expiratory ratio of 1:3. The driving pressure ($P_{driving}$, calculated as the difference between PEEP and peak inspiratory pressure), set at H0 to deliver the target V_T, was kept constant throughout the 6 h of ventilation. Mainstream capnography was used throughout the experiment and respiratory rate was adapted to achieve normocapnia (end-tidal CO_2 of 5.5–6%).

While the PCV consisted in a monotonous pattern, as per conventional use in clinical practice, the PVV pattern, whose characteristics are summarized in **Figure 2** and **Table 1**, reproduced the (physiologically variable) breathing pattern in awake COPD rabbits. To obtain the PVV pattern, whole-body plethysmography was performed in a subgroup of four animals, between experimental days 27 and 30 to repeatedly record their spontaneous breathing after COPD induction. Briefly, a pressure transducer (Honeywell Differential Pressure Sensor model 24PCEFA6D, Charlotte, NC, United States) was connected to a custom-made plexiglass box (external measures of 300 × 300 × 500 mm, internal volume of 37.63 L) and the pressure signal was digitized at 1 kHz (ADInstruments, Powerlab model 8/35 and LabChart 7, Dunedin, New Zealand) with the simultaneous monitoring of movements by a digital camera. The box temperature and humidity were recorded and kept constant with a fresh air supply of 1.5 L/min. Rabbits were placed in the plethysmograph box for 30-min periods for four consecutive days to get accustomed to the handling and the box environment. Ten-minute recordings of spontaneous breathing on the fourth day, when animals were accustomed to the box environment, were used to produce the PVV pattern. Recording segments corresponding to movement artifacts were removed. Replicates of the recorded pattern were created with different average respiratory rates, maintaining the exact ratios of breath-to-breath pressure and frequency for each replicate. The resulting PVV pattern file contained 382 breaths that were reproduced in loop for the duration of the experimental protocol.

Measurement of Respiratory Mechanics

The impedance spectra of respiratory system (Z_{rs}) were measured using forced oscillatory technique, as described in detail previously (Hantos et al., 1992; Peták et al., 2006; Albu et al., 2018). Briefly, 2 cmH_2O peak-to-peak amplitude pseudorandom oscillations (15 non-integer multiples between 0.5 and 21 Hz) were applied for 10 s during end-expiratory pauses by the computer-controlled ventilator turbine. The V′ was measured using a pneumotachograph (PNT 3700 Hans Rudolph Inc., Shawnee, KS, United States) connected to a differential pressure transducer (Honeywell model 24PCEFA6D, Charlotte, NC, United States). A second pressure transducer connected to a side port of the tracheal cannula was used to measure P_{aw}. Z_{rs} ($Z_{rs} = P_{aw}/V'$) was calculated using Fast Fourier Transformation from the 10-s-long recordings with 4-s time windows and 95% overlap.

Three epochs were recorded and averaged in each measurement timepoint. The impedance of the breathing circuit was subtracted from measured impedance spectra. To

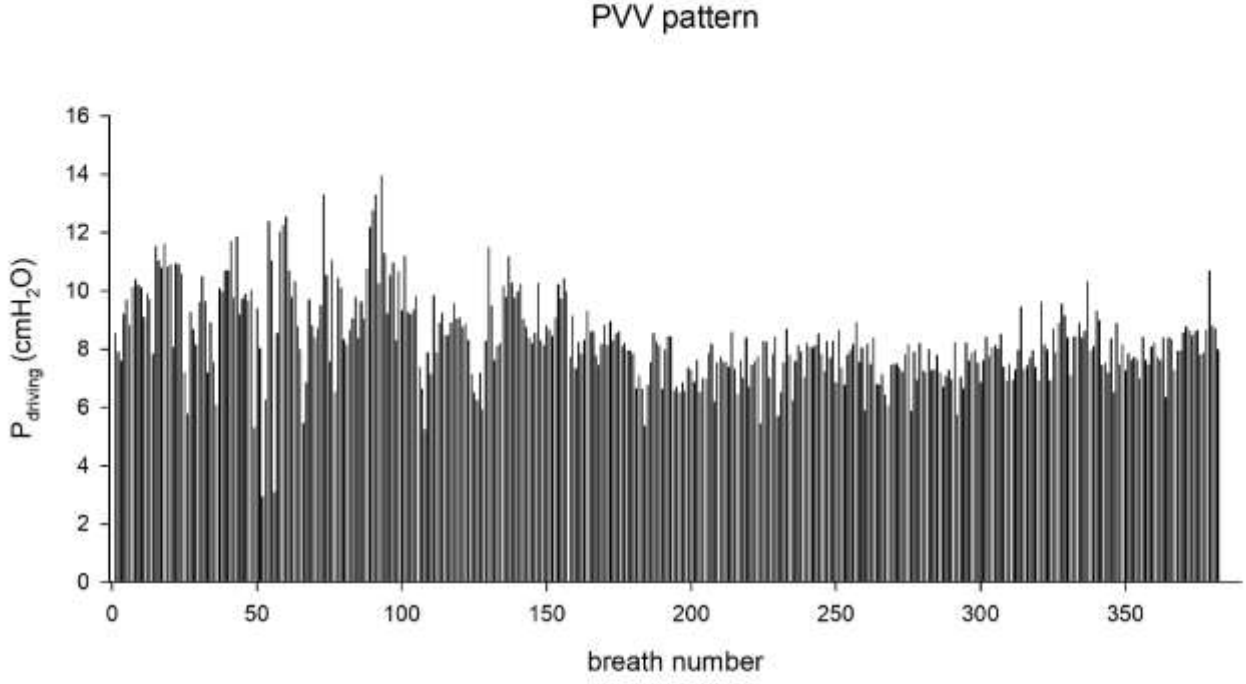

FIGURE 2 | Changes in the driving pressure ($P_{driving}$) during the application of physiological variable ventilation (PVV) pattern, in a representative rabbit.

separate the airway and respiratory tissue mechanical properties, we fitted a well-validated model (Hantos et al., 1992) to the measured Z_{rs} spectra. The model contained airway resistance (R_{aw}) and inertance (I_{aw}), in series with a tissue model including damping (G) and elastance (H). A global optimization procedure was used to minimize the differences between the measured and modeled impedance values.

As previously described (Petak et al., 1997), R_{aw} and I_{aw} reflect the flow resistance and mass inertia of the intrapulmonary gas, respectively. The tissue parameters G and H characterize the energy loss (viscous resistance) and storage (elastance) in the respiratory tissues, respectively.

Measurement of Blood Parameters

Arterial and venous blood was analyzed by a point-of-care blood gas analyzer (i-Stat, Abbott Laboratories, Chicago, IL, United States). Partial pressure of oxygen (PaO_2) and carbon dioxide ($PaCO_2$) were assessed. Oxygenation index was calculated as PaO_2/FiO_2. Intrapulmonary shunt fraction (Qs/Qt) was determined as the ratio of pulmonary end-capillary oxygen content (CcO_2) minus arterial oxygen content (CaO_2), divided by the CcO_2 minus the central venous oxygen content (CvO_2).

$$\frac{Q_s}{Q_t} = \frac{CcO_2 - CaO_2}{CcO_2 - CvO_2}$$

The total and differential white blood cell counts in the plasma were obtained from arterial blood using a pocH-100iV DIFF hematology analyzer (Sysmex Digitana AG, Horgen, Switzerland).

TABLE 1 | Characteristics of the ventilation variables, tidal volume (V_T), and respiratory rate (RR) for pressure-controlled ventilation (PCV) and PVV during the first hour of ventilation.

	PCV		PVV	
	V_T (mL/kg)	RR (1/min)	V_T (mL/kg)	RR (1/min)
Mean	7.2 ± 0.7	24.2 ± 1.4	7.1 ± 0.6	22.9 ± 1.5
CV (%)	1.9 ± 0.8	0.2 ± 0.1	12.6 ± 1.0	12.9 ± 0.4
Minimum	6.8 ± 0.6	24.1 ± 1.4	3.7 ± 0.4	13.1 ± 0.9
Maximum	7.4 ± 0.8	24.3 ± 1.4	10.7 ± 0.7	35.5 ± 2.5

CV, coefficient of variation.

Quantification of Lung Aeration

Structural imaging of the respiratory system was acquired hourly using X-ray fluoroscopic technology (Ziehm Vista C-Arm System, Nuremberg, Germany). The position and distance of the X-ray beam generator and detector were constant during the 6-h ventilation. For each recording, a frame in end-expiration was used for manual segmentation of the lung, based on radiodensity, using pixel counting tool with a custom-made script in MATLAB®. Lung aeration area was calculated in pixels from the segmented two-dimensional images image by a radiologist blinded to group allocations and compared in percentage change to the reference image at H0.

Lung Histological, Cellular, and Protein Assessment

For morphological and morphometric evaluation, the left lung was fixed by infusing 10% neutral-buffered formalin into

the cannulated main bronchus at a hydrostatic pressure of 20 cmH_2O, followed by immersing in a container with the same fixative for > 48 h. The organ was then embedded in agarose, and the tissue was cut into 18 step slices, anterior to posterior, spaced by 3 mm and processed for paraffin-embedding (six from the cranial lobe and 16 from the caudal lobe). Sections (4 μm) were stained with hematoxylin-eosin, digitally scanned in Nanozoomer SQ (Hamamatsu), and visualized in NDP2.view software. Morphological analysis was performed by a pathologist blinded to group allocations and scored following American Thoracic Society guidelines (Matute-Bello et al., 2011). The scoring system included the presence of inflammatory cells in the alveolar and interstitial spaces, hyaline membranes, proteinaceous debris filling the airspaces, and alveolar septal thickening. Overall lung injury score was calculated by averaging the score for each of the 18 lung slices.

Score for emphysema was performed (i) by a pathologist using a five-tier system with a grading scale (0, absent; 1, minimal; 2, mild; 3, moderate; 4, marked), in which classification is based on the most severe lesions, and (ii) by automated measurement of airspace size, performed using mean linear intercept (MLI) method, as previously described (Crowley et al., 2019). The average MLI was calculated from all 18 microscopy fields at 10x magnification, avoiding big vessels and airways, and with the same cranio-caudal distribution of the histological injury score described above. Lung tissue from naive New Zealand White rabbits with comparable age and weight to the current study animals was used as control ($n = 3$).

The cannulated main bronchus of the right lung was flushed with 20 mL warm phosphate buffered saline containing 1% bovine serum albumin, to obtain bronchoalveolar lavage fluid (BALF). Subsequently, the right lung was stored at −80°C, and lung tissue homogenate was obtained by sonication. The BALF was centrifuged at 412 g for 5 min at 5°C and the supernatant stored at −20°C until analysis. The total and differential cell counts in the BALF were analyzed as described in previous work (Walesa et al., 2018). Enzyme-linked immunosorbent assays were performed according to the manufacturer's instructions using frozen lung tissue homogenates and undiluted supernatant of the BALF to quantify the inflammatory cytokines interleukin (IL)-6 and IL-8 (Raybiotech Norcross, GA, United States), tumor necrosis factor (TNF-α, MyBiosource MBS2021700, San Diego, CA, United States), surfactant protein B (SP-B, LSBio LS-F47557, Muttenz, Switzerland), surfactant protein D (SP-D, Blue Gene Biotech E04S0170, Paris, France), and E-cadherin (LSBio LS-F43438, Muttenz, Switzerland).

Study Outcomes

The primary outcomes of the present study were the PaO_2/FiO_2 and H after 6 h of ventilation. Secondary outcomes included mean V_T, Qs/Qt, lung aeration area, mechanical parameters R_{aw} and G, lung histological injury score, plasma and BALF cell counts, and cytokine levels.

Statistical Methods

Data are presented as mean ± standard deviation. Normality of each variable distribution was assessed using the Shapiro–Wilk test. Two-way repeated measures analyses of variances were used to analyze the absolute values of primary and secondary outcomes, using ventilation mode (PCV or PVV) and time (H0 to H6) as between and within subject factors, respectively. In case of significance, Dunnett's *post hoc* test was used to assess significances for ventilation mode (using PCV as reference) and time (using H0 as a reference). Relative changes between H0 and H6 were analyzed using a paired *t*-test or a Mann–Whitney test, depending on normality. Correlation between H and PaO_2/FiO_2 was analyzed using Pearson's correlation test. All statistical tests were performed using SigmaPlot (Version 13, Systat Software, Inc., Chicago, IL, United States). Results were considered significant for a level of $p < 0.05$, and all *p*-values are two-sided.

Sample Size Estimation

The sample size was estimated based on respiratory tissue elastance (H), using data previously obtained under similar conditions in rabbits (Walesa et al., 2018). We aimed at detecting 20% differences between groups, assuming an interindividual variation of 15%, a statistical power of 0.8, and a two-sided alpha error of 0.05. The calculation resulted in a minimum sample size of 10 rabbits per group. Considering a potential 10% drop-out rate, we induced experimental COPD on 22 rabbits.

RESULTS

Study Population

Chronic obstructive pulmonary disease induction was performed on 22 rabbits. Seven animals were not included in the 6-h ventilation protocol due to lethal alveolar hemorrhage at day 0 after the elastase nebulization. Accordingly, 15 rabbits were randomized at day 30 to receive PCV ($n = 7$) or PVV ($n = 8$) (**Figure 1**).

Ventilation Parameters

The ventilatory parameters are summarized in **Figure 3**. Mean inspiratory pressure, PEEP, driving pressure, and FiO_2 were maintained unchanged during the 6-h ventilation with no difference in these parameters between the study groups.

At the onset of the study (H0), PCV and PVV animals were ventilated with identical mean V_T [6.90 ± 0.78 (mean ± SD) *versus* 6.67 ± 0.44 mL/kg in PCV and PVV, respectively]. Despite a constant $P_{driving}$ throughout the 6-h ventilation, there was a significant and progressive reduction in mean V_T in both experimental groups, starting from H1 in the PCV group ($p < 0.001$) and from H2 in the PVV group ($p < 0.001$). Notably, after 6 h of ventilation, mean V_T was significantly lower in animals ventilated with PCV ($p < 0.05$). To target normocapnia, a significant increase in RR was necessary in both experimental groups, in comparison to H0, with no evidence for a statistical difference in RR between the experimental groups. No evidence for intrinsic PEEP or air trapping was observed

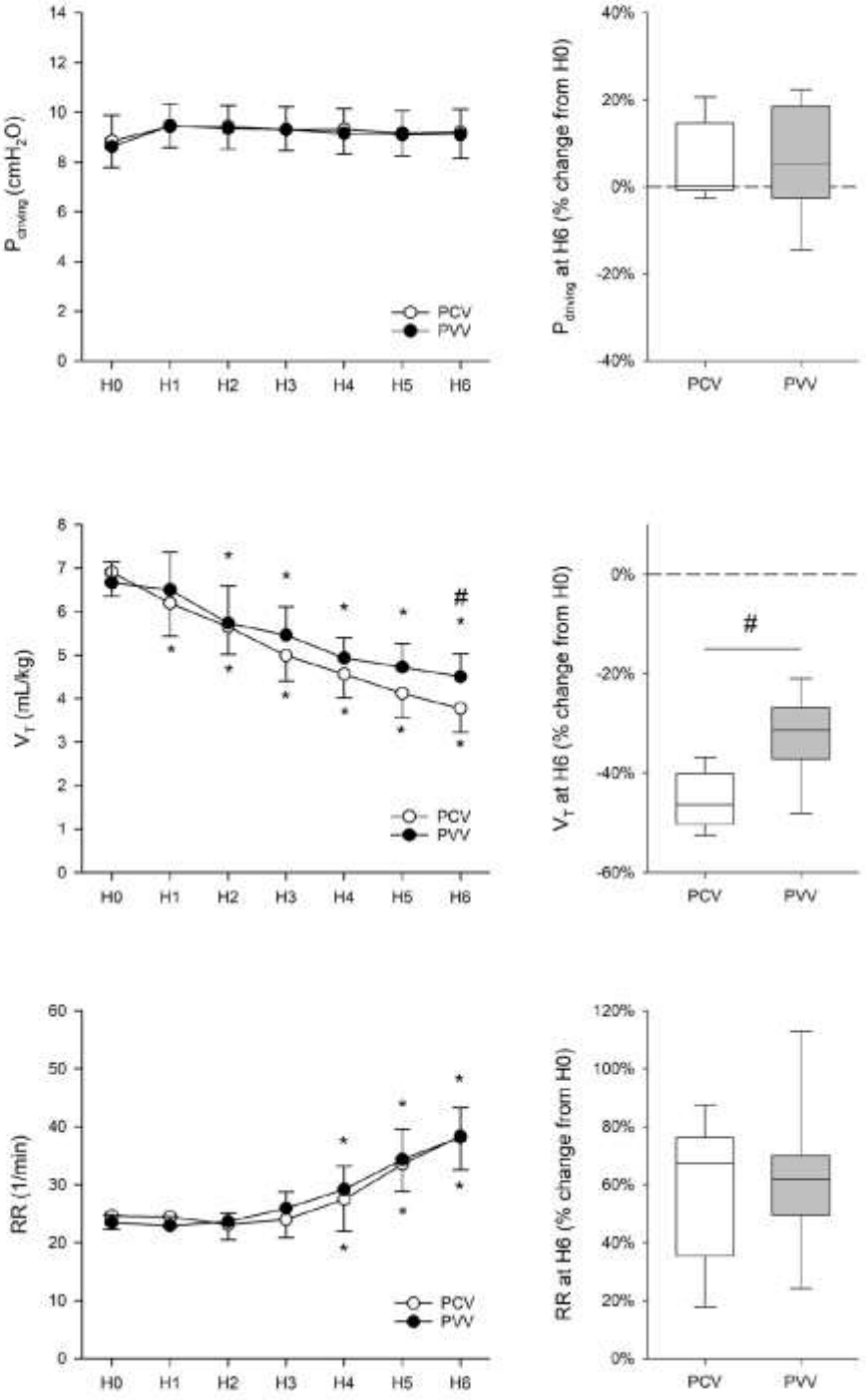

FIGURE 3 | Driving pressure ($P_{driving}$), tidal volume (V_T), and respiratory rate (RR), measured at the onset (H0) and throughout the 6 h of ventilation (H1 to H6). Results from physiologically variable ventilation (PVV, filled circles) and pressure-controlled ventilation (PCV, empty circles) are expressed as mean ± standard deviation. Relative changes compared to H0 after the application of 6-h PCV (white box) or PVV (gray box) are reported on the right panels. *$p < 0.05$ *versus* H0; #$p < 0.05$ *versus* PCV.

during the experimental protocol, as assessed though continuous monitoring of expiratory pressure and flow.

The analysis of the ventilatory pattern revealed differences between the experimental groups (**Table 1**). While animals in the PCV group received nearly constant V_T and RR, those in the PVV group received a variable pattern for V_T and RR with a comparable coefficient of variation of approximately 13%.

Gas Exchange

Changes in PaO_2/FiO_2 and $PaCO_2$ during the study protocol are summarized in **Figure 4**. Despite COPD induction, O_2 and CO_2 levels at H0 were in the physiological range in both groups. While PaO_2/FiO_2 remained constant throughout the 6-h ventilation with PVV, it progressively decreased under PCV, and this decrease became significant after 2 h of ventilation ($p < 0.001$). Subsequently, animals in the PVV group exhibited significantly higher PaO_2/FiO_2 in the second half of the ventilation period ($p < 0.001$).

Despite a lack of difference in RR between the study groups (**Figure 3**), the animals in the PCV group presented a significantly

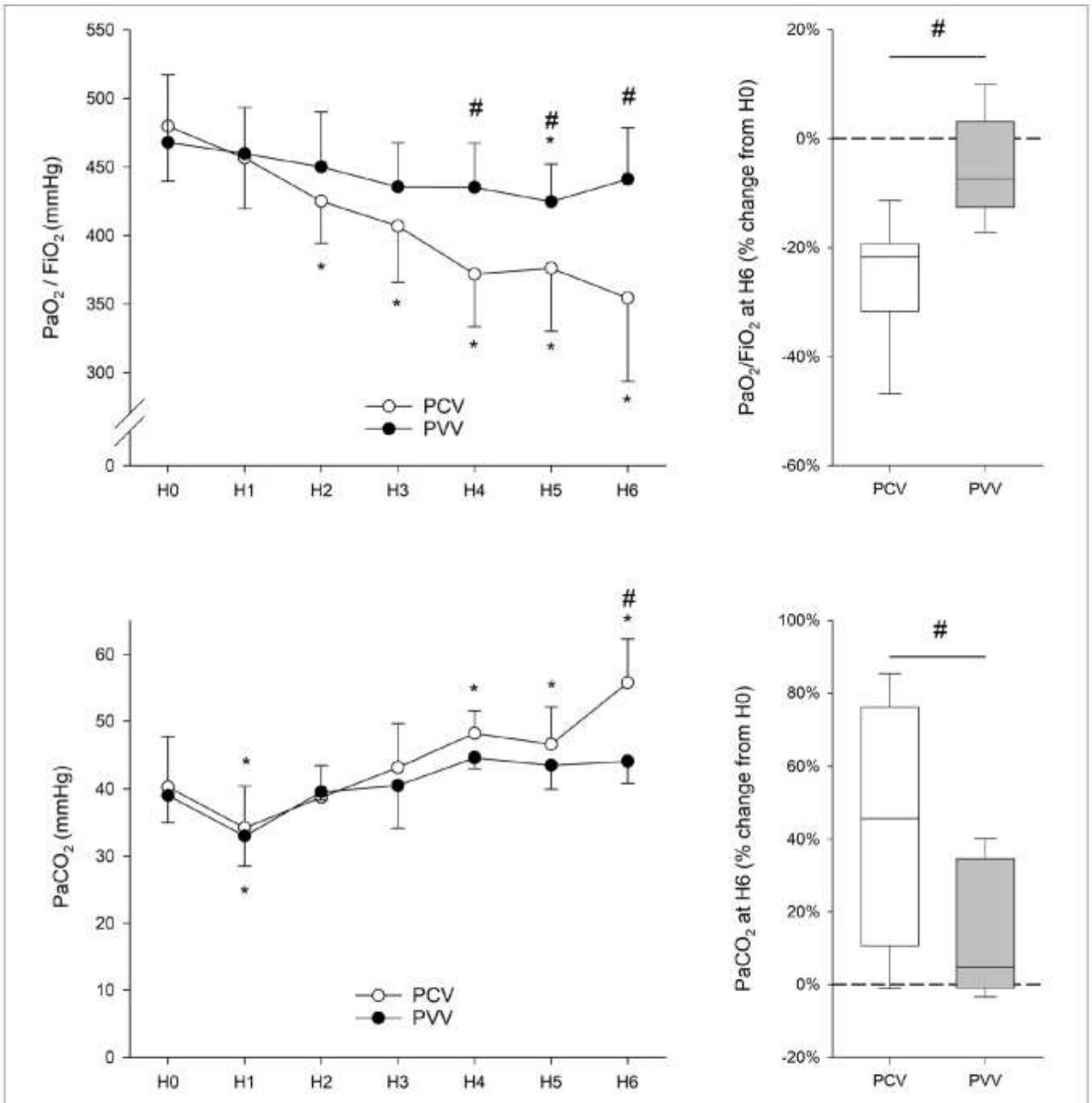

FIGURE 4 | Gas exchange parameters expressed as oxygenation index (PaO_2/FiO_2) and partial pressure of CO_2 in the arterial blood ($PaCO_2$), measured at the onset (H0) and throughout the 6 h of ventilation (H1 to H6). Results from physiologically variable ventilation (PVV, filled circles) and pressure-controlled ventilation (PCV, empty circles) are expressed as mean ± standard deviation. Relative changes compared to H0 after the application of 6-h PCV (white box) or PVV (gray box) are reported on the right panels. PaO_2, arterial partial pressure of oxygen; FiO_2, fraction of inspired oxygen. *$p < 0.05$ *versus* H0; #$p < 0.05$ *versus* PCV.

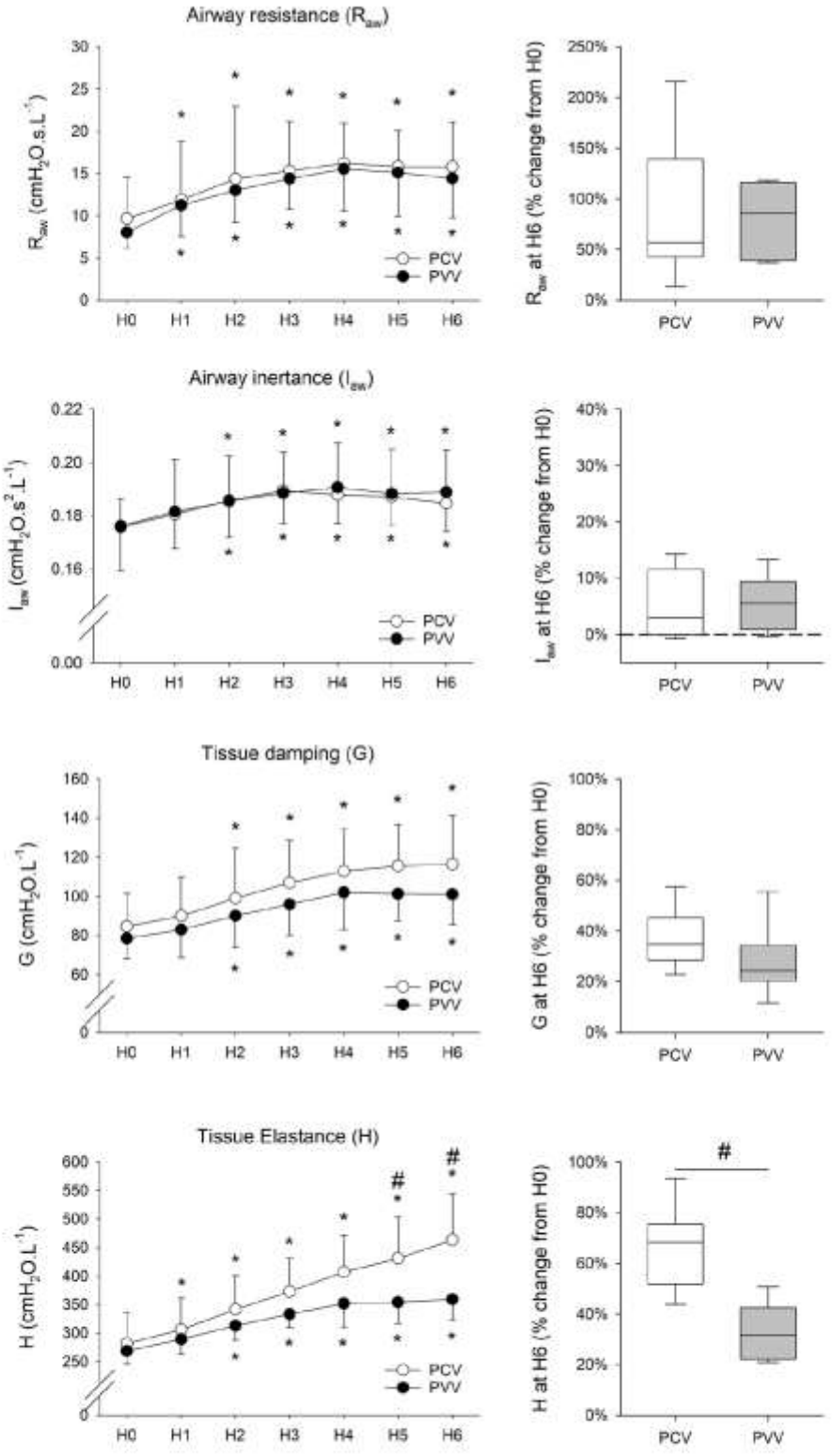

FIGURE 5 | Respiratory mechanical parameters (left panels) in rabbits at the onset (H0) and during 6 h of ventilation (H1 to H6) receiving physiologically variable ventilation (PVV, filled circles) or pressure-controlled ventilation (PCV, empty circles). Values are expressed as mean ± standard deviation. Relative changes compared to H0 after the application of 6-h PCV (white box) or PVV (gray box) are reported in the panels on the right. R_{aw}, airway resistance; I_{aw}, airway inertance; G, respiratory tissue damping; H, respiratory tissue elastance. *$p < 0.05$ *versus* H0; #$p < 0.05$ *versus* PCV.

higher $PaCO_2$ after 6 h of mechanical ventilation than those in the PVV group ($p < 0.001$).

Mean arterial pressure and heart rate elevated during the 6-hour ventilation with no difference between the protocol groups (**Supplementary Figure S1**).

Respiratory Mechanical Parameters

All mechanical parameters (R_{aw}, I_{aw}, G, and H) progressively increased after 6 h of mechanical ventilation, irrespective of the ventilation mode (**Figure 5**). Nevertheless, increases in H were significantly lower after 6 h of mechanical ventilation with PVV compared to PCV ($p = 0.002$). Moreover, there was a significant correlation between H and oxygenation index during the 6 h of ventilation ($r = -0.47$, $p < 0.001$, **Figure 6**).

Lung Aeration Area and Intrapulmonary Shunt Fraction

The relative change in lung aeration area during the 6-h ventilation period is represented in **Figure 7**. While lung aeration remained unchanged during the study period in the PVV group, a significant and progressive deterioration in lung aeration appeared from the first hour of ventilation in the PCV group ($p = 0.02$). This decrease in lung aeration area became significant between the two groups at H6 ($p = 0.007$).

No difference in Qs/Qt was observed at H0 between the protocol groups; however, intrapulmonary shunt fraction was significantly elevated at H6 only in animals ventilated with PCV ($p < 0.001$). This increase resulted in significantly higher Qs/Qt in PCV compared to PVV at H6 ($p = 0.002$).

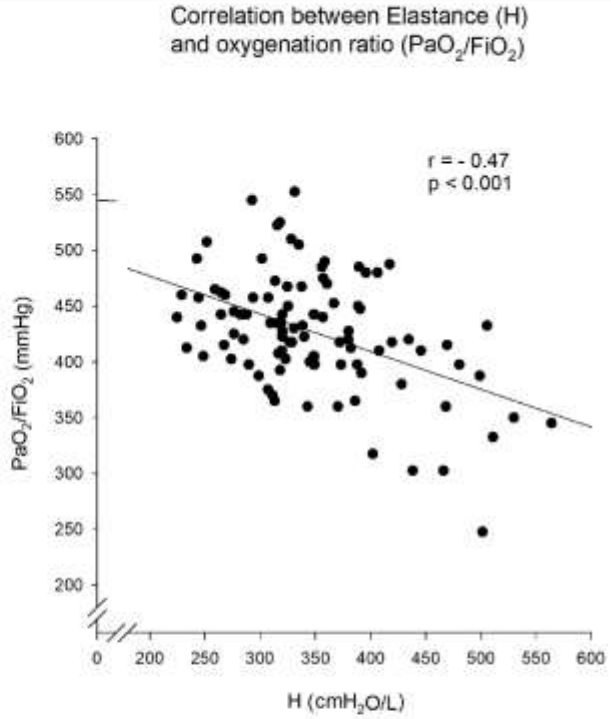

FIGURE 6 | Relationship between the tissue mechanical parameter elastance (H) and the oxygenation index (calculated as partial pressure of oxygen in the arterial blood divided by the fraction of inspired oxygen, PaO_2/FiO_2). The trend line corresponds to the best linear fit. Pearson's correlation coefficient (r) shows statistical significance ($p < 0.001$).

Histological, Cellular, and Protein Assessment

Pathological findings are summarized in **Figure 8**. Although no difference in the WBC count at H0 was observed between groups, a significant increase was detected at H6 in the PCV group ($p = 0.04$). In addition, there was a tendency for a significant increase in the total cell count in the BALF in the PCV group in comparison to PVV ($p = 0.051$). Moreover, the differential cell count in the BALF revealed a significantly higher number of lymphocytes in the PCV group ($p = 0.02$). Proteins and cytokine concentrations in the BALF and in the lung tissue revealed no significant difference between the two groups (**Table 2**). After application of PCV and PVV, there was no evidence for a difference in the overall histological injury score (**Figure 8D**) or in its five histological components (neutrophils in the alveolar and interstitial spaces, hyaline membranes, proteinaceous debris, and alveolar septal thickening) (**Supplementary Figure S2**). Of note, both groups of animals presented similar degrees of lung tissue inflammation and emphysema, as assessed with MLI and pathology scoring system (**Figure 9**).

DISCUSSION

In an experimental model of COPD, mechanical ventilation with a physiologically variable mode had a beneficial effect compared to the conventional pressure-controlled mode by preventing deterioration in oxygenation and respiratory mechanics. In addition, PVV, in contrast to PCV, protected from declines in tidal volume, intrapulmonary shunt fraction, and lung aeration over time. While these beneficial effects were also reflected in the cytology findings, no clear difference was observed in histological injury.

The experimental model applied in the present study aimed at reproducing the hallmark features of COPD, including lung inflammation and emphysematous airspace enlargement, associated with peribronchiolar infiltrates, inflammation, and thickening in the interstitial and alveolar spaces (Wittels et al., 1974; Kuhn et al., 1976; Mahadeva and Shapiro, 2002; Vernooy et al., 2002). Indeed, the histological analysis confirmed the presence of emphysema and inflammation (**Figure 9** and **Supplementary Figure S2**, respectively). Moreover, lung pathology revealed heterogeneous inflammatory cell infiltrates in the alveolar, interstitial, and peribronchiolar spaces, and rare appearance of multifocal hemorrhages and hypertrophy of the muscular media of small pulmonary arteries. These pathological changes were obtained by combining elastase and LPS nebulization, as described previously in rodents (Sajjan et al., 2009; Ganesan et al., 2010). In this experimental protocol, we did not reproduce the characteristics of airway obstruction and intrinsic PEEP that are often observed while ventilating COPD

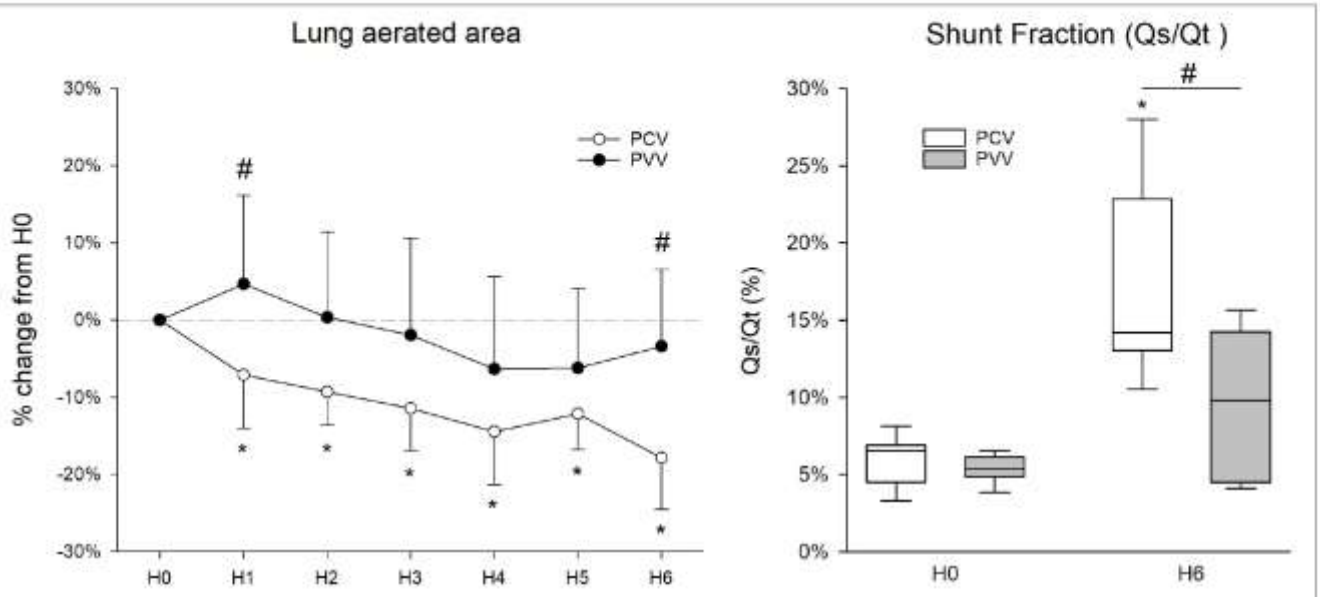

FIGURE 7 | Percentage change of lung aerated area (left graph) obtained from chest X-ray imaging during the 6 h of ventilation (H1 to H6) relative to the lung aerated area at the ventilation onset (H0). Values are expressed as mean ± standard deviation. On the right, shunt fraction (Qs/Qt, in percentage) before and after 6 h of mechanical ventilation (H0 and H6, respectively). Results from physiologically variable ventilation (PVV) and pressure-controlled ventilation (PCV) are represented with filled and empty circles/boxes, respectively. *$p < 0.05$ *versus* H0; #$p < 0.05$ *versus* PCV.

patients, especially during acute exacerbations of the disease. Instead, our model recapitulates a stable COPD scenario (i.e., when a COPD subject requires mechanical ventilation during anesthesia). Despite potential differences between experimental models of COPD and the complex pathophysiology of COPD in humans (Wright et al., 2008), the model applied in the present study presented the main histological features of COPD. Therefore, it can be considered as a reliable model to assess the potential deleterious effects of mechanical ventilation in the presence of COPD.

TABLE 2 | Protein concentration in the supernatant of the bronchoalveolar lavage fluid (BALF), and in frozen lung tissue homogenate are expressed as mean ± standard deviation for pressure-controlled ventilation (PCV) and physiological variable ventilation (PVV).

BALF	PCV	PVV
Total protein (μg/mL)	0.11 ± 0.03	0.13 ± 0.06
TNF-α (pg/mL)	38.8 ± 7.88	41.04 ± 15.88
IL-8 (pg/mL)	238.66 ± 96.68	250.80 ± 82.42
IL-6 (pg/mL)	2.60 ± 2.35	3.79 ± 3.18
SP-B (ng/mL)	0.32 ± 0.37	0.31 ± 0.20
SP-D (ng/mL)	0.28 ± 0.05	0.44 ± 0.36
E-cadherin (ng/mL)	0.40 ± 0.66	0.58 ± 0.69
Lung tissue	**PCV**	**PVV**
TNF-α (pg/mg of protein)	*Out of range**	*Out of range**
IL-8 (pg/mg of protein)	39.49 ± 14.36	39.65 ± 10.29
IL-6 (pg/mg of protein)	9.03 ± 2.44	7.32 ± 1.66
SP-B (ng/mg of protein)	0.82 ± 0.22	0.76 ± 0.23
SP-D (ng/mg of protein)	2.90 ± 0.73	3.89 ± 1.22
E-cadherin (ng/mg of protein)	2.70 ± 0.70	2.38 ± 0.55

*TNF, tumor necrosis factor; IL, interleukin; SP, surfactant protein.*Optical density values were out of the absorbance detection limits, equally in both experimental groups.*

The variable ventilation pattern applied in the present study was recorded in awake rabbits after induction of COPD. We hypothesized that the breathing pattern of healthy subjects would not be adequate for chronically diseased lungs, namely, in this model of COPD. This approach contrasts with previous applications of variable ventilation where the pattern was generated from healthy, spontaneously breathing animals (Lefevre et al., 1996; Walesa et al., 2018) or with random variability (Arold et al., 2002). This difference may explain the lower coefficient of variation obtained in the present study compared to that reported for healthy rabbits (Walesa et al., 2018). This finding is in agreement with available data demonstrating decreased breathing variability in the presence of COPD and restrictive lung diseases (Brack et al., 2002; Huhle et al., 2016). Therefore, we find it a reasonable assumption that the variable ventilation pattern applied in the present study resembles the spontaneous breathing observed in COPD subjects.

One major finding of the present study was the improved gas exchange with PVV compared to conventional PCV. Despite equal mean $P_{driving}$ and RR between the two ventilation modes, prolonged application of PVV prevented the deterioration of both oxygenation and $PaCO_2$. Similar benefits of variable ventilation on gas exchange were reported for models of prolonged ventilation of healthy lungs (Mutch et al., 2000a), atelectasis (Mutch et al., 2000b; McMullen et al., 2006), prematurity (Bartolak-Suki et al., 2017), bronchospasm (Mutch et al., 2007), and acute lung injury with nebulized LPS

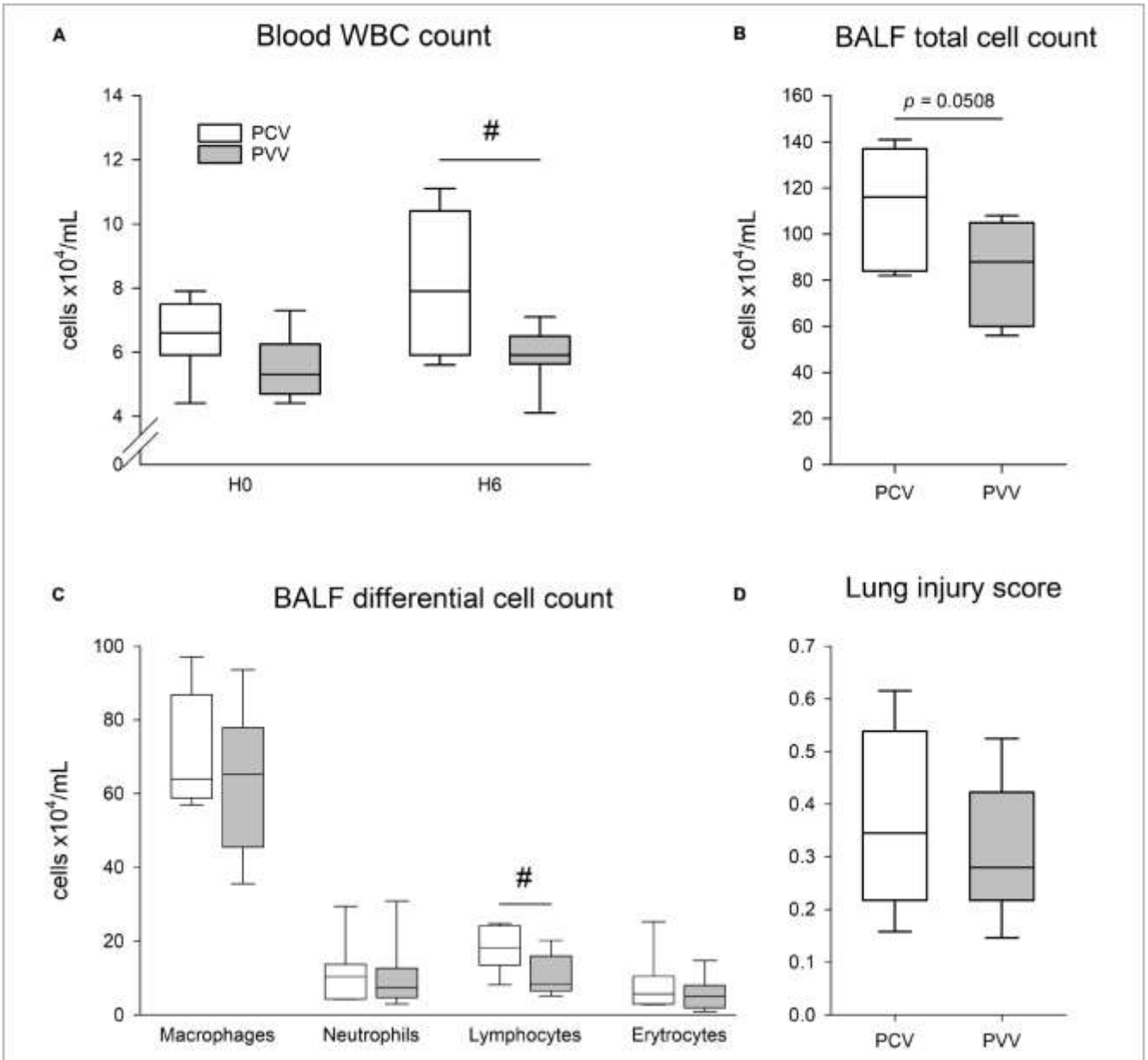

FIGURE 8 | (A) Total white blood cell (WBC) count in the blood at the onset (H0) and at the end of the 6-h period of mechanical ventilation (H6) with pressure-controlled (PCV, white) or physiologically variable modes (PVV, gray). **(B,C)** Total and differential cell counts in the bronchoalveolar lavage fluid (BALF) after 6 h of mechanical ventilation with PCV and PVV. **(D)** Lung injury score (range: 0–1) assessed in the lung histological sections after application of 6 h of PVV or PCV. Data are represented as median and quartiles #$p < 0.05$ *versus* PCV.

(Arold et al., 2002). Concerning CO_2 clearance, animals in the PVV group remained normocapnic throughout the 6-h ventilation period, while those in the PCV group developed progressive hypercapnia. This phenomenon was observed despite a comparable RR between groups. Further increase in RR would have led to development of air trapping and auto-PEEP, despite the prolonged expiratory time with I:E ratio of 1:3.

Regardless of the ventilation mode, the deterioration observed in all respiratory mechanical parameters after mechanical ventilation in supine position can be attributed to progressive lung derecruitment. Since respiratory elastance reflects lung volume loss due to peripheral airway closure (Lutchen et al., 1996; Albu et al., 2013), the limited deterioration in H observed in animals with PVV suggest that application of variable ventilation prevented this alveolar derecruitment. These findings in an experimental model of COPD are in accordance with previous reports in other experimental conditions (Lefevre et al., 1996; Mutch et al., 2000b; Arold et al., 2002; Walesa et al., 2018), where variable ventilation

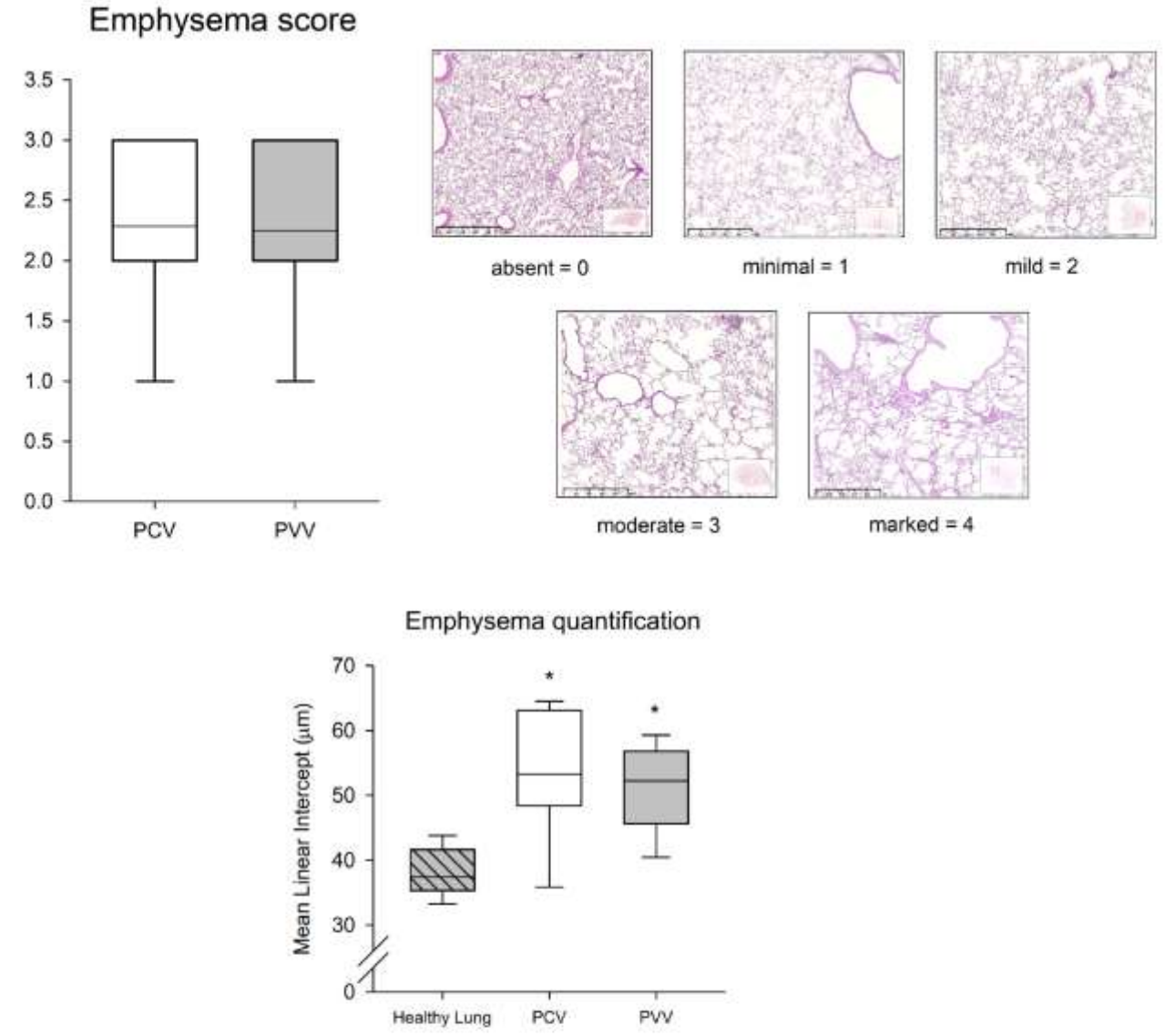

FIGURE 9 | Emphysema pathology score (upper graph), using a five-tier system with 0–4 grading scale: 0, absent; 1, minimal; 2, mild; 3, moderate; and 4, marked. Classification was based on the most severe lesions in 18 lung step slices per animal, anterior to posterior. Example micrographs of each score category are represented in the upper right panels. In the lower graph, airspace enlargement is quantified in histological sections of healthy lungs from control rabbits and PCV and PVV rabbits using MLI (μm). Data are represented as median and quartiles for PCV (pressure-controlled ventilation) and PVV (physiological variable ventilation). *$p < 0.05$ *versus* healthy lung.

improved lung distensibility (H or respiratory compliance). Moreover, the significant correlation obtained between H and PaO_2/FiO_2 further confirms that the increase in H can be attributed to the loss of alveolar units available for gas exchange. The ability of PVV to protect from the deterioration of respiratory elastance was associated with maintenance of lung aeration and a less severe decline in mean V_T, which further support the recruitment effect of PVV in the presence of COPD. This recruitability effect observed for variable ventilation agrees with previous results reported in the presence of ARDS (Graham et al., 2011; Ruth Graham et al., 2011; Huhle et al., 2016).

Another beneficial effect of PVV was manifested in the ameliorated ventilation-perfusion matching that was estimated from the intrapulmonary shunt fraction. The Qs/Qt was in the physiological range for both groups of animals at onset of the ventilation period (Marino, 2014). While intrapulmonary shunt fraction increased after the 6-h ventilation with PCV, physiological ventilation-perfusion matching was preserved in the animals under PVV (**Figure 7**). This finding is in accordance with the improved aeration, lower derecruitment, and maintenance of normocapnia observed in animals ventilated with PVV.

Assessing the effect of PVV on inflammatory cells revealed benefits both in the blood and the BALF. The effects of variable ventilation on lung inflammatory response are a subject of controversy (Huhle et al., 2016). Our results obtained with the COPD model support earlier observations on the benefit of variable ventilation on lung inflammation (Boker et al., 2002; Arold et al., 2003; Kiss et al., 2016). It is worth noting that all the beneficial effects of PVV outlined above were not reflected in the cytokine profiles and lung histological findings. This result can be attributed to the already established lesions induced by LPS and elastase in the lungs, which likely produce more profound injury than that produced by ventilation. Accordingly, the basal cell counts in the BALF and the histological scores measured in the present model of COPD were correspondingly higher and more severe than those reported earlier for healthy rabbits ventilated with PVV or PCV for 7 h (Walesa et al., 2018).

Variable ventilation has been previously studied in a rat model of elastase-induced emphysema (Henriques et al., 2016), using mathematically random variability over the course of 2 h. This study reported a benefit in lung elastance but failed to observe any effect in gas exchange. While the timespan of the experimental ventilation might have been too short to detect effects in oxygenation, also the pattern and extent of variability might not be appropriate to emphysematous lungs. In fact, it has been demonstrated that too much variability can have deleterious effects (Nam et al., 2000; Wierzchon et al., 2017). On the contrary, in the present study, the driving signal of variable ventilation was the pattern of spontaneous breathing recorded in awake rabbits, after COPD features were induced by a combination of elastase and LPS.

This study has a certain number of limitations which warrant consideration. First, a smaller number of animals was included in the 6-h ventilation period that the estimated sample size. Despite the optimization of the COPD induction protocol, one-third of the experimental animals did not survive the elastase nebulization. However, evidence for the benefits of PVV over PCV was already demonstrated with smaller number of animals and thus, requesting additional animals to reach the initially estimated sample size could not be justified in view of the recommendations on the reduction of the use of animals in research (Russell and Burch, 1959). Second, sham-treated rabbits were not included in the present study. Instead, the current study outcomes were compared to previous results obtained in ventilated healthy rabbits from our research group (Walesa et al., 2018). Since these previous experiments were performed under identical conditions, the comparisons are valid and compliant with the recommendations on the reduction of the use of animals in research (Russell and Burch, 1959), as requested by the local animal welfare committee. Third, the timespan of the mandatory ventilation in the present study was limited to 6 h. Thus, extrapolating the present results for longer term ventilation and outcomes may be limited. Of note, a recent clinical trial failed to demonstrate long-lasting benefits when applying a mathematical model of variable ventilation in healthy lungs (Spieth et al., 2018). Finally, the design and sample size calculation of the study were not powered to demonstrate histological and cytokine outcomes.

CONCLUSION

In summary, the comparison of PVV to conventional PCV in an experimental model of COPD revealed that the introduction of physiological variability to mechanical ventilation improves oxygenation, CO_2 clearance, respiratory tissue mechanics, tidal volume, lung aeration, and intrapulmonary shunt fraction. Thus, in a model of COPD, PVV has the ability to prevent alveolar derecruitment, thereby reducing alveolar shear stress with subsequent improvement in gas exchange. Therefore, our results encourage the consideration of PVV as a protective ventilation modality in the context of COPD.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Welfare Committee of the Canton of Geneva and the Experimental Ethics Committee of the University of Geneva, Switzerland (GE 184/18, 2 January 2019).

AUTHOR CONTRIBUTIONS

ADSR, RS, RD, FP, and WH contributed to study design. ADSR, RS, and DB contributed to experimental work. ADSR, RS, DB, MK, TC, FP, and WH contributed to data analyses. ADSR, FP, and WH contributed to manuscript drafting. ADSR, RS, DB, MK, TC, RD, FP, and WH contributed to manuscript review and editing. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2020.625777/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary Material

Benefit of physiologically variable over pressure-controlled ventilation in a model of chronic obstructive pulmonary disease: a randomized study

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Figure S1

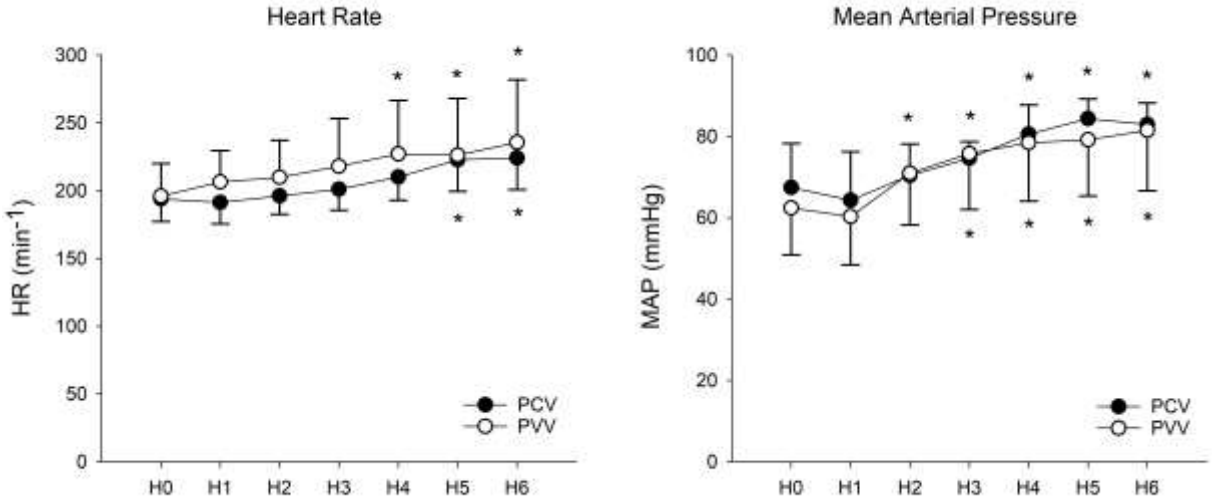

Figure S1. Hemodynamic parameters obtained during the 6-hour ventilation period. Values expressed as mean ± standard deviation. MAP: mean arterial pressure; HR: heart rate; H0 to H6: average value during the corresponding hour of the 6-hour long ventilation period; PCV: pressure-controlled ventilation; PVV: physiological variable ventilation. *: $p < 0.05$ vs. H0

Figure S2

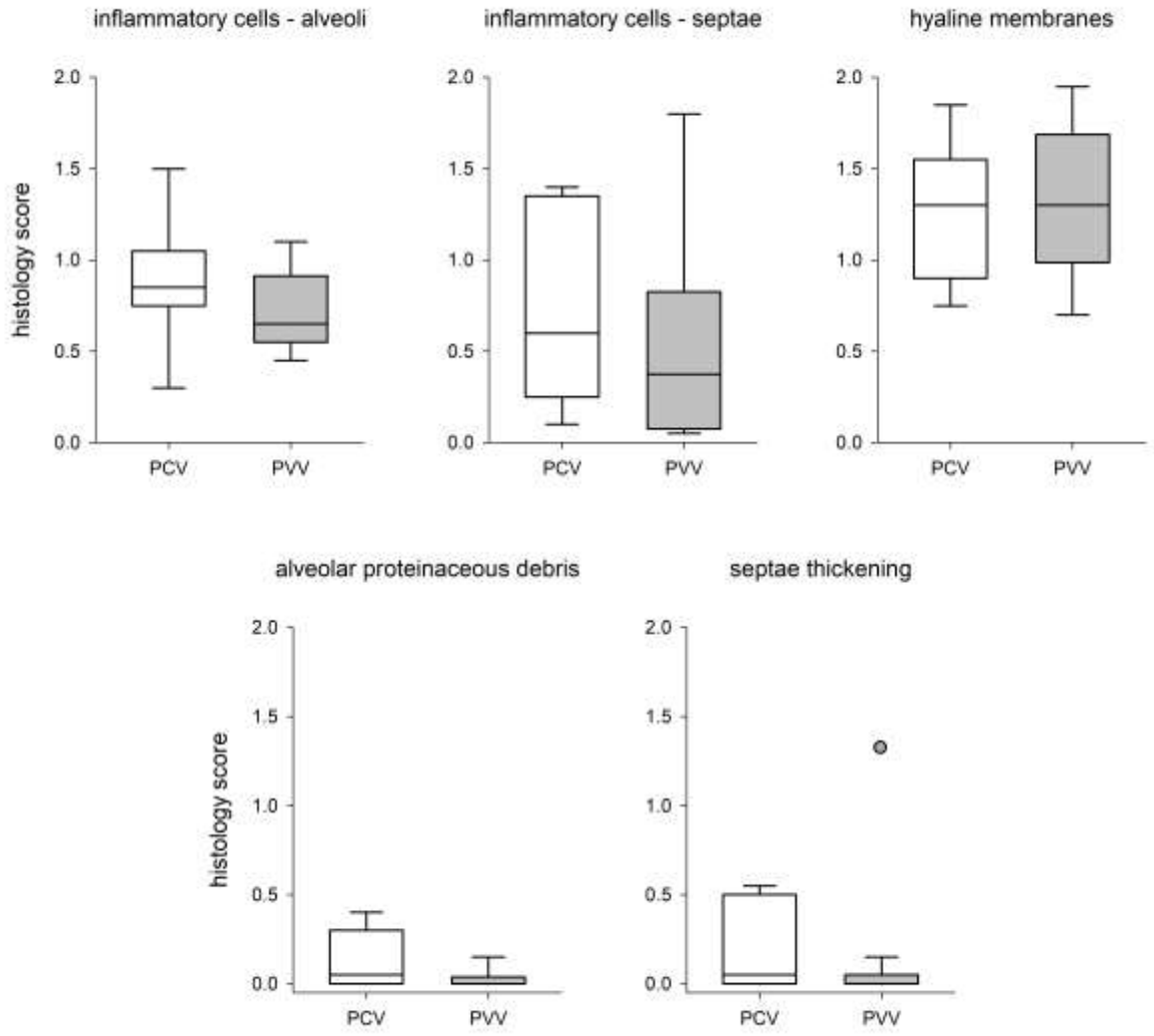

Figure S2. Quantification of the five histological components of the Lung Injury Scoring System, summarized in Figure 6, using the guidelines of the American Thoracic Society (145). Accordingly, a score per field (between 0 and 2) was averaged from 18 lung step slices, anterior to posterior, of the left lung. Data in the box plot is represented as median and quartiles. PCV: pressure-controlled ventilation; PVV: physiological variable ventilation.

Table S1. Animal welfare score, approved by the Animal Welfare Committee of the Canton of Geneva and the Experimental Ethics Committee of the University of Geneva, Switzerland (GE 184/18, January 2nd, 2019), for bi-weekly assessment of rabbit well-being during COPD induction. If the total score was ≥1, rabbits would receive supplementary oxygen. BW: body weight.

Animal Welfare Score (Protocol GE 184/18)

For each parameter, attribute a score of 0 (absent) or 1 (present).

Reduced activity
No grooming
Pale eyes
Folding of the eyelids
Reduction of food/water intake
Weight loss (> 5% BW between 2 evaluations or 10% in one week)
Change of posture, folding of the abdomen, muscle tension
On guard, tendency to hide or be aggressive
Dyspnea (noisy breathing, sneezing, respiratory rate >60/min)

DECLARATIONS

PRESENTATIONS

The scientific results of the current thesis were presented in scientific conferences prior to its publication, as follows:

▪ **Physiologically variable *versus* pressure-controlled ventilation in COPD: a randomized experimental study** - Oral presentation at the Anesthesiology 2020 – the annual conference of the American Society of Anesthesiolgy; 5 October 2020. Washington (virtual), USA.

▪ **Physiologically variable ventilation improves lung mechanics and regional inflammation in a multiple-hit model of ARDS**. Free communication at the Federation for Translational Medicine of Strasbourg, 4 December 2019, Strasbourg, France.

▪ **Regional distribution of lung inflammation in an animal model of ARDS assessed by micro-PET-CT imaging** – Poster presentation at the Annual Congress of the Swiss Society of Anesthesiology and Reanimation, November 2019, Interlaken, Switzerland.

▪ **Benefits of physiological variable ventilation during asthma exacerbations: a randomised experimental study** – Poster Presentation at the European Respiratory Society International Congress. September 2019. Madrid, Spain.

▪ **A rabbit model of chronic obstructive pulmonary disease: how to refine animal experiments in respiratory medicine** – Poster Presentation at the Swiss 3R Day. September 2019. Bern, Switzerland.

▪ **Value of capnography to detect asthma exacerbations during mechanical ventilation - a study in allergen-sensitized rabbits**. Poster Presentation at the Annual Congress of the Swiss Society of Anesthesiology and Reanimation, 8 November 2018, Interlaken, Switzerland.

▪ **Impact of natural sleep, sedation and hypercapnia on physiological ventilation variability: an experimental study**, Poster Presentation at the European Respiratory Society International Congress, September 2018, Paris, France.

PRIZES

The author was awarded the following prizes based on the results of the present thesis:

▪ **Bayer Award – 1st Prize - Best Free Communication**

Attributed to the free communication "Benefits of physiological variable ventilation during asthma exacerbations: a randomised experimental study", during the Annual Congress of the Swiss Society of Anesthesiology and Reanimation, November 2019, Interlaken, Switzerland.

▪ **Bayer Award – 2nd Prize - Poster**

Attributed to the poster "Regional distribution of lung inflammation in an animal model of multiple-hit ARDS assessed by micro-PET-CT imaging", during the Annual Congress of the Swiss Society of Anesthesiology and Reanimation, November 2019, Interlaken, Switzerland.

▪ **Swiss 3R Competence Centre - Best Young Scientist Poster Award**

Attributed to the poster "A rabbit model of chronic obstructive pulmonary disease: how to refine animal experiments in respiratory medicine", during the Swiss 3Rs Day Meeting, September 2019, Bern, Switzerland.

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