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**EFFECTS OF ARTIFICIALLY-INDUCED INTRINSIC
POSITIVE END-EXPIRATORY PRESSURE IN
MECHANICALLY-VENTILATED PATIENTS WITH
ACUTE RESPIRATORY FAILURE**

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ABSTRACT

It has been suggested that, in patients with acute respiratory failure (ARF), relative to conventional mechanical ventilation with application of external positive end-expiratory pressure (PEEPe), mechanical ventilation artificially inducing intrinsic PEEP (PEEPi) worsens arterial oxygen tension (PaO_2) by causing a less homogeneous distribution of inspired gas, even if tidal volume (V_t), respiratory rate (RR) and total PEEP (PEEP_{tot}) are kept invariant.

This hypothesis has been investigated assessing spatial ventilation heterogeneity directly with electrical impedance tomography (EIT), and measuring mechanical and capnographic parameters indirectly related to ventilation unevenness, together with measurements of hemodynamic function and gas exchange.

Seventeen patients with ARF without chronic obstructive pulmonary disease were mechanically ventilated in four experimental conditions, with the same RR and V_t . In one condition, PEEPe was zero (ZEEP); in the others, PEEP_{tot} was constant, but obtained by either applying external PEEP (PEEPe) or shortening the duration of expiration to induce PEEPi. PEEPi was produced either by increasing the duration of the inflation (PEEPi no pause, PEEP_{inp}), or by introducing a post-inspiratory pause while keeping the duration of the inflation constant (PEEPi with pause, PEEP_{iwp}). In all conditions, we measured PaO_2 , respiratory mechanics, cardiac output, physiological dead space (DS_{phys}) and the slope of capnographic phase III, ventral and dorsal ventilated surface, and global inhomogeneity index (GI), an index of spatial heterogeneity of ventilation distribution.

A similar PEEP_{tot} of ~ 7 cmH₂O was obtained at PEEPe, PEEP_{inp} and PEEP_{iwp}. With respect to PEEPe, PaO_2 decreased significantly at ZEEP and both PEEPi conditions. Cardiac output was similarly depressed at PEEPe, PEEP_{inp} and PEEP_{iwp} than at ZEEP. Regarding respiratory mechanics, static elastance did not change among the conditions, while interrupter and additional resistance were respectively lower and higher at PEEP_{inp} than at the other conditions, in line with what expected from the increase in inflation time. Both DS_{phys} and slope of phase III decreased significantly at PEEP_{iwp} than at the other conditions. Ventral ventilated surface did not vary among the conditions and, with respect to ZEEP, the dorsal one increased similarly in all other conditions, while GI was

significantly higher at ZEEP than at the other conditions, but similar between PEEPe, PEEPinp and PEEPiwp.

Our results confirm previous findings of a worse PaO_2 with artificially-induced PEEPi with respect to ventilation with PEEPe. However, none of the measured cardiorespiratory parameters appear to provide a direct explanation for this difference and results of ventilated surface and GI from EIT seem to argue against the hypothesis of an increased ventilation heterogeneity during ventilation with artificial PEEPi. These findings suggest that other mechanisms may be at play and further research is needed to better elucidate the factors behind poorer PaO_2 during this kind of ventilation.

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ABBREVIATIONS

PEEP	positive end-expiratory pressure
PEEPi	intrinsic positive end-expiratory pressure
auto-PEEP	see PEEPi
FRC	functional residual capacity
DH	dynamic hyperinflation
EFL	expiratory flow-limitation
ZEEP	zero end-expiratory pressure
PEEPe	external positive end-expiratory pressure
PEEPinp	PEEPi no pause
PEEPiwp	PEEPi with pause
CPAP	continuous positive airway pressure
IRV	inverse ratio ventilation
ARF	acute respiratory failure
ARDS	acute respiratory distress syndrome
ICU	intensive care unit
BMI	body mass index
APACHE II	Acute Physiology And Chronic Health Evaluation II
SOFA	Sequential Organ Failure Assessment
CT	computed tomography
T_I	duration of inspiration
T_E	duration of expiration

pIp	post-inspiratory pause
pEp	post-expiratory pause
V_t	tidal volume
RR	respiratory rate
F_{insp}	inspiratory flow
F_{exp}	mean expiratory flow
$\dot{V}_E$	minute ventilation
PEEP_{tot}	total PEEP (the sum of external and intrinsic PEEP)
$\Delta EELV$	difference between the actual end-expiratory volume and the relaxation volume of the respiratory system
FiO₂	fraction of inspired oxygen
PaO₂	partial pressure of oxygen in the arterial blood
PaCO₂	partial pressure of carbon dioxide in the arterial blood
HCO₃⁻	bicarbonate concentration in the arterial blood
(A-a)O₂	alveolar-arterial gradient of oxygen
SV	stroke volume
SVV	stroke volume variation
CO	cardiac output
BP_{syst}	systolic arterial pressure
BP_{diast}	diastolic arterial pressure
MAP	mean arterial pressure
CVP	central venous pressure
HR	heart rate

P_{aw}	pressure at airway opening (in an intubated mechanically ventilated patient is the pressure at the Y of the circuit connecting the subject to the ventilator)
P_{alv}	alveolar pressure
P_{es}	esophageal pressure
$P_{max,aw}$	peak inspiratory airway pressure
$P_{max,es}$	peak inspiratory esophageal pressure
$P_{1,aw}$	airway pressure immediately after the rapid interruption of inspiratory flow
$P_{1,es}$	esophageal pressure immediately after the rapid interruption of inspiratory flow
$P_{st,aw}$	airway pressure at the end of a long post-inspiratory pause
$P_{st,es}$	esophageal pressure at the end of a long post-inspiratory pause
$P_{st,L}$	transpulmonary pressure at the end of a long post-inspiratory pause
$P_{ee,aw}$	airway pressure at end-expiration
$P_{ee,es}$	esophageal pressure at end-expiration
$P_{ee,L}$	transpulmonary pressure at end-expiration
$P_{epEp,es}$	esophageal pressure at the end of a long post-expiratory pause
$E_{st,rs}$	static elastance of the respiratory system
$E_{st,cw}$	static elastance of the chest wall
$E_{st,L}$	static elastance of the lung
ΔR_{rs}	additional resistance of the respiratory system
ΔR_{cw}	additional resistance of the chest wall
ΔR_L	additional resistance of the lung
$R_{int,rs}$	interrupter resistance of the respiratory system
$R_{int,cw}$	interrupter resistance of the chest wall
$R_{int,L}$	interrupter resistance of the lung

time-constant product of airway resistance times respiratory system compliance

$\overline{P_{aw}}$ mean airway pressure

$\overline{P_{alv}}$ mean alveolar pressure

P_{ao} airway occlusion pressure

P_{res} resistive pressure drop at end-inspiration

SI stress index

DS_{phys} physiological dead space

DS_{anat} anatomical dead space

DS_{alv} alveolar dead space

PeCO₂ partial pressure of CO₂ in the mixed expiratory air

MDT mean distribution time

EIT electrical impedance tomography

SURF ventilated surface (number of ventilated pixels)

SURF_v ventral ventilated surface (number of ventilated pixels in the ventral part)

SURF_d dorsal ventilated surface (number of ventilated pixels in the dorsal part)

TID tidal impedance difference

TID_v tidal impedance difference in the ventral part

TID_d tidal impedance difference in the dorsal part

GI global inhomogeneity index

GI_v global inhomogeneity index in the ventral part

GI_d global inhomogeneity index in the dorsal part

RVD_{ij} regional ventilation delay of pixel ij

RVD SD standard deviation of RVD_{ij}

RVD	see RVD SD
$\Delta Z_{ei-ee,ij}$	tidal impedance change between end-inspiration and end-expiration in pixel ij
$\overline{\Delta Z_{ei-ee}}$	average pixel tidal impedance change
Z_{ij}	absolute impedance value in pixel ij in a given moment
Z_{tot}	absolute global impedance value in a given moment
$\hat{Z}_{ij}$	absolute impedance value in pixel ij in a given moment normalized to its tidal variation
$\hat{Z}_{tot}$	absolute global impedance value in a given moment normalized to its tidal variation
ANOVA	analysis of variance
SD	standard deviation

INTRODUCTION

When a healthy subject breaths spontaneously at rest in the ambient, at end-expiration, the volume of the respiratory system (that is functional residual capacity, FRC) corresponds, on the average, to the equilibrium volume of the respiratory system, and alveolar pressure (P_{alv}) is equal to the pressure at airway opening (P_{aw}). This is also the case for a healthy paralyzed subject mechanically-ventilated at zero end-expiratory pressure (ZEEP), as long as expiratory duration (T_E) is not set to an unusual low value. When a continuous positive airway pressure (CPAP) is applied to the spontaneously breathing healthy subject, or an external positive end-expiratory pressure (PEEPe) to the mechanically ventilated healthy subject, at end-expiration, equalization between P_{alv} and P_{aw} still occurs, and FRC corresponds to the new equilibrium volume (that is volume predicted by the static pressure-volume relation at that given CPAP or PEEPe).

SPONTANEOUSLY OCCURRING INTRINSIC POSITIVE END-EXPIRATORY PRESSURE

If T_E is not long enough for the respiratory system to return to the equilibrium volume, at end-expiration P_{alv} remains greater than P_{aw} by an amount called intrinsic positive end-expiratory pressure (PEEPi) or auto-PEEP. In this situation, FRC rises progressively (dynamic hyperinflation, DH), until the increased lung recoil generates an expiratory flow sufficient to reach a new equilibrium. At this point, expired volume is again equal to inspired volume, and on the average FRC does not change anymore.

During spontaneous breathing or mechanical ventilation, PEEPi can develop spontaneously because of pathological changes of the respiratory system, such as an increase of airway resistance or a decrease of lung recoil. Alternatively, PEEPi can appear because the time available for expiration is artificially shortened to a point so to limit complete emptying of the system down to equilibrium volume (Laghi and Goyal, 2012).

In both spontaneously breathing and mechanically ventilated patients, the spontaneous occurrence of PEEPi is frequently caused by the presence of global tidal expiratory flow-limitation (EFL) (Alvisi et al., 2012; Armaganidis et al., 2000; Boczkowski et al., 1997; Duguet et al., 2000; Eltayara et al., 1996; Ferretti et al., 2001; Goetghebeur et

al., 2002; Koulouris et al., 2003; Koutsoukou et al., 2000; Pankow et al., 1998; Pecchiari et al., 2020, 2009; Volta et al., 2019), that is the inability of expiratory flow to increase in response to an increase of driving pressure at isovolume. This phenomenon, secondary to pathological changes of airways and/or lung parenchyma, manifests with the formation of choke points along the tracheobronchial tree, causing a marked increase of airway resistance, which in turn prevents complete emptying of the respiratory system. Tidal EFL is a common occurrence in acute respiratory distress syndrome (ARDS) patients ventilated at ZEEP, and can be abolished in this population by application of PEEP_e (Junhasavasdikul et al., 2018; Koutsoukou et al., 2002, 2000).

Spontaneously occurring PEEP_i and DH are responsible of a number of deleterious pathophysiological consequences.

In spontaneously breathing subjects, and in patients treated with ventilatory support, DH and PEEP_i increase the work of breathing and decrease the efficiency of inspiratory muscles contraction, thus contributing to the development of dyspnea and, possibly, muscle fatigue (Bégin and Grassino, 1991; Haluszka et al., 1990; Loring et al., 2009; Pardy and Roussos, 1983). In these subjects, increased intrathoracic pressure during expiration causes preload at the level of the right ventricle to decrease, and the elevation of P_{alv} during expiration decreases the portion of lung parenchyma in West's zone 3. This in turn causes pulmonary vascular resistance to increase, resulting in an increase of the afterload to the right ventricle. Both these effects tend to depress cardiac output (Laghi and Goyal, 2012). These negative hemodynamic effects are present also in mechanically-ventilated paralyzed patients (Gottfried, 1991; Pepe and Marini, 1982). It is important to note that when PEEP_i and DH occur spontaneously, they appear to affect the different lung units¹ with different degrees. Indeed, a study suggested that heterogeneity of regional PEEP_i distribution is associated with a deterioration of gas exchange (Koutsoukou et al., 2002).

ARTIFICIALLY-INDUCED INTRINSIC POSITIVE END-EXPIRATORY PRESSURE

While the deleterious consequences of spontaneously occurring PEEP_i and DH during mechanical ventilation are well-recognized, less is known about the

¹ “Lung unit” is a general expression referring to a portion of lung parenchyma, whose anatomical components are characterized by homogeneous functional properties, in terms of mechanics and gas exchange.

pathophysiological effects of artificially-induced PEEPi, that is the PEEPi intentionally produced by a decrease of expiratory time, in the absence of global tidal EFL, such as during inverse I:E ratio ventilation (IRV) or airway pressure release ventilation.

Few previous studies have investigated the effects on gas exchange of artificially-induced PEEPi relative to PEEPe, in the presence of the same total PEEP ($PEEP_{tot}$), respiratory rate (RR), tidal volume (V_t) and hence ventilation, in patients under mechanical ventilation for acute respiratory failure (ARF) or ARDS, without global tidal EFL. The results of these studies were partially conflicting and data remain inconclusive.

According to a first study conducted by Brandolese and coll. in the early nineties in patients under mechanical ventilation for ARF, for the same $PEEP_{tot}$, arterial oxygen tension (PaO_2) measured during ventilation with an artificially-induced PEEPi was lower than the one measured during ventilation at PEEPe (Brandolese et al., 1993). This conclusion, however, was drawn by the Authors indirectly, from the fact that, relative to ventilation at ZEEP, PEEPe improved PaO_2 , while artificially-induced PEEPi did not.

Another study on the topic, published a few years later by the same group on ARDS patients, was unable to detect a significant difference of PaO_2 when conventional ventilation at PEEPe was compared to IRV producing artificially-induced PEEPi at the same $PEEP_{tot}$ (Zavala et al., 1998).

As a matter of fact, both studies were most probably underpowered to detect small differences in PaO_2 ($n=10$ in (Brandolese et al., 1993) and $n=8$ in (Zavala et al., 1998)). Using data of (Brandolese et al., 1993), it could be estimated that, in order to detect a mean difference in PaO_2 of ~ 7 mmHg with a standard deviation of ~ 12 mmHg (as reported in the study), the sample size should have been at least 30 subjects, three times the one actually used in their work. Thus, the contrasting results among the two studies may be simply due to the limited sample size.

The interest in studying the effects of mechanical ventilation with artificially-induced PEEPi does not primarily stem from the PaO_2 difference relative to ventilation with an equivalent level of PEEPe. Indeed, the PaO_2 difference between these two ventilatory conditions, if present, appears relatively small. Rather, the interest for the effects of an artificially-induced PEEPi arises from the pathophysiological mechanisms determining this PaO_2 difference. If V_t , RR and $PEEP_{tot}$ are kept constant (as done in (Brandolese et al.,

1993) and (Zavala et al., 1998)), the difference in PaO_2 between ventilation at PEEPe and artificial PEEPi cannot be attributed to differences in ventilation or operating volume. Moreover, in (Brandolese et al., 1993), differences in cardiac output could not be found when PEEPe was directly compared with artificial PEEPi, and the same was found by Zavala and coll. when comparing ventilation at PEEPe with that at an artificial PEEPi produced similarly to Brandolese and coll. Thus, data seem to exclude changes of hemodynamics as a possible explanation for the PaO_2 difference between ventilation with PEEPe and artificially-induced PEEPi. These findings prompted Brandolese and coll. to hypothesize that the worse PaO_2 associated with artificially-induced PEEPi could result from an enhanced ventilation heterogeneity produced during ventilation in this condition (Brandolese et al., 1993).

Why should ventilation heterogeneity increase when a given level of PEEP_{tot} is produced by artificially-induced PEEPi rather than by application of PEEPe in subjects without global tidal EFL?

To answer this question we have to consider that, in order to produce PEEPi at a constant RR, T_E must be shortened and, if this is done also keeping V_t constant, this will necessarily result into an increase of the expiratory flow.

As long as the respiratory system behaves as a mechanically homogeneous system characterized by a single time-constant (product of airway resistance times respiratory system compliance), an increase of expiratory flow does not affect ventilation distribution, which remains homogeneous (Otis et al., 1956).

Patients with ARF, however, and, even more pronouncedly with ARDS, tend to present varying levels of mechanical heterogeneity of lung parenchyma (Gattinoni et al., 1988, 1987; Maunder et al., 1986; Swenson et al., 1995), and therefore their respiratory system behaves as if it is made by a number of units with different time-constants. In this situation, an increase of expiratory flow may affect ventilation distribution, and therefore gas exchange. Additionally, in line of principle, ventilation heterogeneity may be exacerbated if EFL, although absent at global level, is present regionally, since the increase of regional airway resistance due to compression further increases the scatter of regional time-constants.

Indeed, a study conducted on a lung model composed of units with different time-constants showed that, when a given level of $PEEP_{tot}$ is obtained by reducing T_E and inverting the I:E ratio, the units with longer time-constants exhibit a higher end-expiratory pressure and volume with respect to when the same $PEEP_{tot}$ is obtained by application of $PEEP_e$ (Kacmarek et al., 1995). The opposite was conversely observed for units with shorter time-constants, in which end-expiratory pressure and volume were lower compared to ventilation at $PEEP_e$. These findings substantiate the notion that, in presence of time-constant heterogeneity, the distribution of volume and pressure is different between conditions in which the same $PEEP_{tot}$ and lung volume are obtained by reduction of the T_E or by application of $PEEP_e$. Finally, parenchymal inhomogeneity in mechanics may impact pulmonary perfusion distribution due to transmission of P_{alv} on the lung vasculature, causing a relative reduction of perfusion in the hyperinflated units with longer time-constant and a relative increase of perfusion in the underinflated units with shorter time-constants. As a result, these phenomena, developing during ventilation with $PEEP_i$, could promote ventilation to perfusion mismatch and cause gas exchange deterioration, in spite of a $PEEP_{tot}$ and lung volume similar to ventilation at $PEEP_e$.

In recent times, a renewed interest for these issues has arisen from insights regarding the process of airway closure/alveolar collapse, a phenomenon responsible for derecruitment. Cyclic recruitment/derecruitment during tidal ventilation generates abnormal stresses at the level of the airways and of the surrounding parenchyma, potentially able to cause ventilator-induced lung injury (VILI) (D'Angelo et al., 2007, 2002; Pecchiari et al., 2012). While in the past airway collapse was believed to take place immediately upon reaching a critical closing pressure, some evidence has been now gathered indicating that not only pressure, but also time has a role in derecruitment. In other words, derecruitment does not take place instantaneously when the critical closing pressure is reached, but rather it occurs with a delay which is proportional to the difference between actual pressure and critical closing pressure (Bates and Irvin, 2002). If it is so, then, in line of principle, at a given transpulmonary pressure, derecruitment can be reduced by shortening T_E , as it is done during airway pressure release ventilation, so that less time is available for the airways to collapse. In this way, however, during deflation the equilibrium volume is not reached, and most of $PEEP_{tot}$ is provided by $PEEP_i$. It is debated whether, in this situation, the possible

negative effects of artificially-induced PEEPi may exceed the beneficial effects of a reduction of recruitment/derecruitment (Habashi et al., 2024; Parhar and Doig, 2024).

In the last decades, electrical impedance tomography (EIT) emerged as a viable mean to assess ventilation distribution, thus offering a unique opportunity to study ventilation distribution in the presence of artificially-induced PEEPi. EIT has progressively gained attention in the setting of intensive care as a non-invasive, radiation-free technique available at the bedside to continuously monitor in real time pulmonary ventilation in mechanically ventilated subjects. Several indexes and parameters have been proposed in the years to capture information regarding spatial and temporal heterogeneity of ventilation, such as global inhomogeneity index (GI) (Zhao et al., 2009b) and regional ventilation delay (RVD) (Wrigge et al., 2008). Literature on the application of EIT-derived parameters in the clinical setting is currently having great expansion and a continuously growing amount of data regarding already existing and new parameters is being accumulated.

AIMS

The aim of the present work is twofold.

First, since the results of previous studies were not univocal, we aimed at verifying the effects on oxygenation of mechanical ventilation with an artificially-induced PEEP_i, relative to mechanical ventilation with PEEP_e, at the same ventilation and PEEP_{tot}, using a sample size of sufficient dimension to detect small differences in PaO₂. To this purpose, artificially-induced PEEP_i was produced as in the previous studies (Brandolese et al., 1993; Zavala et al., 1998), that is by increasing the duration of the inspiration at the expenses of the duration of the expiration (PEEP_i no pause condition, PEEP_{inp}). As this implies a reduction of mean inspiratory flow, and since mean inspiratory flow may be a determinant of ventilation distribution (Santini et al., 2019), artificially-induced PEEP_i was also produced by keeping inspiratory duration and flow constant, while shortening T_E by the introduction of a post-inspiratory pause between inspiration and expiration (PEEP_i with pause condition, PEEP_{iwp}).

Second, the hypothesis proposed by Brandolese and coll., according to which deterioration of gas exchange during ventilation with artificially-induced PEEP_i is caused by a more heterogeneous distribution of ventilation (Brandolese et al., 1993), was investigated using respiratory mechanics and EIT. Patients were also studied during ventilation at ZEEP as a positive control, as at ZEEP both gas exchange and ventilation distribution are supposed to deteriorate with respect to ventilation at PEEP_e, provided that the level of PEEP_e used is not excessive.

METHODS

STUDY POPULATION

Subjects admitted to the intensive care unit (ICU) and under controlled mechanical ventilation due to ARF for at least 24 hours were eligible for enrollment in the study: the need for mechanical ventilation could arise from different causes of ARF, as in the study by Brandolese and coll. To evaluate global patient severity at baseline and the underlying etiology of ARF, at enrollment information regarding age, sex, body weight, height, body mass index (BMI), APACHE II score at ICU admission and duration of intubation before enrollment were collected. SOFA score was obtained at the moment of the data collection to assess patient's conditions at experimental time.

As this study focuses on artificially-induced PEEP_i, patients with spontaneously-occurring PEEP_i ≥ 3 cmH₂O (measured at airway opening on ZEEP) were excluded. In the same line of thought, also patients with a history of chronic obstructive pulmonary disease (Global Initiative for Chronic Obstructive Lung, 2020), asthma ("Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention," 2024) or mixed obstructive-restrictive pulmonary disease were excluded, as in these patients PEEP_i is frequently present (Alvisi et al., 2003; Koutsoukou et al., 2004; Lourens et al., 2001), and it may be difficult to maintain a precise level of artificially-induced PEEP_i. Additionally, patients with severe obesity (BMI > 50 kg/m²) were excluded due to technical limitations in the use of EIT.

Additional exclusion criteria included: age < 18 years, pregnancy, recent pulmonary or cardiothoracic surgery, contraindication to the use of EIT (e.g. presence of pacemaker or automatic implantable cardioverter-defibrillator, thoracic wound or drainage at the site of EIT belt), contraindication to placement of esophageal balloon (e.g. esophageal varices or lesions, epistaxis, recent nasopharyngeal surgery, sinusitis, coagulation disturbances) and hemodynamic instability defined as requirement of more than two vasopressors to maintain a mean arterial pressure ≥ 65 mmHg (Polanco and Pinsky, 2006).

The experimental protocol was approved by the local Ethics Authorities and written informed consent was obtained from the next of kin of all the enrolled patients by one of the physicians involved in the study and in charge of patient care.

Ongoing therapies, hemodynamic support and FiO₂ were not modified throughout the experiment.

MEASUREMENTS

Patients were studied during sedation and paralysis, under volume-controlled mechanical ventilation provided by a Servo-U ventilator (Servo-U v4.4, Maquet, Sölna, Sweden) with V_t (6-8 ml/kg of predicted body weight) and RR set by the attending physician.

An esophageal balloon (Nutrivent, Sidam, Mirandola, Italy) was positioned and inflated in the mid-lower third of the esophagus. Correct positioning was checked by measuring the ratio between esophageal and airway pressure swings ($\Delta P_{es}/\Delta P_{aw}$) produced by repetitive chest compression during a post-expiratory pause, using an ad-hoc function in the ventilator providing the mean $\Delta P_{es}/\Delta P_{aw}$ value during the maneuver. A value between 0.8 and 1.2 was considered to indicate correct positioning and inflation (Higgs et al., 1983; Mauri et al., 2016). For technical reasons, P_{es} was not available in one subject. Carbon dioxide percentage at airway opening was measured with a mainstream transducer connected to the ventilator.

Hemodynamic parameters were monitored throughout all the experimental time using a minimally-invasive approach (FloTrac sensor coupled with HemoSphere monitor, Edwards LifeSciences, Irvine, California, USA). Before starting the experiment and at end of each experimental step, stroke volume (SV), stroke volume variation (SVV), cardiac output (CO), systolic, diastolic and mean arterial blood pressure (BP_{syst}, BP_{diast}, MAP, respectively), central venous pressure (CVP) and heart rate (HR) were recorded.

Parameters related to acid-base balance and blood gases, including pH, PaO₂, PaCO₂, HCO₃⁻ and lactate, were measured with a hemogasalyzer (RAPIDPoint 500e, Siemens Healthineers, Erlangen, Germany) on arterial blood sampled via an intra-arterial catheter.

$\text{PaO}_2/\text{FiO}_2$ was calculated together with the alveolar-arterial O_2 gradient (A-aO_2) (Jones et al., 1998).

Monitoring via EIT (PulmoVista 500, Dräger Medical GmbH, Lübeck, Germany) was obtained with a 16-electrode belt placed on the transverse plane at the 4th-5th intercostal space, with the patient in semi-recumbent position, and connected to the EIT monitor (Dräger/GoeMFII EIT Evaluation Kit 2, Dräger Medical GmbH, Lübeck, Germany). One reference electrode was placed on the abdomen. Proper skin-electrode contact and signal receival was checked before recording. The anti-decubitus mattress was switched off throughout all the experimental time to avoid interference on the EIT signal. EIT data were recorded at 50 Hz sampling frequency, stored on the EIT hardware, and subsequently exported on a computer for offline analysis.

Flow, airway pressure (P_{aw}) and carbon dioxide percentage at airway opening, together with esophageal pressure (P_{es}) connected via a dedicated port to the ventilator, were continuously recorded at 100 Hz sampling frequency by the ServoTracker software (Getinge, Gothenburg, Sweden) on a PC connected to the ventilator throughout all the experiment. V_t was obtained by numerical integration of the flow signal.

PROCEDURES

Subjects were studied during four different ventilatory conditions, in which RR, V_t and minute ventilation ($\dot{V}_E$) were constant. PEEP_{tot} was kept constant in three of the four experimental conditions, in which the same PEEP_{tot} was obtained either by applying PEEP_e (PEEP_e condition) or by shortening T_E to produce PEEP_i (**Fig.1**). T_E shortening at constant RR was performed in two different ways, either 1) by increasing the duration of the inflation (T_I) and reducing inspiratory flow (F_{insp}) (PEEP_{inp} condition), or 2) by keeping T_I and F_{insp} constant, and introducing a long post-inspiratory pause (pIp) (PEEP_{iwp}). We aimed at a PEEP_{tot} as similar as possible to the one targeted in (Brandolese et al., 1993) ($\sim 8 \text{ cmH}_2\text{O}$), compatibly with patient's conditions at the moment of the experiment. The fourth condition was obtained by ventilating the subject with the same timings as in PEEP_e condition, but setting PEEP_e to 0 cmH_2O (ZEEP condition). A short pIp ($\sim 0.1 \text{ s}$) was introduced in PEEP_e , ZEEP and PEEP_{inp} for the purpose of respiratory mechanics assessment.

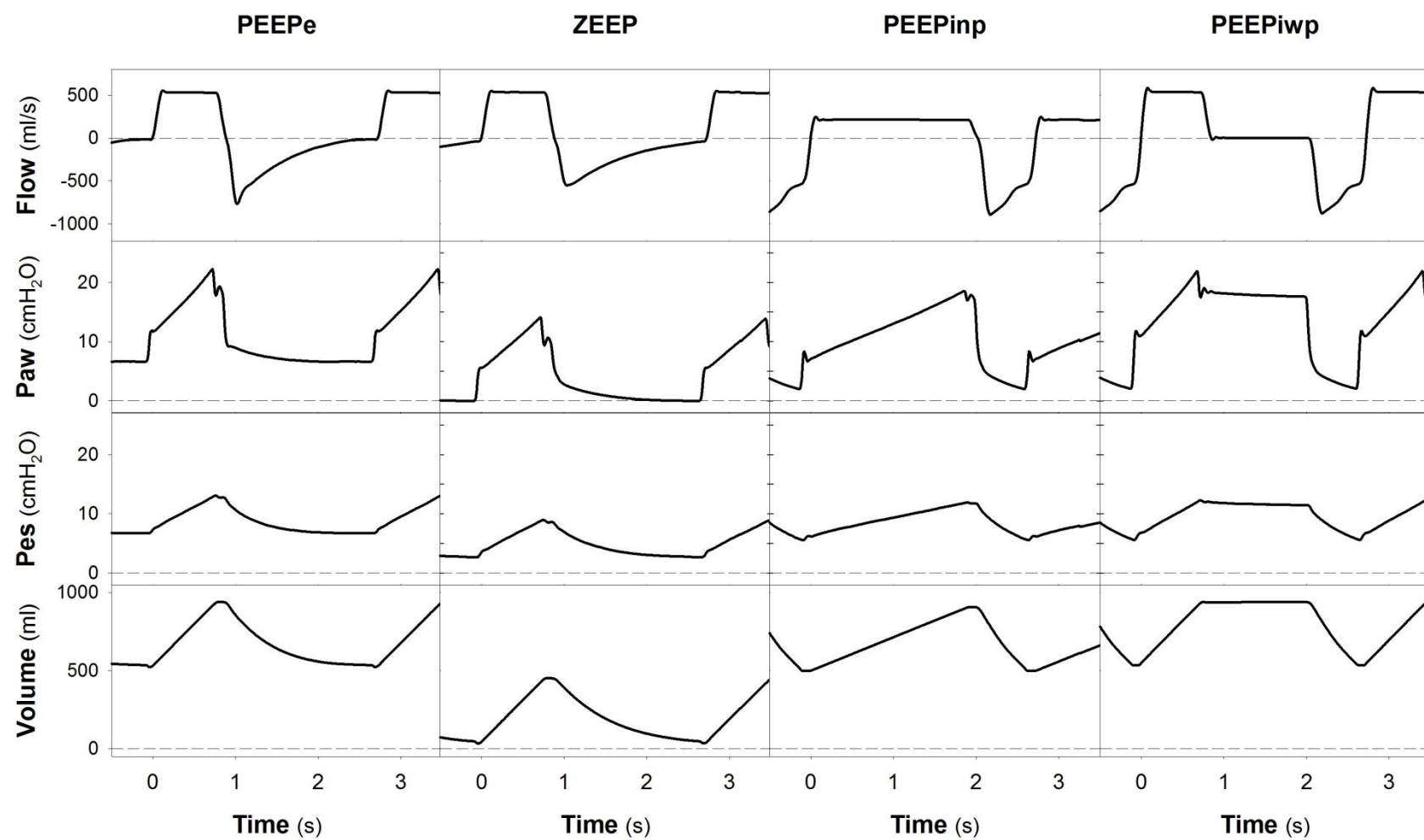


Figure 1. Time course of flow, airway pressure (P_{aw}), esophageal pressure (P_{es}) and volume in a representative patient during the four ventilatory conditions, i.e. PEEPe, ZEEP, PEEPinp, and PEEPiwp. The broken line in the volume diagrams indicates the equilibrium volume of the respiratory system.

The order of experimental conditions was randomized, with each condition lasting ~20 minutes and being preceded by a 10 minutes period of baseline ventilation (as decided by the attending physician), in order to reset volume history before each experimental step and avoid any interference on gas exchange between one session and the following one. The choice of the 10 minutes period was taken based on results of previous studies on the kinetic of PaO₂ changes after PEEPe modifications during mechanical ventilation (Chiumello et al., 2013; Coppola et al., 2013; Tugrul et al., 2005) and on time requirements for patient care.

At the end of each session, a panel of parameters regarding hemodynamics, gas exchange and acid-base balance and respiratory mechanics were measured and recorded, in this specific order.

Respiratory mechanics was measured in each condition during the breathing pattern, and in breaths in which long (>3s) pIp or post expiratory pauses (pEp) were introduced. Thereafter, RR was reduced (6 breath/min) and, in the case of PEEPe, PEEP was set to 0 cmH₂O, in order to measure the volume difference between the actual end-expiratory volume and the relaxation volume of the respiratory system (Δ EELV).

DATA ANALYSIS

Respiratory mechanics

In each experimental condition, respiratory mechanics was assessed as previously described (D'Angelo et al., 1991; Henderson et al., 2017; Laghi and Goyal, 2012; Milic-emili et al., 1990), by combining measurements performed on the breaths where a long pIp or pEp were introduced together with measurements performed on a representative breath, obtained by ensemble averaging all breaths contained in a 2-3 minutes recording during the breathing pattern. This operation considerably decreased the noise due to the cardiac artifact (D'Angelo et al., 1994). All measurements were performed offline using custom-built LabView programs (version 6, National Instruments, Austin, TX, USA).

a) Elastic, resistive and viscoelastic properties

V_t, T_I, pIp and T_E were measured on an average breath of the breathing pattern.

$PEEP_{tot}$ was measured as the P_{aw} at the end of a long pEp, and $PEEP_i$ as the difference between P_{aw} at the end of a pEp ($PEEP_{tot}$) and P_{aw} at the end of a tidal breath ($P_{ee,aw}$) (practically, the difference between $PEEP_{tot}$ and externally applied PEEP) (**Fig.2**).

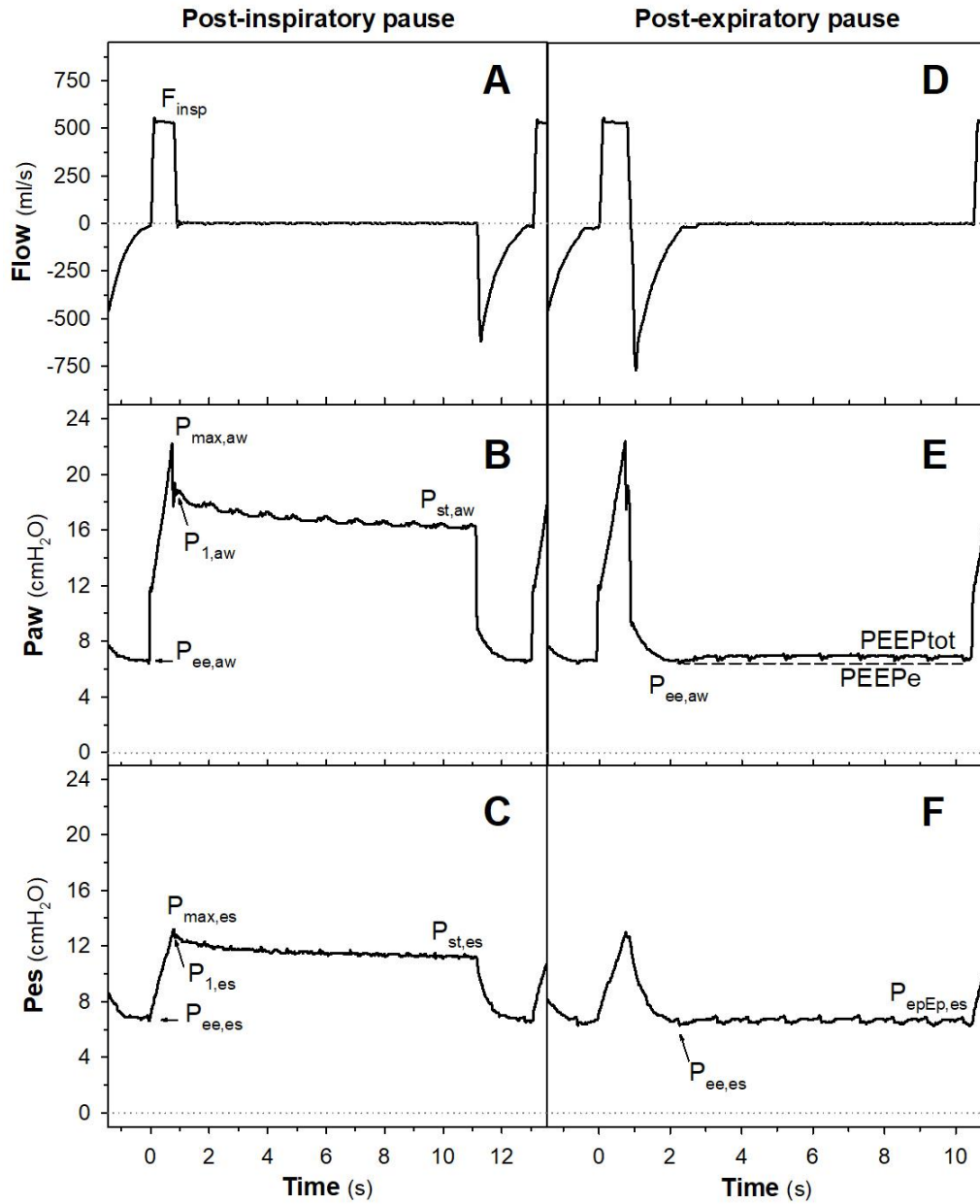


Figure 2. Representative tracings of flow (panels A and D), airway pressure (P_{aw} , panels B and E) and esophageal pressure (P_{es} , panels C and F) in two breaths in which a long pIp (panels A-C) or pEp (panels D-F) were introduced, recorded in $PEEP_{Pe}$ condition. F_{insp} : inspiratory flow; P_{max} : peak pressure immediately before the rapid occlusion; P_1 : pressure immediately after the rapid occlusion; P_{st} : pressure at the end of the pIp; P_{ee} : pressure at end-expiration. $PEEP_{tot}$: total positive end-expiratory pressure measured as P_{aw} at the end of a pEp. $PEEP_i$ is given by the difference between $PEEP_{tot}$ and $PEEP_{Pe}$ (which in the $PEEP_{Pe}$ condition equals $P_{ee,aw}$). $P_{epEp,es}$: P_{es} at the end of the pEp.

Using these values, together with P_{aw} and P_{es} at the end of the long pIp ($P_{st,aw}$ and $P_{st,es}$, respectively), the quasi-static elastic properties of the respiratory system, chest wall and lung were assessed in the form of quasi-static elastance ($E_{st,rs}$, $E_{st,cw}$ and $E_{st,L}$):

$$E_{st,rs} = \frac{P_{st,aw} - PEEP_{tot}}{V_t}$$

$$E_{st,cw} = \frac{P_{st,es} - P_{ep,es}}{V_t}$$

$$E_{st,L} = E_{st,rs} - E_{st,cw}$$

where $P_{ep,es}$ is the P_{es} at the end of the pEp (**Fig.2**).

On the same tracings, measurement of the pressure immediately after the rapid interruption of the inspiratory flow (P_1) allowed calculation of the additional resistance of the respiratory system, chest wall and lung (ΔR_{rs} , ΔR_{cw} , and ΔR_L , respectively, **Fig.2**):

$$\Delta R_{rs} = \frac{P_{1,aw} - P_{st,aw}}{F_{insp}}$$

$$\Delta R_{cw} = \frac{P_{1,es} - P_{st,es}}{F_{insp}}$$

$$\Delta R_L = \Delta R_{rs} - \Delta R_{cw}$$

ΔR_L reflects stress-relaxation of lung tissues, surface phenomena and pendelluft, if present.

Finally, the interrupter resistance of the respiratory system, chest wall and lung ($R_{int,rs}$, $R_{int,cw}$, and $R_{int,L}$, respectively) were measured using peak inspiratory pressure (P_{max}) and P_1 read on the average breath from the breathing pattern:

$$R_{int,rs} = \frac{P_{max,aw} - P_{1,aw}}{F_{insp}}$$

$$R_{int,cw} = \frac{P_{max,es} - P_{1,es}}{F_{insp}}$$

$$R_{int,L} = R_{int,rs} - R_{int,cw}$$

The difference between P_{aw} and P_{es} values at end-expiration during tidal breathing ($P_{ee,aw}$ and $P_{ee,es}$, respectively) and $P_{st,aw}$ and $P_{st,es}$, were used to compute the transpulmonary pressure at end-expiration ($P_{ee,L}$) and at end-pIp ($P_{st,L}$), respectively.

b) Mean airway and alveolar pressure ($\overline{P_{aw}}$ and $\overline{P_{alv}}$)

Mean airway pressure ($\overline{P_{aw}}$) was measured as the average P_{aw} during the representative breath from the breathing pattern in each condition.

The procedure to assess mean alveolar pressure ($\overline{P_{alv}}$) is more elaborate (**Fig.3**). First, flow (**Fig.3, panel A**) was integrated during a breathing cycle in order to obtain volume variations (**Fig.3, panel B**). Second, P_{alv} variations were obtained as the product of volume variations times dynamic elastance, on the assumption that the latter parameter is constant in the V_t range. In the absence of PEEPi, it is easy to transform P_{alv} variations into P_{alv} , since at end-expiration flow is zero and P_{alv} should be equal to P_{aw} . It is not so in dynamic conditions as PEEP_{inp} and PEEP_{iwp}, when P_{aw} and P_{alv} at end-expiration differ by an amount equal to the resistive pressure drop across the tracheobronchial tree and the endotracheal tube. For this reason, in all the experimental conditions, P_{alv} has been equated to P_{aw} immediately after the end of the inflation, that is P_1 (**Fig.3, panel C**). $\overline{P_{alv}}$ was then calculated as the average of the instantaneous P_{alv} values during one respiratory cycle.

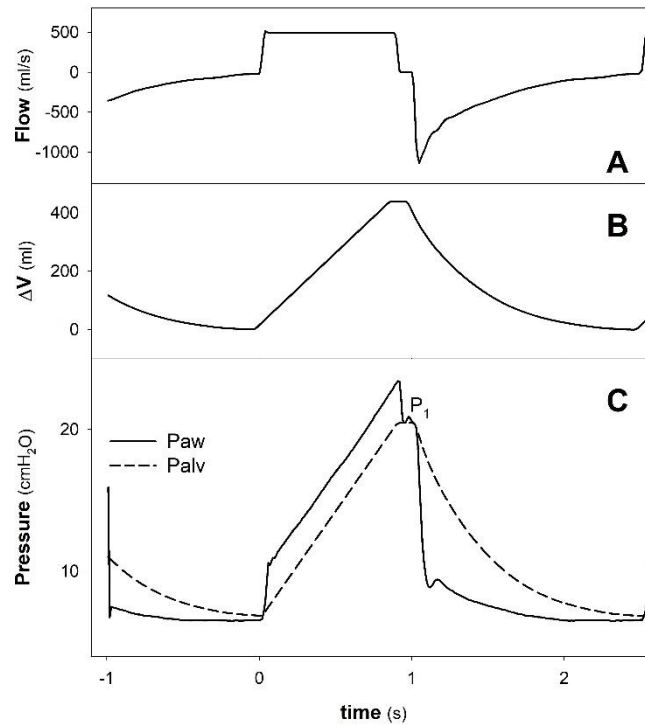


Figure 3. Volume variations (ΔV , panel B), obtained as the integral of flow (panel A) are multiplied by dynamic elastance of the respiratory system, to yield P_{alv} variations. Dynamic elastance is calculated as $(P_{1,aw} - PEEP_{tot})/V_t$. P_{alv} variations are converted into P_{alv} by equaling P_{alv} variations to P_{aw} at P_1 . In panel C, the time course of P_{alv} (broken line) is shown together with that of P_{aw} .

c) Stress index (SI)

Stress index (SI) was measured in all experimental conditions on the average breath as previously described (Grasso et al., 2004; Ranieri et al., 2000). Briefly, the part of the P_{aw} time course where F_{insp} was constant was isolated, and interpolated with the power function

$$P_{aw} = at^{SI} + k$$

where t is time, and a and k constants.

The P_{aw} time course is convex to the time axis if $SI < 1$, a straight line if $SI = 1$, and concave to the time axis if $SI > 1$. In experimental models, SI decreases below 1 in the presence of intratidal recruitment, and increases above 1 when the system approaches the flat part of the quasi-static pressure-volume curve, indicating tidal overdistention. The rationale behind the use of this parameter is that during constant-flow inflation, assuming that airway resistance is constant and viscoelasticity is negligible, the P_{aw} time course mirrors the quasi-static volume-pressure relation, which is almost rectilinear in the mid-volume range, but becomes markedly curvilinear when the pulmonary parenchyma becomes overdistended, or when recruitment during inflation is prominent.

d) Airway occlusion pressure (P_{ao})

Airway occlusion pressure (P_{ao}) is the pressure which should be exceeded in order for the inflation to start. The slow inflation method (Chen et al., 2018), which is the gold standard for this measurement, could not be implemented in our settings, as, with our ventilator it was not possible to produce a slow inflation immediately after a PEEPi breath without intermediate steps, which would have caused a substantial change of PEEPi. Therefore, P_{ao} was assessed with an alternative method not requiring slow inflations (Haudebourg et al., 2023).

The procedure is illustrated in **Fig.4**, which shows the time course of P_{aw} during a breath in the PEEPe condition, in a subject with a P_{ao} and in a subject without it.

For this method to be applied, a short pIp should be produced at the end of a constant-flow inflation, so to measure the rapid end-inspiratory pressure drop (P_{res}) at end-

inspiration (Henderson et al., 2017), from $P_{\max,aw}$ to $P_{1,aw}$. P_{res} has been corrected for the effect of the finite closure time of the inspiratory valve (Zilianti et al., 2024).

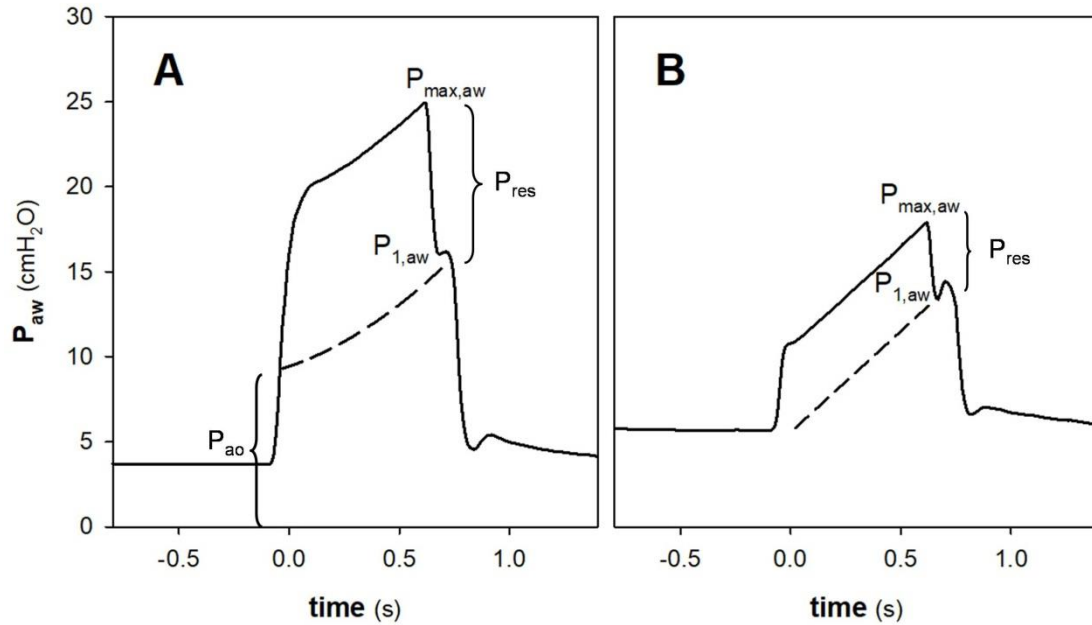


Figure 4. Time course of P_{aw} in a subject with an airway occlusion pressure (P_{ao} , panel A) and a subject without it (panel B), during PEEPe condition. The broken line indicates non-resistive pressure.

The corrected P_{res} , together with flow, allows calculation of respiratory system resistance ($R_{int,rs}$). Assuming that $R_{int,rs}$ is constant during the inflation, P_{res} is subtracted from total P_{aw} , to obtain non-resistive pressure (broken line in **Fig.4**). In the absence of airway closure, P_{res} accounts for most of the initial rapid increase in P_{aw} at the beginning of the inflation, where non-resistive pressure is very close to $PEEP_{tot}$ (**Fig.4, panel B**). To the contrary, if airways are collapsed, gas flows into the respiratory system only after pressure rises to the point at which airways open, and P_{res} is smaller than the initial rapid increase in P_{aw} at the beginning of the inflation. In this case (**Fig.4, panel A**), non-resistive pressure at the beginning of the inspiration is greater than $PEEP_{tot}$, and corresponds to P_{ao} .

Capnography

In each condition, the average breath was analyzed with a custom-built LabView program to assess slope of phase III, anatomical dead space (DS_{anat}), together with the mean expiratory CO_2 in %. DS_{anat} was measured by means of the Fowler's method (Fowler, 1948).

Mean expiratory CO₂ was used to calculate the partial pressure of CO₂ in the mixed expiratory air (PeCO₂), which was then incorporated in the calculation of the physiological dead space (DS_{phys}) using the Enghoff's modification of Bohr's equation (Enghoff, 1938):

$$DS_{phys} = \left(\frac{PaCO_2 - PeCO_2}{PaCO_2} \right) \times V_t$$

The alveolar dead space (DS_{alv}) was calculated as the difference between physiological and anatomical dead space. Finally, the mean distribution time (MDT), an index quantifying the time available for gas diffusion, was computed for each condition, according to (Uttman and Jonson, 2003).

Electrical impedance tomography (EIT)

a) Data extraction

Using Dräger EIT Data Analysis Tool (version 6.3, Drägerwerk, Lübeck, Germany), impedance tracings were exported as binary files, and converted by a custom-made LabView program into a 3D array, made of a sequence of EIT images (32x32) collected 20 ms one from another.

In order to decrease the impedance oscillations due to the cardiac activity, several breaths were ensemble-averaged to obtain a breath representative of each condition in a given subject. All subsequent analysis was performed on these representative breaths.

b) Lung area

Lung area is defined as the group of pixels in a subject which can be ventilated (regardless of whether they are ventilated or not in a particular condition), and calculated with the procedure described by EIT Diag-User Manual (Rev. 1.0 June 16th 2015). Briefly, for all pixels and for all experimental conditions, tidal impedance change between end-inspiration and end-expiration ($\Delta Z_{ei-ee,ij}$) was measured, and the maximal value determined. If $\Delta Z_{ei-ee,ij}$ of a pixel in any condition is greater than 15% of the maximal $\Delta Z_{ei-ee,ij}$ (even if temporarily), the pixel is assigned to the lung area, otherwise the pixel is excluded. All the parameters that will be described in the subsequent sections were calculated using only pixels belonging to the lung area.

From a practical standpoint, it is useful to define a mask operation m for each pixel of row i and column j :

$$m_{ij} = \begin{cases} 1 & \text{if the pixel is ventilated} \\ 0 & \text{if the pixel is not ventilated} \end{cases}$$

c) Regional subanalysis

Analysis has been performed on the pixels corresponding to the global area of the lung, or on the pixels corresponding to the ventral and dorsal parts of the lung area. A pixel is arbitrarily defined as ventral if it belongs to the upper half of an EIT image, and dorsal if it belongs to the lower half of an EIT image.

From a practical standpoint, it is useful to define a ventral part operation v for each pixel of row i and column j :

$$v_{ij} = \begin{cases} 1 & \text{if the pixel is ventral} \\ 0 & \text{if the pixel is not ventral} \end{cases}$$

and a dorsal part operation d for each pixel of row i and column j :

$$d_{ij} = \begin{cases} 1 & \text{if the pixel is dorsal} \\ 0 & \text{if the pixel is not dorsal} \end{cases}$$

d) Ventilated surface (SURF)

Ventilated surface (SURF) is defined as the group of pixels in a subject which are ventilated in a given condition. SURF is calculated according to EIT Diag-User Manual as the number of pixels whose $\Delta Z_{ei-ee,ij}$ is greater than the 15% of the maximal $\Delta Z_{ei-ee,ij}$ recorded in a given condition.

Ventral and dorsal SURF ($SURF_v$ and $SURF_d$) are given by the number of ventilated pixels in the ventral part and in the dorsal part, respectively.

e) Tidal impedance difference (TID)

Tidal impedance difference (TID) is the total impedance variation from end-expiration to end-inspiration in the lung area. In a given subject, TID is proportional to V_t , as impedance changes closely reflect volume changes assessed with a variety of techniques, including computed tomography (CT) (Meier et al., 2008; Wrigge et al., 2008), radionuclide

scanning (Kunst et al., 1998), electron beam CT (Frerichs et al., 2002) and single-photon emission CT (SPECT) (Hinz et al., 2003). A good correlation was also found between impedance changes and volume changes assessed with strain gauge plethysmography (Marquis et al., 2006) or the supersyringe method (Adler et al., 1997; Nopp et al., 1997). The proportionality between impedance measured volume changes and true lung volume changes fades if the belt is placed at a level at which the diaphragm can enter, even if only temporarily, the measurement field of the EIT belt (6th-7th rib), causing a reduction of the ventilated tissue (Bikker et al., 2011). In line of principle, the proportionality between impedance changes and lung volume changes decreases when the respiratory system does not behave as a mechanical system with a single degree of freedom: in this case the inhaled air may distribute differently in the different parts of the system (such as more cranially or caudally with respect to the EIT field), depending on a variety of conditions.

TID has been calculated as described in EIT Diag-User Manual as the sum of $\Delta Z_{ei-ee,ij}$ in the lung area. In symbols:

$$TID = \sum_{i=0}^{31} \sum_{j=0}^{31} m_{ij} \Delta Z_{ei-ee,ij}$$

Ventral and dorsal TID (TID_v and TID_d , respectively) are given by

$$TID_v = \sum_{i=0}^{31} \sum_{j=0}^{31} m_{ij} v_{ij} \Delta Z_{ei-ee,ij}$$

$$TID_d = \sum_{i=0}^{31} \sum_{j=0}^{31} m_{ij} d_{ij} \Delta Z_{ei-ee,ij}$$

f) Global inhomogeneity index (GI)

Global inhomogeneity index (GI) is an index of spatial ventilation heterogeneity, and it has been calculated as previously reported (Zhao et al., 2014, 2009a, 2009b) as:

$$GI = \frac{\sum_{i=0}^{31} \sum_{j=0}^{31} m_{ij} |\Delta Z_{ei-ee,ij} - M(\Delta Z_{ei-ee,ij})|}{TID}$$

where $M(\Delta Z_{ei-ee,ij})$ is the median ΔZ of the lung area.

As the average pixel tidal impedance change ($\overline{\Delta Z_{ei-ee}}$) is

$$\overline{\Delta Z_{ei-ee}} = \frac{TID}{\sum_{i=0}^{31} \sum_{j=0}^{31} m_{ij}}$$

GI can be rewritten as

$$GI = \frac{\sum_{i=0}^{31} \sum_{j=0}^{31} m_{ij} |\Delta Z_{ei-ee,ij} - M(\Delta Z_{ei-ee,ij})|}{\overline{\Delta Z_{ei-ee}}}$$

showing that GI is the average deviation of $\Delta Z_{ei-ee,ij}$ from the median value, normalized to $\overline{\Delta Z_{ei-ee}}$.

Dräger EIT Data Analysis Tool has implemented a regional version of GI, ventral and dorsal (GI_v and GI_d , respectively), so defined:

$$GI_v = \frac{\sum_{i=0}^{31} \sum_{j=0}^{31} m_{ij} v_{ij} |\Delta Z_{ei-ee,ij} - M(\Delta Z_{ei-ee,ij})|}{TID}$$

$$GI_d = \frac{\sum_{i=0}^{31} \sum_{j=0}^{31} m_{ij} d_{ij} |\Delta Z_{ei-ee,ij} - M(\Delta Z_{ei-ee,ij})|}{TID}$$

Thus,

$$GI = GI_v + GI_d$$

g) Regional ventilation delay (RVD SD)

Regional ventilation delay (RVD_{ij}) is a parameter expressing the temporal relation between the time course of the impedance of one pixel relative to the time course of global impedance. Its dispersion in the lung area (RVD SD) is an index of temporal ventilation heterogeneity. RVD SD, calculated on slow inflations, is often used to detect the presence of recruitment/derecruitment (Muders et al., 2012; Wrigge et al., 2008; Yang et al., 2021). If it is measured during a normal breath, i.e. in dynamic conditions as in the present ones, this parameter also reflects the presence of units with different time-constants (Otis et al., 1956). A positive RVD_{ij} corresponds to units with long time-constants (pixel impedance lags behind global impedance), a negative one to units with short time-constant (pixel impedance anticipates global impedance). RVD_{ij} has been measured as described in EIT Diag-User Manual and previous works (Muders et al., 2012; Wrigge et al., 2008; Yang et al., 2021). The inspiratory time course of impedance has been obtained for each pixel and normalized

relative to its amplitude, so that impedance value Z_{ij} was 0 at the beginning of the inspiration and 1 at the end ($\hat{Z}_{ij}$). The same normalization was made for the inspiratory time course of global impedance ($\hat{Z}_{tot}$). For each pixel, regional ventilation delay (RVD_{ij}) was defined as the time interval between the instant at which normalized $Z_{ij}=0.4$ and the instant at which normalized $Z_{tot}=0.4$, divided by T_I . In symbols:

$$RVD_{ij} = \frac{t_{\hat{Z}_{ij}=0.4} - t_{\hat{Z}_{tot}=0.4}}{T_I} \times 100$$

The standard deviation of RVD_{ij} in the lung area (RVD SD) was calculated as

$$RVD\ SD = \sqrt{\frac{\sum_{i=0}^{31} \sum_{j=0}^{31} m_{ij} \left(RVD_{ij} - \frac{\sum_{i=0}^{31} \sum_{j=0}^{31} m_{ij} RVD_{ij}}{\sum_{i=0}^{31} \sum_{j=0}^{31} m_{ij}} \right)^2}{\left(\sum_{i=0}^{31} \sum_{j=0}^{31} m_{ij} \right) - 1}}$$

Ventral and dorsal RVD SD (RVD SD_v and RVD SD_d, respectively) were calculated as

$$RVD\ SD_v = \sqrt{\frac{\sum_{i=0}^{31} \sum_{j=0}^{31} m_{ij} v_{ij} \left(RVD_{ij} - \frac{\sum_{i=0}^{31} \sum_{j=0}^{31} m_{ij} v_{ij} RVD_{ij}}{\sum_{i=0}^{31} \sum_{j=0}^{31} m_{ij} v_{ij}} \right)^2}{\left(\sum_{i=0}^{31} \sum_{j=0}^{31} m_{ij} v_{ij} \right) - 1}}$$

$$RVD\ SD_d = \sqrt{\frac{\sum_{i=0}^{31} \sum_{j=0}^{31} m_{ij} d_{ij} \left(RVD_{ij} - \frac{\sum_{i=0}^{31} \sum_{j=0}^{31} m_{ij} d_{ij} RVD_{ij}}{\sum_{i=0}^{31} \sum_{j=0}^{31} m_{ij} d_{ij}} \right)^2}{\left(\sum_{i=0}^{31} \sum_{j=0}^{31} m_{ij} d_{ij} \right) - 1}}$$

STATISTICS

Statistical analysis was performed using SPSS v.29.0.1.0 (SPSS Inc., Chicago, IL, USA). Normality of data distribution was assessed with the Shapiro-Wilk test. Data are reported as mean±SD. Comparisons between experimental conditions were tested using repeated measurements ANOVA. When appropriate, post-hoc pairwise comparisons were carried out with Bonferroni's correction. The overall within-individual relationship among paired measures assessed on two or more occasions for multiple individuals was

investigated with repeated measurements correlation (rmcorr) (Bakdash and Marusich, 2017; Bland and Altman, 1995).

Sample size was calculated using the G*Power free software (version 3.1.9.6) (Faul et al., 2007). Alpha error and power were set at 5% and 90%, respectively. The values of PaO₂ at PEEPe and PEEPi reported by (Brandolese et al., 1993) were used for estimation of the mean difference and standard error (-7.5 and 11.8 mmHg, respectively). Based on these parameters, a sample size of 28 subjects was obtained. To allow for possible subject exclusion after collection due to data incompleteness, we aimed at a final sample size of 32 subjects. The present work represents a preliminary analysis of data from the 17 subjects recruited by the end of the PhD program (September 30th 2024).

RESULTS

PATIENTS POPULATION

Anthropometric and clinical characteristics of the 17 patients investigated are reported in **Table 1**. The reason for intubation and mechanical ventilation was predominantly pulmonary in 7 patients (bilateral pneumonia in 5, ischemic stroke with massive pulmonary embolism in 1 and 1 localized pneumonia complicated by septic shock) and predominantly non-pulmonary in 10 patients (cardiogenic pulmonary edema in 6, cardiac arrest or myocardial ischemia in 3 and myasthenic syndrome complication in 1). Three patients met criteria for ARDS (Matthay et al., 2024). Eleven subjects died during the stay in the ICU, while 6 were discharged alive.

BREATHING PATTERN

The relevant parameters regarding breathing pattern during the four experimental conditions are reported in **Table 2**. RR was the same for all conditions, while V_t was trivially less (~ 5 ml) during PEEPiwp than in the other conditions. T_I was similar at PEEPe, ZEEP and PEEPiwp, and longer at PEEPinp. p_{Ip} was greater at PEEPiwp than in the other conditions. T_E was the same in the PEEPi conditions, and much longer in PEEPe and ZEEP. F_{insp} was lower at PEEPinp and similar between PEEPe, ZEEP and PEEPiwp, while mean expiratory flow (F_{exp}) was greater in PEEPinp and PEEPiwp than in PEEPe and ZEEP. As can be observed from the I:E ratio, PEEPinp and PEEPiwp were obtained by producing IRV. FiO_2 was kept unchanged throughout all the experiment.

Table 1: Anthropometric data and clinical severity of disease at admission and experimental time.

Subject	Age (yrs)	Sex (M/F)	BMI (kg/m ²)	Intubation time (days)	APACHE II	SOFA	cause of ARF	ARDS (yes/no)	Outcome (D/S)
1	74	F	25.4	2.7	21	7	Aspergillus pneumonia in immunodepressed subject (long-standing corticosteroid therapy for eosinophilic pneumonia)	yes	D
2	67	M	29.4	20.7	20	5	Right pneumonia complicated with pleural effusion and empyema treated surgically, followed by cardiogenic pulmonary edema during weaning trial	no	D
3	79	F	35.2	3.9	22	7	Cardiogenic pulmonary edema due to acute mitral regurgitation	no	D
4	83	F	27.3	5.0	23	7	COVID-19 pneumonia	yes	D
5	55	F	34.9	15.4	24	6	Cardiac arrest and coma due to acute myocardial ischemia	no	S
6	46	F	26.2	2.1	19	4	Coma from drug intoxication with COVID-19 infection	no	S
7	84	M	25.6	2.8	28	8	Influenza pneumonia and acute myocardial ischemia	no	S
8	37	F	21.3	2.8	19	7	Influenza pneumonia complicated with alveolar hemorrhage	yes	S
9	56	M	35.9	1.5	29	10	Cardiogenic pulmonary edema due to acute myocardial ischemia	no	D
10	77	M	28.6	1.7	34	8	Cardiogenic pulmonary edema due to acute-on-chronic aortic regurgitation	no	D

Table 1-continued

Subject	Age (yrs)	Sex (M/F)	BMI (kg/m ²)	Intubation time (days)	APACHE II	SOFA	cause of ARF	ARDS (yes/no)	Outcome (D/S)
11	66	M	31.1	3.3	30	10	Cardiac arrest and ab-ingestis pneumonia	no	D
12	73	M	35.2	4.3	22	8	Cardiogenic pulmonary edema due to acute-on-chronic heart failure	no	D
13	60	M	24.9	17.3	29	9	Ischemic stroke and massive pulmonary embolism	no	D
14	67	F	18.4	1.2	31	7	Localized pneumonia complicated with septic shock	no	D
15	75	F	34.0	2.5	21	7	Cardiogenic pulmonary edema due to acute heart failure in patient with obesity-related hypoventilation syndrome	no	S
16	71	M	23.1	2.2	31	7	Cardiac arrest	no	D
17	70	M	24.2	8.2	31	7	Respiratory arrest in patient with myasthenic syndrome	no	S
mean±SD	67±13	9M:8F	28.3±5.4	5.7±6.0	26±5	7±2		3Y:13N	11D:6S

Table 1. BMI, body mass index. Intubation time refers to the duration of intubation before the experiment. APACHE II, Acute Physiology And Chronic Health Evaluation score II; SOFA, Sequential Organ Failure Assessment score. APACHE II score was assessed at ICU admission, while SOFA score at the moment of the experiment. ARF; acute respiratory failure. Presence of ARDS was assessed according to the ARDS Network criteria (Matthay et al., 2024). Outcome refers to ICU discharge (D, dead; S, survived).

Table 2: Ventilatory parameters at PEEPe, ZEEP, PEEP_{Pin} and PEEPi_w

	PEEPe	ZEEP	PEEP _{Pin}	PEEPi _w	p-value
V_t (ml)	421±62	421±62	422±62	417±61	*§‡ <0.001
(ml/kg PBW)	6.8±0.5	6.8±0.5	6.8±0.5	6.7±0.5	*§‡ <0.001
T_I (s)	0.78±0.15	0.77±0.15	1.88±0.33	*§ 0.76±0.15	‡ <0.001
(%T _{tot})	29±4	28±4	69±7	*§ 28±4	‡ <0.001
pIp (s)	0.11±0.03	0.12±0.03	0.11±0.03	1.22±0.22	*§‡ <0.001
(%T _{tot})	4±1	4±1	4±1	45±5	*§‡ <0.001
T_E (s)	1.83±0.35	1.83±0.35	0.73±0.27	*§ 0.75±0.27	*§ <0.001
I:E	0.50±0.10	0.50±0.09	2.94±0.79	*§ 2.82±0.75	*§ <0.001
RR (breath/min)	23±4	23±4	23±4	23±4	0.332
F_{insp} (ml/s)	580±161	586±160	238±62	*§ 596±154	‡ <0.001
F_{exp} (ml/s)	236±48	236±47	614±132	*§ 587±124	*§ <0.001
Ṁ_E (L/min)	9.7±1.9	9.7±1.9	9.7±1.9	9.5±1.9	*§‡ <0.001
FiO₂	0.45±0.13	0.45±0.13	0.45±0.13	0.45±0.13	1.000

Table 2. V_t, tidal volume; PBW predicted body weight; T_I, duration of the inflation; pIp, duration of the post-inspiratory pause; T_E, expiratory time; I:E, total inspiratory to expiratory time ratio; RR, respiratory rate; F_{insp}, inspiratory flow; F_{exp}, mean expiratory flow; Ṁ_E, minute ventilation; FiO₂, fraction of inspired oxygen. p-value: probability of a difference between the four experimental conditions (repeated measurements ANOVA). Results of post-hoc analysis are indicated as: *, significantly different (p<0.05) from PEEPe; §, significantly different from ZEEP; ‡, significantly different from PEEP_{Pin} (indicated for PEEPi_w only).

RESPIRATORY MECHANICS

PEEP and ΔEELV

PEEP_{tot} and ΔEELV were similar among PEEPe, PEEP_{Pin} and PEEPi_w (for PEEP_{tot} 6.9±1.3, 6.6±1.4, 6.7±1.4 cmH₂O, respectively, p>0.465; for ΔEELV 417±193, 426±198, 452±211 ml, respectively, p>0.270) (**Fig.5, panel A and B**). PEEPi was not different between PEEP_{Pin} and PEEPi_w (5.1±0.9 and 5.3±0.9 cmH₂O, p=1.000).

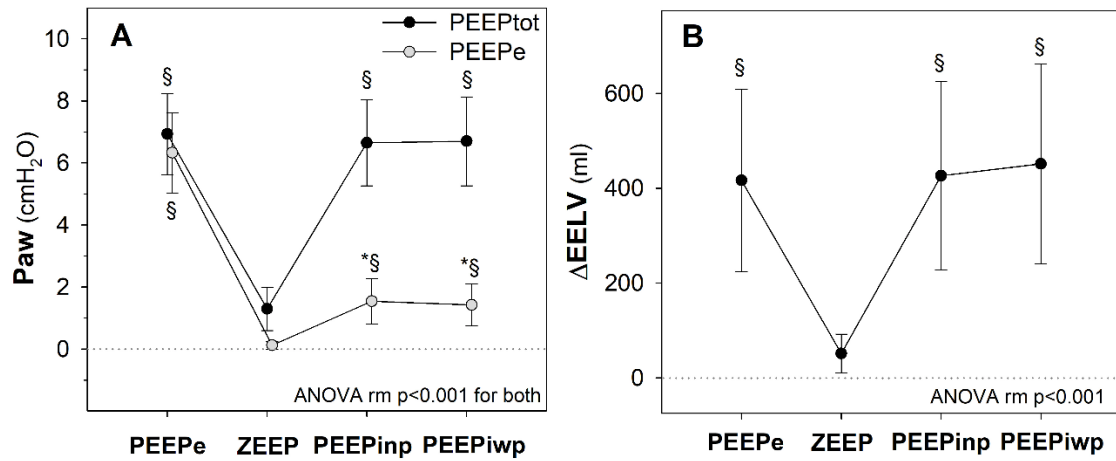


Figure 5. Total and external PEEP (PEEP_{tot} and PEEPe, panel A) and variations in end-expiratory lung volume (ΔEELV, panel B) in the four experimental conditions. The vertical distance between PEEP_{tot} (black) and PEEPe (grey) represents PEEPi. Error bars correspond to one SD. p-value: probability of a difference between the four experimental conditions (ANOVA rm, repeated measurements). Results of post-hoc analysis are indicated as: *, significantly different (p<0.05) from PEEPe; §, significantly different from ZEEP.

Elastic properties

Respiratory system, chest wall and lung recoil at the end of a long pIp (P_{st,aw}, P_{st,es} and P_{st,L}, respectively) and at the end of a long pEp (PEEP_{tot}, P_{epEp,es} and PEEP_{tot}-P_{epEp,es}, respectively) have been represented in **Fig.6** as a function of the relative volume above equilibrium volume. P_{st,aw}, P_{st,es} and P_{st,L} were lower at ZEEP (10.9±3.7, 7.1±3.2 and 3.3±3.1 cmH₂O, respectively) compared to the other three conditions (p<0.001 for all comparisons), in which values were similar (16.7±4.4, 9.3±3.3, 7.0±4.3 cmH₂O at PEEPe; 17.0±3.6, 9.4±3.4, 7.3±4.0 cmH₂O at PEEP_{inp}; 16.8±3.3, 9.3±3.1, 7.2±3.1 cmH₂O at PEEPiwp, p=1.000 for all comparisons).

The inverse of the slopes of the lines joining the end-expiratory and end-inspiratory points in **Fig.6** corresponds to E_{st}. E_{st,cw} did not change among the conditions (8.2±2.9, 8.0±3.5, 8.2±3.6, 8.4±3.3 cmH₂O/L, at PEEPe, ZEEP, PEEP_{inp} and PEEPiwp, respectively, p=0.712), while E_{st,L} tended, although not significantly, to be lower at ZEEP (14.2±7.0 cmH₂O/L) compared to PEEPe, PEEP_{inp} and PEEPiwp (15.7±7.8, 15.6±7.6, 15.4±6.6 cmH₂O/L, respectively). As a result, also E_{st,rs} tended to be lower at ZEEP (23.6±10.0 cmH₂O/L) compared to the other conditions (25.1±9.4, 24.9±9.4, 25.0±9.5 cmH₂O/L at PEEPe, PEEP_{inp} and PEEPiwp, respectively).

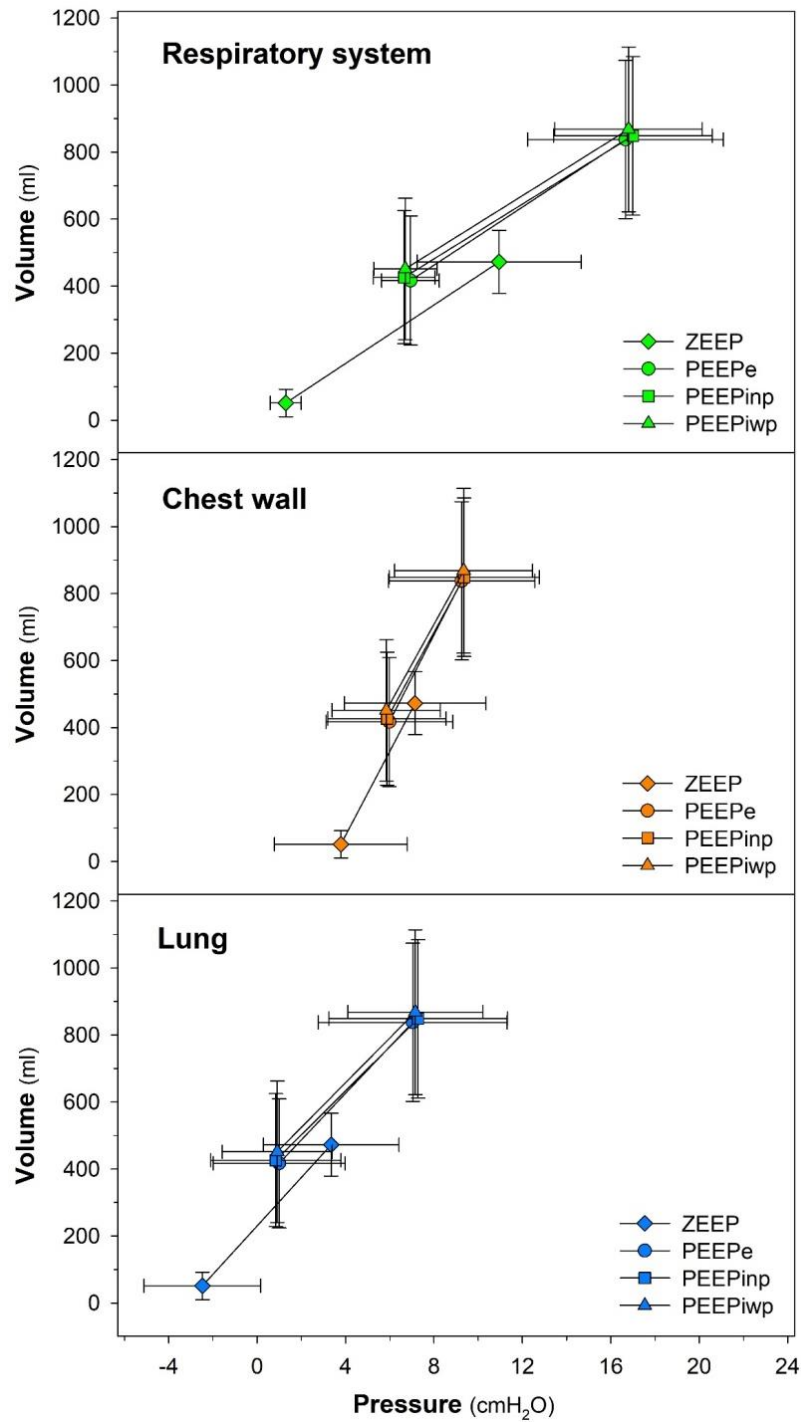


Figure 6. Values of volume and pressure at the end of a pEp and at the end of a pIp in the four experimental conditions (*diamond*, ZEEP; *circle*, PEEPe, *square*, PEEPinp, *triangle*, PEEPiwp), for the respiratory system (top panel in green), the chest wall (middle panel in orange) and the lung (bottom panel in blue). In the volume scale, the zero value refers to the equilibrium volume of the respiratory system. The volume change between end-expiration and end-inspiration is V_t . Vertical and horizontal error bars correspond to one SD.

Resistive and viscoelastic properties

$R_{int,rs}$ was measured in all recruited patients ($n=17$); $R_{int,cw}$ and $R_{int,L}$ instead were computed from 12 subjects only, as in one patient P_{es} was not available and in other 4, noise from cardiac artifact on the P_{es} tracing prevented clear identification of P_1 . $R_{int,rs}$ and $R_{int,L}$ were lower at PEEP_{inp} (6.93 ± 3.31 and 6.38 ± 2.63 cmH₂O/L, respectively) compared to the other three conditions (for $R_{int,rs}$ and $R_{int,L}$, respectively, 8.89 ± 2.46 and 8.41 ± 2.21 cmH₂O/L at PEEPe; 9.48 ± 2.60 and 8.94 ± 2.29 cmH₂O/L at ZEEP; 9.11 ± 2.41 and 9.39 ± 2.44 cmH₂O/L at PEEPiwp, $p < 0.050$ for both). With respect to PEEPe and ZEEP, which had similar values (0.42 ± 0.21 and 0.50 ± 0.29 cmH₂O/L, respectively, $p = 0.904$), $R_{int,cw}$ tended to increase at PEEP_{inp} (0.63 ± 0.30 cmH₂O/L, $p < 0.089$), and decreased at PEEPiwp (0.34 ± 0.17 cmH₂O/L, $p < 0.047$) (**Fig.7 panel A**).

Regarding viscoelastic properties, ΔR_{rs} , ΔR_{cw} and ΔR_L increased at PEEP_{inp} (7.65 ± 3.06 , 2.38 ± 1.73 , 4.85 ± 2.04 cmH₂O/L, respectively) compared to the other three conditions (for ΔR_{rs} , ΔR_{cw} and ΔR_L , respectively, 5.08 ± 2.28 , 1.20 ± 0.81 , 3.63 ± 1.84 cmH₂O/L at PEEPe; 4.84 ± 2.56 , 1.00 ± 0.64 , 3.58 ± 2.22 cmH₂O/L at ZEEP; 3.95 ± 1.76 , 1.24 ± 0.67 , 2.58 ± 1.83 cmH₂O/L at PEEPiwp, $p < 0.032$ for all comparisons) (**Fig.7 panel B**); moreover, ΔR_{rs} and ΔR_L were lower at PEEPiwp compared to PEEPe ($p < 0.011$).

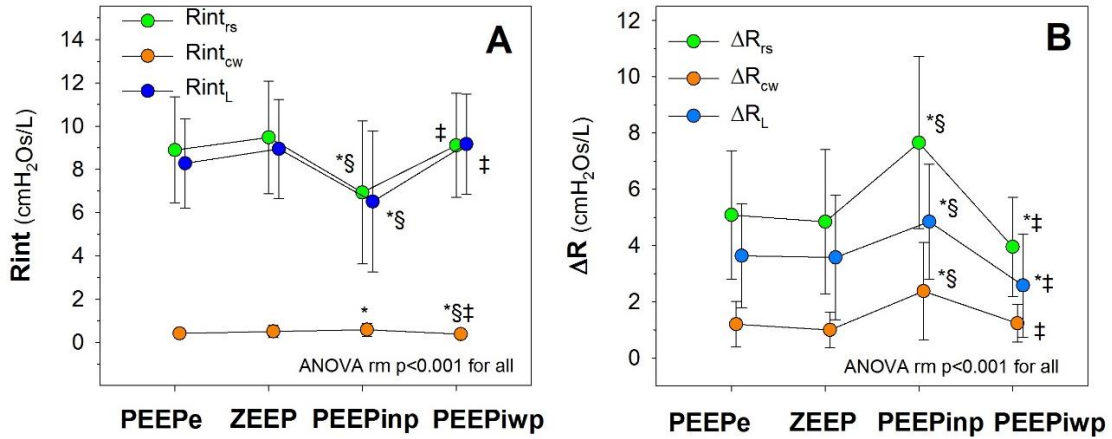


Figure 7. Interrupter resistance (R_{int} , panel A) and additional resistance (ΔR , panel B) in the four experimental conditions for the respiratory system ($R_{int,rs}$ and ΔR_{rs} , green), chest wall ($R_{int,cw}$ and ΔR_{cw} , orange) and lung ($R_{int,L}$ and ΔR_L , blue). Error bars correspond to one SD. p-value: probability of a difference between the four experimental conditions (ANOVA rm, repeated measurements). Results of post-hoc analysis are indicated as: *, significantly different ($p < 0.05$) from PEEPe; §, significantly different from ZEEP; ‡, significantly different from PEEP_{inp} (indicated for PEEPiwp only).

Mean airway and alveolar pressure ($\overline{P_{aw}}$ and $\overline{P_{alv}}$)

$\overline{P_{aw}}$ was 11.0 ± 1.8 , 5.0 ± 1.2 , 11.2 ± 1.9 and 13.7 ± 2.4 cmH₂O during PEEPe, ZEEP, PEEPinp, and PEEPiwp, respectively ($p < 0.001$). It was lower at ZEEP ($p < 0.001$) than in all other conditions, not different between PEEPe and PEEPinp ($p = 1.000$), and greater at PEEPiwp than at PEEPe and PEEPinp ($p < 0.001$). $\overline{P_{alv}}$ was 11.3 ± 2.1 , 5.3 ± 1.3 , 12.7 ± 2.4 and 14.9 ± 2.8 cmH₂O during PEEPe, ZEEP, PEEPinp, and PEEPiwp, respectively ($p < 0.001$) (**Fig.8**). It was lower at ZEEP ($p < 0.001$) than in all other conditions, greater at both PEEPiwp and PEEPinp than at PEEPe ($p < 0.002$), and at PEEPiwp than at PEEPinp ($p < 0.001$).

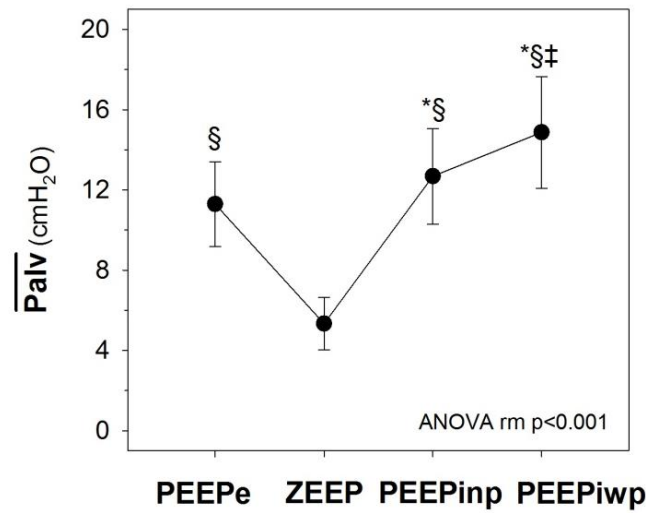


Figure 8. Mean alveolar pressure ($\overline{P_{alv}}$) in the four experimental conditions. Error bars correspond to one SD. p-value: probability of a difference between the four experimental conditions (ANOVA rm, repeated measurements). Results of post-hoc analysis are indicated as: *, significantly different ($p < 0.05$) from PEEPe; §, significantly different from ZEEP; ‡, significantly different from PEEPinp (indicated for PEEPiwp only).

Stress index (SI)

SI was close to one in all experimental conditions, being 1.02 ± 0.09 , 0.99 ± 0.12 , 0.97 ± 0.09 and 1.04 ± 0.13 during PEEPe, ZEEP, PEEPinp, and PEEPiwp, respectively ($p = 0.028$). Significant differences were detected only between PEEPinp and PEEPe ($p = 0.004$) or PEEPiwp ($p = 0.038$).

Airway occlusion pressure (P_{ao})

According to published criteria (Haudebourg et al., 2023), P_{ao} was present in all experimental conditions in only one patient, in whom P_{ao} was 8.9, 8.4, 6.2 and 7.5 cmH₂O at PEEPe, ZEEP, PEEP_{inp}, and PEEP_{iwp}, respectively. Two other subjects displayed P_{ao} only at ZEEP (5.0 and 3.7 cmH₂O). Overall, PEEP_{tot} and non-resistive pressure at the beginning of the inflation were not statistically different ($p=0.440$).

HEMODYNAMICS

Results regarding hemodynamic parameters are reported in **Table 3**. SV was higher at ZEEP compared to PEEPe, PEEP_{inp} and PEEP_{iwp} ($p<0.041$). CO showed a trend similar to that of SV and SVV an opposite one. CVP tended to be lower at ZEEP compared to the other conditions (post-hoc analysis $0.05>p>0.10$). BP_{syst}, BP_{diast}, MAP and HR did not vary significantly.

Table 3: Hemodynamic parameters at PEEPe, ZEEP, PEEP_{inp} and PEEP_{iwp}

	PEEPe		ZEEP		PEEP _{inp}		PEEP _{iwp}		p-value
SV (ml)	82±28	§	88±31		81±26	§	80±24	§	<0.001
SVV (%SV _{mean})	7.9±4.3	§	5.7±2.8		7.1±2.9		8.4±4.0	§	<0.001
HR (beat/min)	77±24		77±22		76±22		77±23		0.874
CO (L/min)	6.1±2.7	§	6.6±3.0		6.0±2.2		6.0±2.4		0.031
BP_{syst} (mmHg)	133±17		136±18		129±18		131±15		0.083
BP_{diast} (mmHg)	54±10		55±8		53±9		54±9		0.584
MAP (mmHg)	80±11		81±8		78±12		78±11		0.179
CVP (mmHg)	9.2±4.1		7.9±4.7		9.7±3.9		9.1±3.9		0.009

Table 3. SV, stroke volume; SVV, stroke volume variation; CO, cardiac output; BP_{syst}, systolic arterial pressure; BP_{diast}, diastolic arterial pressure; MAP, mean arterial pressure; CVP, central venous pressure; HR, heart rate. p-value: probability of a difference between the four experimental conditions (repeated measurements ANOVA). Results of post-hoc analysis are indicated as: §, significantly different ($p<0.05$) from ZEEP.

BLOOD GAS ANALYSIS

PaO₂ was significantly higher at PEEPe (95.3±17.0 mmHg) compared to the other three conditions (85.0±20.3, 90.2±14.9, 87.0±22.6 mmHg at ZEEP, PEEP_{Pin} and PEEPiwp, respectively, $p<0.050$) (**Fig.9, panel A**). PaCO₂ was lower at PEEPiwp (35.2±6.1 mmHg) compared to PEEPe, ZEEP and PEEP_{Pin} (38.4±6.2, 38.0±6.3, 37.7±4.4, respectively, $p<0.025$) (**Fig.9, panel B**). (A-a)O₂ followed an opposite behavior with respect to PaO₂, being lower at PEEPe (175.0±92.7 mmHg) compared to all other conditions (185.8±94.7, 180.9±97.8 and 187.2±96.3 mmHg at ZEEP, PEEP_{Pin} and PEEPiwp, respectively, $p<0.043$). PaO₂/FiO₂ followed a trend similar to the one of PaO₂ (232±84, 208±85, 222±86, and 214±96 mmHg at PEEPe, ZEEP, PEEP_{Pin} and PEEPiwp, respectively).

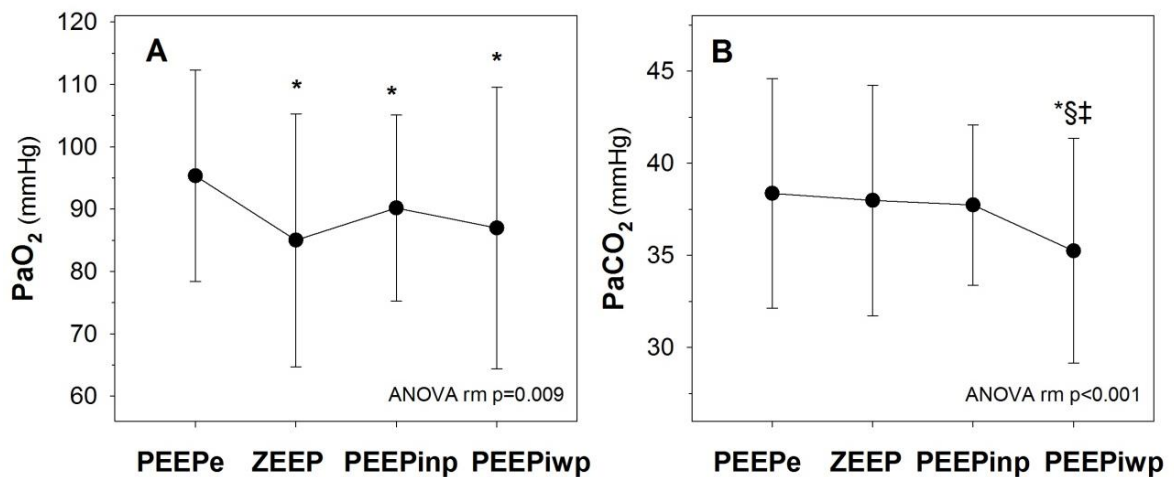


Figure 9. PaO₂ (panel A) and PaCO₂ (panel B) in the four experimental conditions. Error bars correspond to one SD. p-value: probability of a difference between the four experimental conditions (ANOVA rm, repeated measurements). Results of post-hoc analysis are indicated as: *, significantly different ($p<0.05$) from PEEP; \$, significantly different from ZEEP; ‡, significantly different from PEEP_{Pin} (indicated for PEEPiwp only).

pH was greater at PEEPiwp (7.451±0.085) than at PEEPe, ZEEP, and PEEP_{Pin} (7.422±0.086, 7.430±0.077, and 7.426±0.083, respectively, $p<0.050$). Lactate remained constant around 1.3±0.5 mmol/L during the whole experiment ($p=0.566$).

CAPNOGRAPHY

Overall, compared to PEEPe, DS_{phys} , DS_{anat} and DS_{alv} did not change at PEEP_{Pin} ($p>0.398$), while decreased significantly at PEEPiwp ($p<0.002$ for all) (**Fig.10, panel A**).

Indeed, DS_{phys} was lower at PEEPiwp (184 ± 55 ml) compared to the other three conditions, which had similar values (214 ± 57 , 209 ± 52 , 214 ± 52 ml at PEEPe, ZEEP and PEEP_{Pin}, respectively, $p<0.001$). DS_{anat} was similar at ZEEP and PEEPiwp (137 ± 30 and 137 ± 32 ml, respectively, $p=1.000$) and lower compared to PEEPe and PEEP_{Pin} (151 ± 31 and 155 ± 32 ml, respectively, $p<0.002$). Relative to PEEPe and ZEEP (64 ± 55 and 72 ± 52 ml, respectively, $p=0.351$), DS_{alv} decreased at PEEPiwp (47 ± 48 ml, $p<0.002$). Comparable results were obtained for all dead space parameters also when expressed in $\%V_t$. MDT was similar at PEEPe and ZEEP (both 0.51 ± 0.08 s, $p=1.000$), and increased significantly at PEEP_{Pin} and PEEPiwp ($p<0.001$). Additionally, MDT was higher at PEEPiwp than at PEEP_{Pin} (1.58 ± 0.28 and 1.05 ± 0.18 s, respectively, $p<0.001$).

Results for the slope of phase III were similar to those for DS_{phys} , with lower values at PEEPiwp (2.0 ± 1.0 $\%CO_2/L$) compared to the other three conditions (3.5 ± 1.9 , 3.3 ± 1.1 , 2.9 ± 1.1 $\%CO_2/L$, at PEEPe, ZEEP and PEEP_{Pin}, respectively, $p<0.004$) (**Fig.10, panel B**).

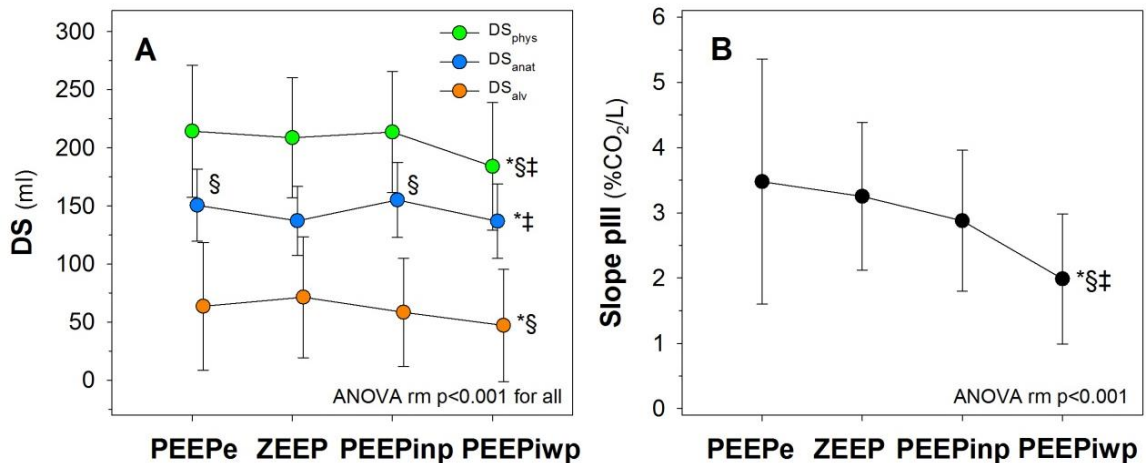


Figure 10. Physiological, anatomical and alveolar dead space (DS_{phys} , green, DS_{anat} , blue, DS_{alv} , orange, panel A) and slope of phase III (slope pIII, panel B) in the four experimental conditions. Error bars correspond to one SD. p-value: probability of a difference between the four experimental conditions (ANOVA rm, repeated measurements). Results of post-hoc analysis are indicated as: *, significantly different ($p<0.05$) from PEEPe; §, significantly different from ZEEP; ‡, significantly different from PEEP_{Pin} (indicated for PEEPiwp only).

ELECTRICAL IMPEDANCE TOMOGRAPHY (EIT)

Results of SURF and TID are reported in **Table 4**. SURF decreased at ZEEP relative to the other experimental conditions, due to a decrease of SURF_d ($p < 0.001$ for both). In all conditions TID was similar ($p = 0.071$), and prevalently distributed to the ventral parts of the lung ($p < 0.001$). The ratio TID/SURF increased at ZEEP with respect to the other conditions, due to an increase of TID/SURF at ventral level ($p < 0.001$ for both).

Table 4: SURF and TID

	PEEPe		ZEEP		PEEPinp		PEEPiwp		p-value
SURF (n° pixels)	386±72	§	357±79		380±73	§	384±74	§	<0.001
SURF_v (n° pixels)	217±38		214±45		213±37		214±38		0.512
SURF_d (n° pixels)	169±46	§	143±46		167±45	§	170±48	§	<0.001
TID (A.U.)	2419±743		2541±822		2382±722		2368±734		0.071
TID_v (A.U.)	1549±560	§	1765±633		1530±533	§	1499±555	§	0.002
TID_d (A.U.)	870±326	§	776±324		852±315		869±320	§	0.007
TID/SURF	6.27±1.64	§	7.10±1.62		6.29±1.75	§	6.17±1.64	§	<0.001
TID/SURF_v	7.08±1.95	§	8.14±1.94		7.16±2.15	§	6.94±1.96	§	<0.001
TID/SURF_d	5.12±1.31		5.35±1.39		5.08±1.36		5.11±1.29		0.246

Table 4. Ventilated surface (SURF), tidal impedance difference (TID) and TID/SURF at global, ventral (subscript _v) and dorsal level (subscript _d) in the four experimental conditions. A.U.: arbitrary units. TID/SURF is expressed in A.U./pixel. p-value: probability of a difference between the four experimental conditions (repeated measurements ANOVA). Results of post-hoc analysis are indicated as: *, significantly different ($p < 0.05$) from PEEPe; §, significantly different from ZEEP; ‡, significantly different from PEEPinp (indicated for PEEPiwp only).

GI, GI_v and GI_d were all higher at ZEEP (0.54 ± 0.09 , 0.32 ± 0.07 and 0.22 ± 0.05 , respectively) compared to the other conditions (for GI, GI_v and GI_d, respectively, 0.45 ± 0.07 , 0.26 ± 0.05 , 0.18 ± 0.04 at PEEPe; 0.47 ± 0.07 , 0.28 ± 0.06 , 0.19 ± 0.04 at PEEPinp; 0.45 ± 0.06 , 0.27 ± 0.05 , 0.19 ± 0.04 at PEEPiwp, $p < 0.001$ for all) (**Fig.11, panel A**).

RVD SD, RVD SD_v and RVD SD_d were all significantly lower at PEEPe (5.70±2.22, 4.23±2.35 and 6.16±2.60, respectively) compared to the other conditions (for RVD SD, RVD SD_v and RVD SD_d, respectively, 8.11±3.46, 6.83±3.85, 8.54±3.52 at ZEEP; 8.15±3.38, 6.63±4.15, 8.47±3.51 at PEEPinp; 7.19±2.46, 5.75±2.98, 7.54±2.68 at PEEPiwp, $p<0.007$ for all) (**Fig.11, panel B**).

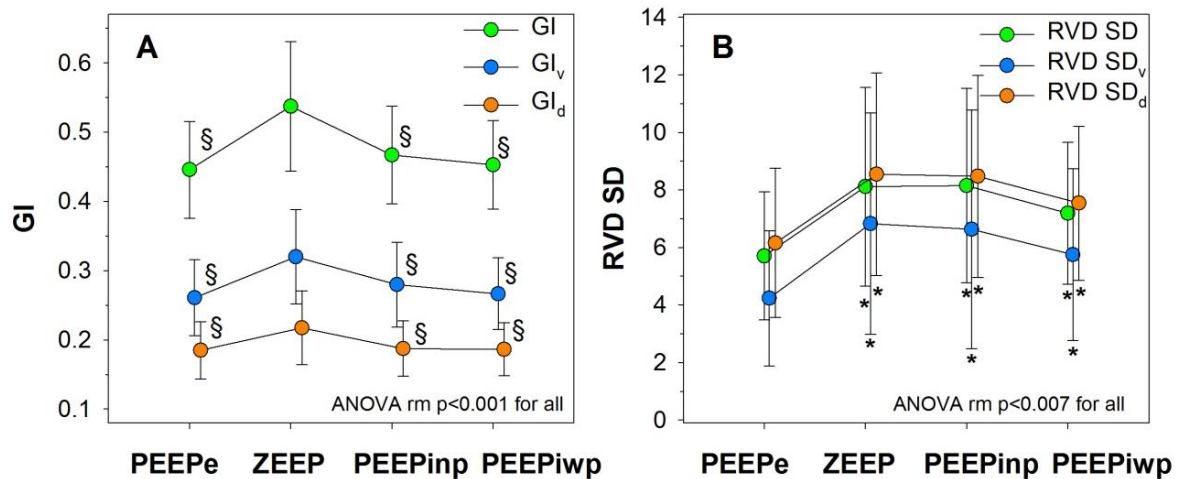


Figure 11. Global inhomogeneity index (GI, panel A) and regional ventilation delay standard deviation (RVD SD, panel B) at global (green), ventral (blue) and dorsal level (orange) in the four experimental conditions. Subscripts _v and _d indicate ventral and dorsal. Error bars correspond to one SD. p-value: probability of a difference between the four experimental conditions (ANOVA rm, repeated measurements). Results of post-hoc analysis are indicated as: * significantly different ($p<0.05$) from PEEPe; §, significantly different from ZEEP.

DISCUSSION

Our work confirmed previous finding showing that, in ARF patients ventilated at constant RR, and V_t , PaO_2 is lower if the same $PEEP_{tot}$ is produced by artificially-induced $PEEP_i$ rather than by $PEEP_e$. Against the original hypothesis of Brandolese and coll., this happened without strong direct or indirect evidence of increased heterogeneity of ventilation distribution. No finding from mechanics, capnography or EIT suggested that spatial ventilation distribution was different when $PEEP_{tot}$ was provided by $PEEP_i$ rather than by $PEEP_e$. Intriguingly, EIT data showed a greater scatter of the temporal pattern of inflation of the different lung units at $PEEP_i$, but the role of this phenomenon in the measured alteration of gas exchange is doubtful, although worth of further investigation.

EXPERIMENTAL DESIGN

The experimental design used in the present work is similar to that adopted in the original work of Brandolese and coll. (Brandolese et al., 1993), in which three conditions equivalent to our $PEEP_e$, ZEEP and $PEEP_{inp}$ were studied. The decision to introduce a fourth experimental condition ($PEEP_{iwp}$) was taken considering that inspiratory flow may have an impact on the distribution of ventilation (Santini et al., 2019) and also on the distribution of stresses in the lung parenchyma (D'Angelo et al., 2004). This is potentially able to affect oxygenation levels, especially in the presence of pre-existing parenchymal heterogeneity, such as in ARF and ARDS patients. Note that in our study both $PEEP_{inp}$ and $PEEP_{iwp}$ were produced by volume-controlled IRV.

Given the limited number of previous works to which our results can be compared, the introduction of the $PEEP_{iwp}$ condition has also the advantage to allow the comparison of our results with those by Zavala and coll. (Zavala et al., 1998). These Authors compared the effects on gas exchange of volume-controlled ventilation at $PEEP_e$ and at ZEEP with those of volume-controlled IRV (similar to our $PEEP_{inp}$) and those of pressure-controlled IRV. In pressure-controlled ventilation, the time it takes for pressure to reach a plateau value (and for V_t to be delivered) depends on total resistance

and compliance of the system (including ventilator tubings and endotracheal tube). If the product of resistance times compliance is small, pressure and volume will remain constant after plateau pressure has been reached, as it happens during a pIp in volume-controlled ventilation. Using the data provided by Zavala and coll., and assuming a reasonable value of resistance (D'Angelo et al., 1997), it can be calculated that plateau pressure was reached in the first second of the 2.79 s of their T_I , corresponding to a pIp (~53% of the T_{tot}) very similar to the one we used during PEEPiwp. Thus, in terms of inflation time and duration of pIp, Zavala's pressure-controlled IRV can be substantially equated to our PEEPiwp condition.

The works of Brandolese and Zavala were both published before the seminal paper supporting the use of low V_t in acute lung injury patients (Acute Respiratory Distress Syndrome Network, 2000), so it is not surprising that the V_t used in their studies were high (~0.70-0.75 L), relative to those currently employed. These higher V_t have allowed the authors to produce a $PEEP_{tot}$ similar to the one in our study with much lower RR (13-14 in Brandolese and 15-17 in Zavala). We chose not to replicate this feature of the older studies, as large V_t have now little clinical use, and we ventilated patients with the low V_t set by the attending physician, even if this choice implied the need of further shortening T_E in comparison with previous investigations.

HEMODYNAMICS

In our subjects, relative to ventilation at ZEEP, ventilation at PEEPe or artificially-induced PEEPi had similar hemodynamic effects, that is a reduction of SV, which, together with an unchanged HR, resulted in a reduction in CO. These results are in line with previous reports (Brandolese et al., 1993; Mercat et al., 1993; Neumann et al., 2000). Indeed, both PEEPe and PEEPi are able to depress CO by several mechanisms related to elevation of intrathoracic pressure and increased pulmonary vascular resistances, which manifest with a decrease of the preload and an increase of the afterload of the right heart (Cournand et al., 1947; Kreit, 2022; Luecke and Pelosi, 2005; Pick et al., 1982). As it can be expected in the presence of a rightward shift of the cardiac function curve due to increased intrathoracic pressure, with respect to ZEEP, CVP tended to increase, although not significantly, in all PEEP conditions (Neumann et

al., 2000; Shojaee et al., 2017). SVV was similar at PEEPe, PEEP_{inp} and PEEPi_w, and greater than at ZEEP. PEEPe has been previously shown to increase SVV (Kubitz et al., 2006; Nakashima et al., 2020), by decreasing the end-diastolic volume of the right ventricle, so that the ventricle operates on the flat compliant part of the end-diastolic pressure-volume relation, where the cyclic changes of preload with breathing produce relevant changes of SV (Dhainaut et al., 1986; Pinsky, 1997).

The substantial similarity of the hemodynamic effects of PEEPe and of PEEPi took place although mean P_{alv} at PEEP_{inp} was slightly (but significantly) greater (12.7 ± 2.4 cmH₂O) than at PEEPe (11.3 ± 2.1 cmH₂O), and even more at PEEPi_w (14.9 ± 2.8 cmH₂O). It should be noted, however, that the corresponding mean P_{es} were even closer to each other (7.2 ± 2.7 , 7.8 ± 3.0 and 8.5 ± 2.9 cmH₂O at PEEPe, PEEP_{inp}, and PEEPi_w, respectively), and thus probably not sufficiently different to produce detectable changes of hemodynamic parameters.

RESPIRATORY MECHANICS

Only one patient out of seventeen showed the presence of substantial airway closure at the beginning of the inspiration, when assessed according to (Haudebourg et al., 2023). Even in this patient, airway closure at end-expiration was probably not complete, as indicated by the transmission of the cardiac oscillations on P_{aw} during pEp. The prevalence of substantial airway closure in ARDS patients is rather high, about 25-33% (Chen et al., 2018), but only three out of the seventeen patients enrolled so far in this study met criteria for ARDS.

On turning from ZEEP to PEEPe, lung volume increased in excess to what predicted by the quasi-static characteristics of the respiratory system at ZEEP (**Fig.6**), indicating recruitment of previously collapsed lung units. This is in agreement with the increase of ventilated surface measured when passing from ZEEP to PEEPe (**Table 4**). Recruitment of previously collapsed areas at PEEPe did not cause $E_{st,rs}$ and $E_{st,L}$ to fall as expected, rather, they tended to increase (although not significantly), suggesting that, in addition to modest recruitment, PEEPe induced some moderate level of overdistension in the lung.

In our patients, intratidal recruitment was minimal, as the SI was 1.02 ± 0.09 at PEEPe and 0.99 ± 0.12 at ZEEP, very close to unity.

PEEP_{tot} value in the present study (~ 7 cmH₂O) was similar to that in the study of Brandolese and coll. and Zavala and coll. (8 cmH₂O). PEEP_{tot} during PEEP_{in} and PEEP_{iwp} was comparable to the one during PEEPe. In line with the substantial constancy of PEEP_{tot}, $\Delta EELV$ was similar in all the three PEEP conditions. As by experimental design, the level of PEEPi was close to zero at PEEPe and increased to ~ 5 cmH₂O during ventilation at PEEPi, with no difference between PEEP_{in} and PEEP_{iwp}.

There was no difference in terms of quasi-static elastances in all PEEP conditions. This is not surprising, as quasi-static elastance is measured as the pressure difference between the end of a long pIp and the end of a long pEp, after pendelluft had all the time to equilibrate eventual regional pressure differences created by PEEPi. Our values of $E_{st,rs}$ were similar to those reported in the works of Brandolese and coll. and Zavala and coll. (Brandolese et al., 1993; Zavala et al., 1998); however, compared to ZEEP, in Brandolese and coll. $E_{st,rs}$ did not change at PEEPe or PEEP_{in}, while in Zavala and coll., $E_{st,rs}$ decreased at both PEEPe and conditions similar to our PEEP_{in} and PEEP_{iwp}. Most probably, the experimental population of Brandolese can be considered similar to ours since, as in our patients, $E_{st,rs}$ did not change between ZEEP and PEEP conditions. In contrast, in the patients of Zavala and coll., the recruitment component most probably predominated at both PEEPe and PEEP_{in} or PEEP_{iwp}, causing $E_{st,rs}$ during PEEP conditions to decrease relative to ZEEP.

In the presence of a PEEPi enhancing the heterogeneity of regional volumes and ventilations, it is reasonable to expect changes of the dynamic properties of the respiratory system, namely $R_{int,rs}$ and ΔR_{rs} . However, no relevant difference between these parameters has been detected. $R_{int,rs}$ did not change between PEEPe and PEEP_{iwp}, arguing against increased functional or anatomical derecruitment in the latter condition. At PEEP_{in}, $R_{int,rs}$ was significantly lower than at PEEPe, but this cannot be attributed to any mechanical effect of PEEPi. Rather, inspiratory flow in this condition was less than half than in the others, and this should have decreased the level of turbulence

provided by the endotracheal tube, the resistance of which is included in our measurement (Flevari et al., 2011; Wilson et al., 2009).

Our values of $R_{int,cw}$ were slightly higher to those previously reported in ARDS patients during mechanical ventilation at ZEEP and PEEP 5 cmH₂O (0.3 cmH₂O/L) (D'Angelo et al., 1997). According to our results, $R_{int,cw}$ was highest at PEEP_{in} and lowest at PEEP_{iw}. We are not able to provide an explanation for this behavior. Without doubt, our measurement of R_{int} is rather approximate, because of the long closing time of ventilators inspiratory valves which can heavily affect this measure (Zilianti et al., 2024). In any case, considering that the contribution of $R_{int,cw}$ to $R_{int,rs}$ never exceeded 10%, the impact of its variation on overall $R_{int,rs}$ remains quite limited.

ΔR_{rs} and ΔR_L are expected to increase in the presence of substantial ventilation heterogeneity, as, in this case, during pIp air redistributes between lung units with different time-constants leading to an increase in the pressure drop from P_1 to P_{st} (Bates et al., 1985; Milic-emili et al., 1990). Against this expectation, no increase of ΔR_{rs} or ΔR_L was found at PEEP_{iw} relative to PEEP_e, strongly arguing against increased ventilation heterogeneity in the former condition. An increase of ΔR_{rs} and of ΔR_L was found at PEEP_{in} relative to the other groups, but this cannot be attributed to an increase of pendelluft, as it is well-known that, in the absence of gas redistribution, additional resistance measured after delivery of the same V_t exhibits an exponential rise to maximum with increasing duration of the T_1 (D'Angelo et al., 2002, 1989; Zilianti et al., 2023).

ELECTRICAL IMPEDANCE TOMOGRAPHY (EIT)

EIT showed that, in all experimental conditions, ventilation was prevalently distributed in the ventral regions. PEEP_e application or PEEP_i production caused recruitment of units which were not ventilated at ZEEP. In line with previous research, at least for what regards PEEP_e (Hinz et al., 2005), the recruited units belonged to the dorsal part of the lung, because no difference in SURF_v was detected, while both SURF_d and TID_d increased with respect to ZEEP. As a result, in the PEEP conditions, ventilation was more evenly distributed, even if ventral regions remained relatively

hyperventilated with respect to the dorsal ones (**Table 4**). No difference in the amount of ventilated surface recruitment was detected between PEEPe, PEEP_{inp} or PEEPi_w.

Spatial heterogeneity of ventilation distribution was quantified using global inhomogeneity index (GI), a parameter which reflects the scatter of the magnitude of tidal impedance changes at pixel level in the ventilated area (Zhao et al., 2014, 2009a, 2009b). The results are shown in **Fig.11, panel A**, and indicate an elevation of spatial heterogeneity of ventilation distribution at ZEEP, but no differences among the three PEEP conditions.

By itself, GI does not distinguish between intraregional small-scale and interregional large-scale ventilation heterogeneity. Indeed, even if ventilation is completely uniform within the dorsal and ventral parts of the lung, but different between the two, GI would increase. As ventilation distribution between the ventral and dorsal parts of the lung changed from ZEEP to PEEP, we wondered if the observed elevation of GI at ZEEP was uniquely due to this phenomenon. To this aim, local inhomogeneity index (LI), a parameter reflecting intraregional ventilation heterogeneity, has been calculated as previously reported (Zhao et al., 2009b), that is by measuring the difference of tidal impedance changes between each pixel and those surrounding it. LI was greater at ZEEP (0.20 ± 0.03) compared to the other conditions (0.19 ± 0.03 , 0.19 ± 0.30 , 0.19 ± 0.02 at PEEPe, PEEP_{inp} and PEEPi_w, respectively, $p < 0.001$). These results indicate that spatial heterogeneity of ventilation distribution was greater at ZEEP relative to the PEEP conditions due to both an increase of interregional large-scale and intraregional small scale ventilation distribution heterogeneity. However, neither interregional or intraregional heterogeneity differences were observed when the same level of PEEP_{tot} was produced by application of PEEPe or by the production of PEEPi.

One of the mechanisms potentially leading to a heterogeneous distribution of ventilation in the present settings is the occurrence of regional EFL due to the increased expiratory flow which is generated in the PEEPi conditions (**Table 2**). However, it is unlikely that significant regional EFL occurred in our settings. In fact, if flow-limitation develops in a significant number of lung units, the consequent increase of effective resistance should blunt the increase of flow elicited by an increase of driving pressure,

and the measured change of mean expiratory flow should be less than that predicted from the change of driving pressure. The difference between expected and measured change of expiratory flow when the subject was switched between PEEPe and PEEPinp, or between PEEPe and PEEPiwp, was just 14 ± 20 and $18 \pm 24\%$, respectively, of the mean expiratory flow at PEEPe. This small difference can be easily accounted for by the increase of resistance which takes place with increasing flow due to turbulence developing at the level of the endotracheal tube, and suggests that regional EFL, if any, involved just a small number of lung units.

Contrary to GI, RVD SD, an index of temporal ventilation heterogeneity, was elevated, relative to PEEPe, both at ZEEP and in the two PEEPi conditions (**Fig.11, panel B**), indicating that, in presence of PEEPi, the temporal pattern of inflation becomes less uniform in the ventilated surface.

While it is easy to imagine why PaO_2 may decrease with increasing spatial heterogeneity of ventilation distribution, it is more difficult to do the same regarding temporal heterogeneity.

In the presence of spatial heterogeneity, some units can be hypoventilated (or not ventilated at all) relative to perfusion, and the consequent shunt-like effect may lead to hypoxemia. To the contrary, a delay in the inflation of one region is supposed to have more limited effects on oxygenation. Indeed, at PEEPinp and PEEPiwp, MDT was actually longer than at PEEPe, and was thus potentially able to better compensate, in terms of gas exchange, for a increase in ventilation delay.

Correlations between EIT parameters and PaO_2 are of limited help in assigning a definite functional role to the observed increase of temporal heterogeneity at PEEPi, as the regional perfusion, a primary determinant of the effect of ventilation heterogeneity on blood gases, could not be assessed. We reasoned that ventilation heterogeneity should have a greater impact on PaO_2 if it involves the dorsal parts of the lung, where perfusion should be elevated. Actually, according to repeated measures correlation, both GI_d and RVD SD_d were negatively correlated with PaO_2 ($R_m = -0.336$, $p = 0.015$ for GI_d ; and $R_m = -0.376$, $p = 0.006$ for RVD SD_d). However, if the same analysis is performed on PEEP conditions only, excluding ZEEP, these relations disappear ($R_m = -0.140$,

$p=0.421$ for GI_d ; and $R_{tm}=-0.26$, $p=0.134$ for $RVD\ SD_d$), questioning the role of temporal heterogeneity in the fall of PaO_2 at PEEP_{inp} and PEEP_{iwp} relative to PEEPe.

DEAD SPACE AND SLOPE OF CAPNOGRAPHIC PHASE III

Compared to PEEPe, DS_{anat} and DS_{phys} , together with slope of phase III, were significantly lower when PEEP_i was produced by shortening T_E and introducing a prolonged post-inspiratory pause at the end of the inflation (PEEP_{iwp}). To the contrary, no difference regarding these parameters was detected when PEEP_i was produced by shortening T_E while increasing the duration of inflation (PEEP_{inp}). In line with this finding, pH and $PaCO_2$ were significantly higher and lower, respectively, in PEEP_{iwp} with respect to the other conditions, as dead space reduction apparently promoted CO_2 elimination in this condition only. The small but significant increase of pH at PEEP_{iwp} should be related to the decrease of $PaCO_2$, because lactate was invariant.

The invariance of dead space and slope of phase III at PEEP_{inp} relative to PEEPe and their decrease at PEEP_{iwp} argues against the hypothesis by Brandolese and coll. (Brandolese et al., 1993) that artificially-induced PEEP_i enhances ventilation heterogeneity and ventilation to perfusion mismatch.

These parameters have multiple determinants, operating simultaneously in our experimental conditions, with potential opposing effects on them. DS_{phys} is the sum of DS_{anat} and DS_{alv} . DS_{anat} depends on lung volume (which was similar at PEEPe, PEEP_{inp} and PEEP_{iwp}), and on the time available for gas diffusion. To the contrary, DS_{alv} mainly reflects the presence of hyperventilated units relative to the perfusion, even if this parameter is also affected by shunt (Robertson, 2015). The slope of phase III is thought to reflect the asynchronous emptying of lung units with different ventilation to perfusion ratios, and is therefore used as an index of ventilation heterogeneity (Fletcher et al., 1981).

On one hand, an increase of the heterogeneity of ventilation distribution is expected to exacerbate the effects of time-constants inequalities present in the diseased lungs (Otis et al., 1956), causing DS_{alv} and slope of phase III to increase.

On the other hand, when T_I is prolonged and V_t kept constant, inspiratory flow necessarily decreases (as in PEEP_{inp} condition). A reduced inspiratory flow tends to

reduce ventilation heterogeneity during inspiration. Indeed, a previous study in mechanically-ventilated ARDS patients found that a high inspiratory flow is associated with increased pendelluft (Santini et al., 2019). More importantly, when the flow profile is square, as in the present conditions, a longer T_I allows more time for diffusion, which tends to reduce dead space and decrease the slope of phase III. Previous research in animals and humans (Aboab et al., 2007; Aguirre-Bermeo et al., 2016; Devaquet et al., 2008; Uttman and Jonson, 2003) showed that DS_{anat} reduction and CO_2 elimination are markedly related with the time available for diffusive mixing of gas during inspiration, indexed by MDT.

In our experiments, at PEEP_{in}, MDT doubled relative to PEEPe. At PEEPiwp, inspiratory flow was the same that at PEEPe, but inflation was followed by the long pIp required to maintain a constant RR with a reduced T_E , and MDT was more than three times the value at PEEPe.

On this ground, one may speculate that, whatever the effects of the alteration of ventilation distribution suggested by the increase of RVD SD at PEEP_{in} and PEEPiwp, they were fully compensated at PEEP_{in} by the concomitant doubling of MDT, and more than compensated at PEEPiwp by an even further rise of MDT, so that, in that latter condition, dead space and slope of phase III not only did not change relative to PEEPe, but even decreased (**Fig.10**).

The reduction in DS_{phys} at PEEPiwp stems from a reduction of both DS_{anat} and DS_{alv} (**Fig.10, panel A**). The prolonged pIp should have allowed a greater movement in proximal direction of the interface between the air contained in exchanging lung units and the air in the conducting airways (Bowes et al., 1985; Fletcher et al., 1981; Uttman and Jonson, 2003). Additionally, the continuous delivery of CO_2 at capillary level during the pIp increases the gradient for CO_2 diffusion, further moving the interface in a central direction (Aboab et al., 2007). Both mechanisms likely promoted a significant reduction of DS_{anat} . Regarding the changes of DS_{alv} during PEEPiwp, its reduction cannot result from a decrease of temporal mismatch between ventilation and perfusion (Fletcher et al., 1981), which appears instead enhanced in this condition, as suggested by our results on RVD SD, but should be due to an improvement in diffusive gas mixing and/or in ventilation to perfusion mismatch at a small-scale level. Slope of phase

III behaved essentially as DS_{alv} . This is in line with previous research demonstrating a reduction in the slope of phase III with pIp prolongation both in healthy (Uttman and Jonson, 2003) and acute lung injury patients (Devaquet et al., 2008).

DS_{anat} at ZEEP was very similar to that at PEEPiwp and lower than at PEEPe and PEEPinp. However, contrarily to PEEPiwp, the reduction of DS_{anat} at ZEEP can be entirely explained by the reduction in lung volume, since according to MDT, which was just one third the value at PEEPiwp, DS_{anat} should have increased.

The present results agree with the few previous studies dealing with artificially-induced PEEPi and IRV in which $PEEP_{tot}$ was carefully controlled. In mechanically ventilated ARF patients, no change of $PaCO_2$ was found when the same level of $PEEP_{tot}$ was produced by applying PEEPe or prolonging T_1 without introducing a pIp (PEEPinp) (Brandolese et al., 1993; Zavala et al., 1998). Relative to the PEEPe, a decrease of $PaCO_2$ and DS_{phys} was observed by Zavala and coll. only when PEEPi was induced by a ventilation modality roughly comparable with our PEEPiwp (Zavala et al., 1998).

ARTERIAL BLOOD OXYGEN TENSION

Compared to ventilation at PEEPe, PaO_2 decreased at both PEEPinp and PEEPiwp, despite the same $PEEP_{tot}$, ventilation and operating lung volume (**Fig.9, panel A**). As expected, removal of PEEP (ZEEP) led to a fall of PaO_2 .

These findings confirm those reported in the work of Brandolese and coll. (Brandolese et al., 1993), in which PEEPi was produced as in our PEEPinp conditions. Zavala and coll. detected a reduction of PaO_2 in a condition similar to our PEEPiwp (pressure-controlled IRV), but not in one similar to PEEPinp (volume-controlled IRV) (Zavala et al., 1998). A lower PaO_2 at conditions close to our PEEPiwp, relative to PEEPe, was reported also by other studies (Lessard et al., 1994; Mercat et al., 1993).

The actual determinant of the decrease of PaO_2 due to artificially-induced PEEPi remains puzzling.

In line of principle, at constant ventilation and composition of inspired air, hypoxemia can be produced by true shunt, ventilation to perfusion mismatch and diffusion limitation. The latter mechanism is a rare occurrence in ARF and ARDS patients (Melot, 1994).

In our subjects, the ventilated surface was the same in all PEEP conditions, a finding which makes unlikely a difference of true shunt due to a difference in the amount of recruited respiratory units.

Also spatial heterogeneity of ventilation distribution was similar in all PEEP conditions, and therefore hypoxemia cannot be attributed to an increase of ventilation to perfusion mismatch driven by increased ventilation heterogeneity. Enhanced ventilation heterogeneity during inverse 3:1 ratio ventilation relative to normal 1:2 ratio ventilation with PEEPe has been suggested by CT measurements performed in a pig model of acute lung injury, in which the decrease of PaO_2 during IRV was associated with an enhancement of the gravity-dependent (ventro-dorsal) density gradient (Neumann et al., 2000). As perfusion distribution was unchanged, the increase of physiological shunt was attributed to the change of ventilation distribution only. However, this kind of phenomenon was absent in our patients according to our EIT data.

To our claim that spatial heterogeneity of ventilation distribution is not affected by artificially-induced PEEPi, one may object that the spatial resolution of impedance tomography is limited (in the range of few cm), and that heterogeneity could still have increased at PEEPi at a smaller scale. This possibility is real, but its investigation is beyond the reach of currently employed EIT devices. The measured constancy (at PEEPinp) or decrease (at PEEPiwp) of the slope of phase III with respect to PEEPe do not support or disprove this hypothesis. Indeed, even if heterogeneity occurs at acinar level or at the level of groups of acini, probably it cannot be detected as a rise of phase III slope, because of gas mixing at bronchiolar level.

Assuming that ventilation distribution does not change with artificially-induced PEEPi, an increase of shunt or of ventilation to perfusion mismatch can still take place if artificially-induced PEEPi is able to modify the perfusion, rather than ventilation.

It has been reported that depression of cardiac output can be a mechanism of shunt reduction in both humans and animal models of ARDS (Dantzker et al., 1980; Lynch et al., 1979). However, relative to ZEEP, usually cardiac output either decreases similarly at PEEPe and at PEEPi, or tends to decrease more with PEEPi than with PEEPe (Brandolese et al., 1993; Cole et al., 1984; Mercat et al., 1993; Zavala et al., 1998). Nevertheless, PaO_2 is greater at PEEPe than with artificially-induced PEEPi. As

a matter of fact, in the works in which shunt was measured, relative to ZEEP, shunt was significantly reduced only at PEEPe, and not at PEEPi (Brandolese et al., 1993; Zavala et al., 1998). This was probably the case also in the present experiments, as (A-a)O₂ was higher at both PEEP_{in} and PEEP_{iw} relative to PEEPe, and this despite similar cardiac output values during the three conditions. Thus, changes of shunt related to cardiac output modifications cannot explain the results of previous works or our findings.

Another factor potentially able to modify the shunt fraction, and therefore to explain the differences of PaO₂ measured between PEEPe and the PEEPi conditions, is the modification of perfusion distribution between different lung units mediated by local P_{alv}. Indeed, P_{alv} may affect perfusion distribution, as it is the pressure acting on the outside of pulmonary capillaries. An increase in mean P_{alv}, not paralleled by a concomitant recruitment of lung units, is potentially able to divert perfusion from ventilated to unventilated or poorly ventilated units. In our patients, mean P_{alv} was slightly higher at PEEP_{in} compared to PEEPe and higher than PEEP_{in} and PEEPe at PEEP_{iw}. Indeed, mean P_{alv} correlated with (A-a)O₂ when the analysis was restricted to the three PEEP groups ($R_{\text{rm}}=0.660$, $p<0.001$). Moreover, the correlation persisted when the PEEPe group was removed, and the analysis repeated on PEEP_{in} and PEEP_{iw} conditions only ($R_{\text{rm}}=0.510$, $p<0.039$). Thus, this mechanism may have contributed to create the (A-a)O₂ and therefore the decrease of PaO₂. However, for the same experimental conditions, the direct correlation between mean P_{alv} and PaO₂ failed to reach significance ($R_{\text{rm}}=0.280$, $p<0.269$), suggesting that other factors also may have had a role in the deterioration of PaO₂ during artificially-induced PEEPi.

The idea that an increase of mean P_{alv} may cause an increase of shunt fraction are in line with the findings of Zavala and coll. (Zavala et al., 1998), but in contrast to what found by Neumann and coll.: in this work, mean P_{aw} during pressure-controlled IRV (similar to our PEEP_{iw}) was not different relative to PEEPe, but the shunt fraction was more than doubled (Neumann et al., 2000). However, interpretation is complicated by use of mean P_{aw} instead of mean P_{alv} and, even more, by the fact that PEEP_{tot} was lower during ventilation at PEEPi compared to PEEPe, and so partial derecruitment cannot be excluded as a contributing factor.

LIMITATIONS

This study has several limitations.

Some studies used as comparisons with our results were performed on ARF patients with ARDS. However, the number of ARDS patients in our patient population, so far, is very limited (3 out of 17). Indeed, about half of our subjects were in ARF due to cardiogenic pulmonary edema, which excludes, by definition, the diagnosis of ARDS. Thus, comparisons should be done with caution.

On the same line of thought, we should acknowledge that our patient population presents a high degree of heterogeneity in terms of underlying conditions and cause of ARF. As a matter of fact, previous research has not been free from this issue. Even if some of previous studies focused on a specific type of population, namely ARDS, also this syndrome has been shown to encompass a wide variety of different phenotypes, in terms of clinical, radiological, physiological and biological parameters (Matthay et al., 2020). Different patient subgroups may have different responses to the four ventilatory modalities and patient stratification can help to better understand the pathophysiological mechanisms at play. This kind of analysis will be performed as soon as the complete dataset will be achieved.

Another difference with the preceding literature is that, in most of the cited works, measurements were taken within 48 hours from ICU admission (Brandolese et al., 1993; Mercat et al., 1993; Zavala et al., 1998). In studies where an animal model of acute lung injury was employed, data were collected as early as 1-2 hours after induction of injury (Mang et al., 1995; Neumann et al., 2000). In our patient population, experiments were carried out 5-6 days after ICU admission, when clinical stability was reached and disease evolution was most probably in a later stage.

Similarly to the study of Brandolese and coll., patients with obstructive lung disease were excluded, as these subjects often exhibit high levels of spontaneous PEEPi. For this reason, our results cannot be applied to this patient population. In these subjects, mechanical heterogeneity is expected to be already high at baseline condition.

Another limitation, which is in common with many previous works investigating the effects of IRV, is the relative short duration of the experimental conditions (~20

minutes). Although it appears that most of the PaO_2 change occurs early after modification of the breathing pattern, we cannot exclude that a longer duration of application of artificially-induced PEEP_i may provide different results, although in a study, no progressive change in PaO_2 was detected when pressure-controlled IRV generating PEEP_i was kept for 24 hours or more (East et al., 1992).

CONCLUSIONS

In conclusion, our results show that, when an ARF patient is mechanically ventilated and $PEEP_{tot}$ is produced by artificially inducing $PEEP_i$, PaO_2 is lower than if the same $PEEP_{tot}$ is produced by $PEEP_e$ application, even if V_t and RR are kept constant. Contrary to previously a proposed hypothesis, this happens without evidence of gross alterations of spatial distribution of ventilation, as assessed by electrical impedance tomography. Rather, indirect evidence suggests that the decline of PaO_2 is related to a change of the distribution of perfusion due to the increased mean P_{alv} , which is not paralleled by concomitant recruitment. In the clinical perspective, this phenomenon, if confirmed by additional data collection, stresses the importance of taking into account recruitment when titrating PEEP in individual patients. The functional relevance of the alterations in the temporal pattern of lung unit inflation suggested by our results from EIT-derived parameters is dubious, and further investigation is needed to better elucidate its role in gas exchange.

These results have been obtained with moderate levels of total PEEP, and caution should be exercised when extrapolating our conclusions to predict what could occur when higher levels of artificially-induced $PEEP_i$ are generated such as during airway pressure release ventilation, since, in this instance, the maximal expiratory flows may be reached, and, at that point, spatial heterogeneity of ventilation distribution can actually appear.

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