Lung regional stress and strain as a function of posture and ventilatory mode

Gaetano Perchiazzi,1,4 Christian Rylander,2 Antonio Vena,3 Savino Derosa,1 Debora Polieri,1 Tommaso Fiore,1 Rocco Giuliani,1 and Göran Hedenstierna4

1Emergency and Organ Transplant, Bari University, Bari, Italy; 2Anaesthesiology and Intensive Care Medicine, Sahlgrenska University Hospital, Göteborg, Sweden; 3Intensive Care Unit, SS Annunziata Hospital, Taranto, Italy; and 4Medical Sciences–Clinical Physiology, Uppsala University, Uppsala, Sweden

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Perchiazzi G, Rylander C, Vena A, Derosa S, Polieri D, Fiore T, Giuliani R, Hedenstierna G. Lung regional stress and strain as a function of posture and ventilatory mode. J Appl Physiol 110: 1374–1383, 2011. First published March 10, 2011; doi:10.1152/japplphysiol.00439.2010.—During positive-pressure ventilation parenchymal deformation can be assessed as strain (volume increase above functional residual capacity) in response to stress (transpulmonary pressure). The aim of this study was to explore the relationship between stress and strain on the regional level using computed tomography in anesthetized healthy pigs in two postures and two patterns of breathing. Airway opening and esophageal pressures were used to calculate stress; change of gas content as assessed from computed tomography was used to calculate strain. Static stress-strain curves and dynamic strain-time curves were constructed, the latter during the inspiratory phase of volume and pressure-controlled ventilation, both in supine and prone position. The lung was divided into nondependent, intermediate, dependent, and central regions; their curves were modeled by exponential regression and examined for statistically significant differences. In all the examined regions, there were strong but different exponential relations between stress and strain. During mechanical ventilation, the end-inspiratory strain was higher in the dependent than in the nondependent regions. No differences between volume- and pressure-controlled ventilation were found. However, during volume control ventilation, prone positioning decreased the end-inspiratory strain of dependent regions and increased it in nondependent regions, resulting in reduced strain gradient. Strain is inhomogeneously distributed within the healthy lung. Prone positioning attenuates differences between dependent and nondependent regions. The regional effects of ventilatory mode and body positioning should be further explored in patients with acute lung injury.

Address for reprint requests and other correspondence: G. Perchiazzi, Dept. of Emergency and Organ Transplant, Section of Anaesthesia and Intensive Care Medicine, Univ. of Bari, c/o Centro di Rianimazione–Policlinico Ospital, Piazza Giulio Cesare, 11, 70124 Bari, Italy (e-mail: gperchiazzi@rianima.uniba.it).

FOR THE LAST TWO DECADES, mechanical ventilation (MV), while keeping the role of life-saving tool, has been undergoing a methodical review of its repercussions on the lungs. Ventilation in acute respiratory distress syndrome has been one of the main research interests: it has been reported that application of low tidal volumes can reduce mortality (34) while transient beneficial effects (35, 39) were observed by using high end-expiratory positive pressures and by systematic application of recruitment maneuvers (RM). Positioning the patient prone has beneficial effects on gas exchange in most patients and on mortality in the most hypoxemic ones (33). However, patients affected by acute respiratory distress syndrome are a minority of the ones admitted to intensive care and to artificial ventilation. A large number of articles have been published about the impact that MV has on healthy lungs both in intensive care and during general anesthesia for surgery, focusing mainly on titration of tidal volumes (32). Because prospective studies about MV application on healthy lungs show dissimilar results, a reconsideration of lung mechanical response to ventilation is needed. The link between mechanical ventilation and biological damage is the structural deformation of parenchyma (37). Deformation can be studied in terms of stress and strain acting on lung structures. They stem from the application of the Hookian law of force/elongation of a spring to the three-dimensional structure of the lung (43). Strain is the elongation of the structure, having its resting point at the functional residual capacity (FRC). Strain can be expressed as the ratio between increment of volume in respect to the FRC. The lung stress is the force acting across airway and alveolar walls and corresponds roughly to the transpulmonary pressure (Ptp). Assessment of potential parenchymal injury must take into account stress and strain as the real physical quantities acting on the lung structures (41). Recently, Chiumello and et al. (6) studied the global stress/strain relation in different groups of patients and concluded that “the stress-to-strain relationship was the same” in all the subgroups and that “it was linear or nearly linear” in the explored range of pressure and volume. However, classical physiology taught that, according to a gravitational vector, the lung shows different degrees of distension and volumetric increment during ventilation (42). Although gravitational effects may not be the only or even major cause of ventilation inhomogeneity (12), the fact that in healthy conditions dependent lung areas undergo greater regional ventilation is well established (22). Moreover, even in healthy conditions, the lung exhibits a spatial and temporal heterogeneity of regional ventilation (27) and lung inflation is accompanied by anisotropic alveolar expansion as proven by studies using in vivo microscopy (31).

Therefore, considering the importance of stress and strain to the generation of lung injury and the known inherent inhomogeneity of the lung, the aims of the present study were to measure, model, and compare 1) the regional stress-to-strain curves of the lung during static conditions (breath hold maneuvers), and 2) the regional strain to time curves of the lung during dynamic conditions (ongoing mechanical ventilation) in two patterns of breathing and in two different body positions by analyzing computed tomography (CT) images and Ptp of anesthetized and mechanically ventilated piglets.

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MATERIALS AND METHODS

Preparation

The study was approved by the local Uppsala University Animal Ethics Committee, and it was performed according to the National Research Council’s Guide for the Principles of Laboratory Animal Care (NIH Publication No. 85-23, revised 1985). All preparations were undertaken with the animals in the supine position. In five healthy piglets (~2 to 3-mo-old, weight 28.3 ± 2.4 kg, in the following called pigs), general anesthesia was induced by intramuscular injection of atropine (0.04 mg/kg), tiletamine-zolazepam (5 mg/kg Zoletil; Boeringer Ingelheim; Copenhagen, Denmark), and medetomidine (5 μg/kg Dormitor vet; Orion Pharma, Sollentuna, Sweden). After intravenous injection of Fentanyl (5 μg/kg), the pigs were intubated via a tracheostomy using a cuffed tube (6.0 Hi-Contour; Mallinckrodt Medical, Athlone, Ireland). Anesthesia was maintained by intravenous infusion of ketamine (20 mg·kg⁻¹·h⁻¹ Ketaminol; Vetpharma, Zurich, Switzerland), fentanyl (5 mg·kg⁻¹·h⁻¹ Pharma- link, Spånga, Sweden), and pancuronium (0.24 mg·kg⁻¹·h⁻¹ Pavulon; Organon Teknika, Gothenburg, Sweden) in buffered glucose 2.5% (Rehydrex; Fresenius Kabi, Uppsala, Sweden) delivered at a rate of 7 ml·kg⁻¹·h⁻¹. For hemodynamic monitoring, an 18-gauge catheter was inserted into the left carotid artery and a floating tip pulmonary artery (PA) catheter (Swan-Ganz thermodilution SP5107H, 7F, 110 cm; Baxter, Irvine, CA), together with another 18-gauge catheter, were introduced into the right internal jugular vein. The position of the PA catheter was confirmed by the pressure traces on the connected bedside monitor (SC 9000 XL; Siemens Medical Systems, Danvers, MA). Cardiac output was measured in triplicate by injection of iced saline randomly during the respiratory cycle. A thermistor in the PA catheter allowed continuous monitoring of blood temperature. Measurement of oxyhemoglobin saturation (SaO₂) was performed by a transcutaneous sensor, and end-tidal carbon dioxide (CO₂) was monitored by means of a mainstream sensor. A urinary catheter was surgically inserted into the bladder. An esophageal balloon (oesophageal catheter; Erich Jaeger, Höchberg, Germany), applying the principles described by Baydur et al. (3), was introduced into the distal third of the esophagus during continuous pressure recording and positioned to minimize cardiac artifacts. Mechanical ventilation was maintained throughout the entire experiment using a mobile ventilator (Servo-I; Maquet, Solna, Sweden). Baseline ventilation was set in proportion to their IC [VT = (IC/12) *1, (IC/12)*2, (IC/12)*3 up to IC] and were administered as monotonic increasing volumes. Before the sequence, we performed a RM to standardize volume history as Chiumello et al. (6) did. Each IHM was separated from the preceding 1 by 2 min or more of tidal breathing to restore steady-state ventila-

![Study protocol](image-url)

Fig. 1. Study protocol. Illustration of the sequence of respiratory maneuvers and the simultaneous image acquisition by computer tomography (CT). FRC, functional residual capacity; IC, inspiratory capacity; RM, recruitment maneuver.

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tion. During each IHM, a spiral scan of the entire lung (120 KV, 80 mA) lasting 5–6 s was performed.

For collection of data from dynamic conditions, we chose a fixed transverse level between heart and diaphragm and scanned it repeatedly without moving the CT table during ongoing ventilation. Reference to the chosen plane was achieved by placing ECG metal electrodes in correspondence to the laser beam projection on the animal thorax and by identifying anatomical landmarks on the CT images. Single plane scans with a rotation time of 0.50 s and a 0.25-s reset in between were repeated covering several tidal breaths and stopped after 100 exposures.

We delivered two dynamic patterns, separated by baseline tidal ventilation: volume control mode, VT = 9 ml/kg, inspiratory time = 3 s, I:E = 1:1, no inspiratory pause, and RR 10/min [volume control ventilation (VCV)], and the pressure control mode, set to reach a VT = 9 ml/kg, inspiratory time = 3 s, I:E = 1:1, no inspiratory pause and RR 10/min [pressure control ventilation (PCV)].

Another RM was performed before the animal was left to expire freely by lung recoil forces at zero end expiratory pressure (ZEEP). While the lung was at ZEEP, a spiral scan of the entire lung was completed to estimate FRC. The whole protocol, as described above (RM, 12 IHMs, dynamic patterns of MV, FRC measurement), was performed in each animal in the supine and in prone position.

Estimation of Strain from CT Images

Each CT exposure during the hold maneuvers generated a stack of images covering the whole lung at a constant lung volume. We chose to analyze images pertaining to the same transverse plane that was scanned during the two dynamic patterns. They were 12 images at end inspiration and 1 at end expiration, per animal, per posture. Images from other parts of the lung than the chosen plane were discarded. All calculations were performed by using scripts for the Image Processing Toolbox for MatLab R2008a (MatLab, The MathWorks, Natick, MA), purposely written by one of the authors (G. Perchiazz). The DICOM-standard image files produced by the CT scanner were directly passed to the MatLab software.

The lung parenchyma of the CT slices was manually outlined. Then, each value of the matrix [containing a measure of X-ray attenuation, expressed as Hounsfield units (HU)] was translated in the equivalent measure of gas content \( V_{\text{gas}} \), by applying the following equation:

\[
V_{\text{gas}} = V_{\text{vox}} \cdot \frac{-\text{HU}}{1000} \tag{1}
\]

This way, we composed a gas volume map of the outlined lung from the volume of each voxel \( V_{\text{vox}} \) and its attenuation value (HU). This made it possible to calculate the gas content of a single region of interest (ROI) or of the entire lung appearing in the slice: \( V_{\text{gas, ROI}} \) by summing the gas content of voxels belonging to a specific region, using the following standard formula (15):

\[
V_{\text{gas, ROI}} = \sum_{i=1}^{n_{\text{vox}}} V_{\text{vox}} \cdot \frac{-\text{HU}}{1000} \tag{2}
\]

where \( n \) is the number of elements in the outlined ROI in the 512 \( \times \) 512 matrix.

Each lung image to be studied was divided into four different ROIs. Landmarks for division in both postures (see Fig. 2) were horizontally the dorsal limit of the heart and the ventral limit of para-vertebral big vessels; vertical limits were the lateral limits of the heart. The compartments were labeled as nondependent (NDP), intermediate (MID), and dependent (DEP) ROI, while the ROI positioned under the heart was identified as central (CEN) ROI. In the prone position, it is not possible to identify a CEN, because the heart lies on the sternum. In the same animal and in the same posture, the ROI limits on the CT slice were consistent and easily reproducible. However, a ROI defined this way can contain different gas volumes, depending on the phase of the respiratory cycle and/or the chosen limits of the ROI.

The strain of a ROI is:

\[
\text{Strain}_{\text{ROI}} = \frac{V_{\text{ROI}} - FRC_{\text{ROI}}}{FRC_{\text{ROI}}} \tag{3}
\]

where \( FRC_{\text{ROI}} \) is the volume measured at the resting position of the respiratory system at ZEEP.

To avoid possible flaws of strain calculations, deriving from the changing dimensions of a ROI during the respiratory cycle, all the gas volumes were weighted by the number of voxels composing each ROI:

\[
\text{Strain}_{\text{ROI,ave}} = \frac{V_{\text{gas,ROI}} - FRC_{\text{ROI}}}{FRC_{\text{ROI}}} \cdot \frac{n_{\text{ROI}}}{n_{\text{gas,ROI}}} \tag{4}
\]

where \( n_{\text{v}} \) is the number of voxels in the ROI at volume V and \( n_{\text{FRC}} \) the number of voxels in the ROI at FRC. From now onwards, when referring to strain calculated in the present study, we will allude to formula labeled as Eq. 4.

Estimation of Stress

Stress was estimated in terms of Ptp during plateau pressure conditions. Strictly speaking, because both airway and esophageal pressures were referenced to their end-expiratory values, we used a
measure of delta transpulmonary pressure ($\Delta P_{tp}$) as done by Chiumello et al (6):

$$\Delta P_{tp} = (P_{aw\text{plateau}} - P_{aw\text{EE}}) - (P_{es\text{plateau}} - P_{es\text{EE}})$$  \hspace{1cm} (5)

where $P_{aw\text{plateau}}$ is airway pressure during IHM; $P_{es\text{plateau}}$ is the esophageal pressure at the same time; and $P_{aw\text{EE}}$ and $P_{es\text{EE}}$ are, respectively, the airway and the esophageal pressures at the end of expiration.

Data Analysis and Statistics

(See Supplemental Data for a more detailed description of statistical methods; Supplemental Material for this article is available online at the J Appl Physiol website.)

At the end of the preparations, we obtained during static conditions the stress-to-strain relation in two postures over the IC. Similarly, we obtained for the dynamic conditions, the strain to time plot over the tidal volume in two postures and two patterns of ventilation. Each relation was obtained for the individual ROIs and for the entire lung slice.

The stress vs. strain functions were modeled (Curve Fitting Toolbox for Matlab 7.0; The MathWorks) by applying the following equation:

$$y = a \cdot e^{b \cdot x}$$  \hspace{1cm} (6)

that in physical terms becomes:

$$P_{tp} = a \cdot e^{b \cdot \text{strain}}$$  \hspace{1cm} (7)

computing the coefficients $a$ and $b$. Moreover, by applying the statistical $F$-test, we computed the $P$ value for each regression. For this analysis and throughout all the subsequent statistical tests, we set a level of significance $\alpha$ at 0.05.

We studied the models describing NDP, MID, CEN (in supine), DEP, and the entire slice by applying the $F$-test (23). We examined the stress/strain relation of these ROIs to verify whether any difference attributable to their anatomical location or animal posture was detectable. In dynamic conditions, as a preliminary analysis, we tested whether the amount of volume of gas in the entire lung slice was equal passing between modalities of ventilation and between postures at end inspiration and end expiration. We applied for this and for the following evaluations on dynamic lung conditions the Kolmogorov-Smirnov test, setting $\alpha = 0.05$.

In dynamic conditions, we used the strain/time curves to evaluate: curve morphology, the end-inspiratory strain value of the single ROIs (EIS), the maximum difference of strain between two ROIs of the same lung at end-inspiration (EIDS), and the difference in strain existing in a ROI between end-inspiration and end-expiration (EI-EE). We examined EIS, EIDS, and EI-EE to verify whether any difference attributable to animal posture or ventilatory pattern was detectable. Moreover, we analyzed EIS and EI-EE of the ROIs to check whether any difference related to ROI anatomical location was noticeable.

RESULTS

(The report of results is labeled to comply with the numbering used in the Supplementary Material.)

All five animals survived the entire protocol.

Static Conditions

Supine (H1). The analysis of the single stress-to-strain relations in the four ROIs yielded exponential regressions (Fig. 3), always exhibiting statistical significance and a statistically distinct course for each ROI (see Supplemental Tables S1 and S2). The dependent ROIs (DEP) were characterized by the lowest $b$ coefficient with the other ROIs, indicating that DEPs had the lowest stress-to-strain ratio. The NDPs were the ones with the highest $b$ coefficient, implying that the NDPs had the highest stress-to-strain ratio. Moreover, in supine position the CENs had a higher stress-to-strain ratio than the MIDs, both being located at the same vertical height. Their

![](http://jap.physiology.org/)[Fig. 3. Stress-to-strain relation in static conditions of the single ROIs in the 2 postures with regression curves (continuous line: regression curve in supine position; dotted line: regression curve in prone position). WS, whole slice.](http://jap.physiology.org/)
Fig. 4. Difference in strain between dependent and nondependent lung regions reached at different levels of delta transpulmonary pressure (ΔPtp). Data were obtained by calculating the difference between strains on the regression curves belonging to dependent and nondependent lung regions at specific ΔPtp. Plot represents the mean of the differences found in the 5 animals.

stress-to-strain curves were positioned between the curves of the NDPs and the DEPs. The difference in strain between ROIs of the same lung tended to increase with ΔPtp (Fig. 4).

**Prone** (H1). In the prone position, in all the animals, the stress-to-strain ratios of the different ROIs became more similar, with the slope of the DEP becoming steeper and that of the NDP flatter than in supine position. However, there was still a significant difference between NDP and DEP but not between MID and DEP. Again, the difference in strain between ROIs tended to increase with ΔPtp but less than in the supine position (Fig. 4).

**Supine vs. prone** (H2). Each ROI significantly changed its curve parameters when comparing it was compared in the supine and prone posture (Supplemental Table S2).

**Dynamic Conditions**

The gas volume in the lung section included in the CT slice was not different when comparing the two ventilation modalities (keeping the same posture) and comparing the different postures at the same ventilatory mode. This held true both at end expiration and end inspiration. (see Supplemental Table S3 for a summary of statistical analysis on dynamic conditions).

**Effect of ROI location** (H3). During VCV, all ROIs had statistically different end-inspiratory strains except for CENs and MIDS; in PCV a difference was detected only between NDPs and DEPs (Fig. 5). In the prone position, no significant difference was detected between the ROIs, with equal pattern of breathing. EI-EE was smaller in NDP than in DEP in both VCV and PCV (H8). Moreover, with VCV, MID and CEN differed from NDP and DEP while supine. This differed from prone position where no statistical difference was found in EI-EE between the lung ROIs.

**VCV vs. PCV.** Plotting the strain over time (Fig. 6) during ongoing MV, showed that strain was higher in the DEPs than in the NDPs. In VCV, strain increased monotonically with time; PCV reached a higher strain faster than VCV. (H5) When studying the EIS of a same ROI in the same posture but with a different pattern of ventilation, there were no statistical
differences. The maximum gradients of strain (Fig. 7) were not different when comparing VCV and PCV in the same posture (H7). The change of ventilation mode, in the same ROI and keeping an identical posture, did not change the EI-EE (Fig. 5) (H10).

Supine vs. prone. The strain vs. time shape was similar in supine and prone. However, in prone lower end-inspiratory strains were reached than in the supine posture. There was a decrease of DEP EIS and an increase of NDP EIS when turning from supine to prone (H4). MID and CEN followed similar courses supine and prone and did not show any statistical difference when ventilated with different patterns. A change from supine to prone lowered EIDS significantly during VCV but not during PCV (H6). Proning lowered EI-EE in DEP and increased in NDP during VCV (H9).

DISCUSSION

The main purpose of the present study has been to track the changes of stress and strain of passive lungs at a regional level in two postures in static conditions and during ongoing mechanical ventilation.

The main findings are that in healthy conditions the NDPs and DEPs of the lung follow different stress-to-strain courses. In both prone and supine postures, the stress-to-strain relations of the ROIs can be described by exponential models. During mechanical ventilation, in the supine posture, the end-inspiratory strain is higher in the DEPs than in the NDPs. In prone postures, the end-inspiratory strain values aggregated. In fact during volume-control ventilation, prone positioning decreases the end-inspiratory strain of dependent ROIs and increases the EIS of NDPs, thus bringing the values closer to each other and reducing the vertical strain gradient in the lung.

Static Conditions

In static conditions, the relevant findings of this study are that the stress-to-strain courses were well described by exponential regressions and they differed between ROIs. We found a strong exponential relation between stress and strain that differs from the linear relationship proposed by Chiumello et al. (6). An exponential profile of the curve might be expected in view of classical studies on respiratory mechanics, stating that “the lung parenchyma acts as a nonlinear spring” and that the “spring constant (the ratio of force to displacement) increases with increasing force, resulting in a relationship in which the force is approximately an exponential function of the stretch” (10, 28). In their study, Chiumello et al. address the problem of the difference between the driving mechanisms acting on the lung parenchyma (stress and strain) and the parameters that can be set on a mechanical ventilator. They showed that plateau pressure (P_plat) and tidal volume (V_t), widely used for titrating ventilation, are not adequate surrogates for lung stress and strain. In fact, they report that the same V_t per kilogram weight can generate different strains in different patients and that the same P_plat can develop variable stresses, depending on the individual patient. Their key message is that it is necessary to be aware of this difference and to
try to ameliorate the approximation of stress and strain acting on the lung. We have approached this problem under a different angle. We have studied an animal model of mechanical ventilation and focused on a single slice. We have tracked, using anatomical landmarks, four ROIs in a transverse section of the lung. We observed that the behavior of the entire lung slice was the effect of quite different regional mechanical behaviors. The NDP is on a steep curve; the most DEP has a more gradual increase. At the same applied stress, they elongate very differently; our measurements show that in the supine position, at a stress of 20 cmH₂O, NDP has a strain of 25% and DEP has a strain of 133%, that is a fivefold difference: a global s/s relation (measured on the entire slice, regression data in Supplemental Table S1) would show a strain of 48%; thus less than what we see in DEP. The fact that healthy alveoli are placed in different positions along the gravitational vector and are differently exposed to traction and compression forces can explain per se this inhomogeneity. This should not come as a surprise when considering the curved pressure-volume relationship of the lung that has been known for years (22, 24). Our regional analysis attribute a stress-to-strain relation to a certain area of the lung, expressing the average behavior of the contained voxels. It is necessary to be aware that when comparing different ROIs, in the same slice and time, the spatial resolution of this approach cannot distinguish whether the gradient is uniformly and gradually distributed along the line connecting the two ROIs or whether there is a step change between a high and a low strain front. However, visual evaluation of CT images excludes a relevant anisotropism in the space of a few voxels on these ideal lines. We share with other authors (6, 14, 30, 38) the idea of strain involvement in the generation of lung damage. However, it is difficult to define safe limits for mechanical ventilation without correlations having been demonstrated between stress/strain and morphological damage and possibly inflammatory response.

The use of a single slice to infer the behavior of the entire lung has been questioned in different occasions. However, in this study we are not trying to extend the quantification of stress and strain to the remaining lung. We are confirming a qualitative knowledge of lung mechanics: the lung is inherently inhomogeneous. The CEN located below the heart and resting on the spine presents a different mechanical behavior from the isogravitational MID during static conditions. It consists of a less strained parenchyma at the same ΔPtp than the parenchyma in the MID. This can be the effect of a regional perturbation. The heart is located at the top of the CEN: by its weight force, it interacts with lung inflation (2, 16, 20) although this interference is not strictly limited to the parenchyma below it (4). Also, the main vascular structures embedded in the lung parenchyma appearing in the CEN can locally alter lung inflation, modifying the isotropic expansion of the CEN (18). The present study was not designed to quantify this last effect. Moreover, analyzing the CT scans coming from more caudal planes, we observed that the top of the diaphragm dome corresponded to the CEN. This might suggest that pressure transmission from the abdominal side through a paralyzed diaphragm could have played a role (13).

### Dynamic Conditions

The initial analysis of equality of slice volumes was performed because we wanted to check for any effect attributable to dynamic hyperinflation (at end expiration) or to erroneous volume setup on the ventilator (at end inspiration). This would have made comparisons between ventilator modes and body positions more difficult. However, volumes were similar when comparing modes and positions and thus allowed us to proceed with our analysis.

**Effect of ROI location.** The different ROIs, during the same pattern of breathing, are characterized in supine posture, by an inhomogeneous behavior. This is more evident during VCV, when NDP, MID and CEN, and DEP regions exhibit statistically different end-inspiratory strain and intra-tidal strain differences. During PCV, the difference between NDP and DEP persists; however, the difference is smaller and it is not possible to detect a statistical difference with the two MIDs. In prone position, no significant differences were seen.

This result confirms our finding obtained in static conditions that even in healthy conditions the supine lung is inherently inhomogeneous. Notably, the area under the heart (CEN) and MID show the same strain during ongoing mechanical ventilation, while in static conditions they reveal different stress-to-strain curves (Fig. 3). This may be explained by two possible conditions: 1) the two ROIs are exposed to a too low stress in dynamic conditions to elicit a significant departure between the two stress-to-strain curves, or 2) the partition of inflation volume in CEN and MID (higher in MID) is compensated by the lower FRC of CEN. Physiologically speaking, this can result by more favorable flow resistive properties of MID (accepting higher volumes) and the compressive effects of the heart on the CEN that lowers the regional FRC. Prone position attenuates inhomogeneity and brings the mechanical behavior of the ROIs closer, confirming the results found in static conditions.

**Effect of ventilatory pattern.** The application of the different ventilatory patterns, maintaining the same posture and referring to the same ROI, did not show any statistically significant difference in EIS, EIDS, and EI-EE. A tendency towards lowering EIS in PCV could be noticed on the plot (Fig. 5); however, it did not reach significance. This means that each ROI gets the same amount of volume when ventilated in VCV or in PCV. Considering that in our setup the difference between VCV and PCV is the airway flow profile, we could reason that at regional level it does not affect ventilation distribution at end inspiration. To our knowledge, the impact of ventilatory mode on lung strain at subsectional level of the lung has not been addressed before. However, studies have been done on global and regional ventilation distribution. Thus Prella et al. (25) found a more homogeneous distribution of ventilation during PCV than VCV in acute lung injury patients, keeping timing and delivered volumes constant in the two patterns. However, they worked in static conditions and took CT scans at end expiration: the slightly more even distribution of ventilation with PCV was only seen inside a lung-apical slice. Edibam et al. (8) compared VCV and PCV with an identical 1:2 I:E ratio in a CT study of five patients and did not find any difference in voxel HU distribution of transverse lung slices. Roth et al. (29) studied the whole lung volume in terms of HU profile by electron beam tomography and did not find...
any difference between VCV and PCV, except for a tendency to increase overinflated compartments during PCV.

**Effect of posture.** Our results show that posture has an impact on strain distribution during ongoing mechanical ventilation. When turning to prone position during VCV, EIS and EI-EE are increased in NDPs and lowered in the DEPs. The net effect of this shift is the reduction of the maximal between-ROI strain (EIDS), thus supporting the idea that prone positioning induces a more homogeneous distribution of regional strain. In PCV, although there is a clear tendency towards an analogous result (Fig. 5), the magnitude of the effect did not reach statistical significance.

Valenza et al. (38) dealt with the effect of prone positioning on lung strain during mechanical ventilation. They estimated lung strain by its length, width, and height from five rats undergoing CT scans. In line with our results, they inferred a more homogeneous strain distribution in prone position. However, differently from us, they did not use CT regional analysis for strain estimation and according to the technical specifications reported, CT exposures were performed in static conditions. On the contrary, our estimation of end-inspiratory and end-expiratory strain data were picked from continuous exposure during uninterrupted mechanical ventilation. Our findings support the results of the experiments performed by Broccard et al. (5): they demonstrated in an animal model that the prone position created the conditions for a less severe and more homogeneous distribution of lung injury due to the mechanical forces developed by artificial ventilation.

**Perspective.** The focus on the impact of mechanical ventilation on the lung has progressively moved from the injured to the healthy lungs (32), also in consideration of the number of procedures in general anesthesia and the number of patients with healthy lungs undergoing MV per year. Applying the study scheme of comparing groups ventilated at “high” vs. “low” tidal volumes and measuring inflammation markers, different prospective studies have been published. Some reports did not show any difference in inflammatory mediators release between high and low tidal volumes (17, 45, 46) during surgery, while others did (7, 26, 44, 48). The clinical problem is whether high strains entail a lung injury. Several studies (41) have been performed in this area of research. Each level of resolution has limitations bound to the instruments used for the study. A notable example is that initial inflation maneuvers can generate a lower than expected cell stretch (36), supporting the idea that in rat lungs the process of inflation involves an initial phase of septal unfolding. As a consequence, if the lung is studied using methods based on volume estimation, the calculated strain could be higher than at the cellular level. The conclusion is that macroscopic (spirometry and CT methods) and microscopic strains are coupled as soon as cells are put in tension. The interplaying roles by amplitude, frequency, and basal stretch and its application time (preconditioning) have been overviewed and brought to results compatible to clinical evidence (9, 37, 40). By this reason, we chose to include in our analysis not only the end-inspiratory strain but also its difference to the end-expiratory strain (EI-EE), which estimates the entity of deformation due to respiratory cycling. Moreover in our study we have introduced the concept of strain gradient (EIDS) between lung ROIs. This value depends on the difference of ROI mechanical properties and it is related to pulmonary inhomogeneity. It measures the difference in expansion (referred to the equilibrium points, i.e., regional FRC) of the different ROIs and yields an estimation of strain inhomogeneity.

**Technical Issues and Limitations**

The calculation of strain has been based on changes in radio-opacities, and this can be the effect of an increase of gas or a decrease of tissue content.

Our estimation of strain is based on the assumption of conservation of tissue mass. In other words, we hypothesized as in Fuld et al. (11) that the volume changes seen at the CT are due to the increase in gas volume and that this volume distributes uniformly inside the studied ROI. Under this assumption, Fuld et al. demonstrated that sVol (the change in lung gas volume normalized by the initial gas volume) depends directly on CT density with no necessity of further correction for tissue. Nevertheless, conservation of tissue mass may not be achieved, since during ventilation there can be a shift of blood between the lung and surrounding tissues. However, the approach by Fuld et al. correlated well with the methods based on Xe-CT and implanted markers for measuring regional specific ventilation.

The number of alveoli populating one voxel is presumably dissimilar between NDPs (lower number) and DEPs (higher number), because of their different dimensions. However, in one ROI a certain number of alveoli will share the gas volume at FRC and then the gas volume of the tidal volume. With the strain being a ratio between the increment in volume and the FRC, the effect of alveolar dimensions in the same ROI can be considered negligible. This results from the fact that in the present study the ROIs were defined to have a lower width on the gravitational axis than on the horizontal one, so including a population of alveoli presenting homogeneous diameters.

The fixed design (prone following supine position) may be considered a limitation of the present study. The reason of this choice has been the attempt of simulating the same sequence that is usually performed in clinical practice, when a supine ventilated patient is put prone.

We decided to fit a two-parameter exponential model to the data coming from the ROIs of pooled data and from single animals. Although we observed a strong exponential relation, it cannot be excluded that other curvilinear functions could have exhibited a good quality of fit on the same data sets.

We chose to study a single plane, defined by its projection on the CT bed. A potential drawback of this choice is that the lung moves through this plane when increasing volumes are delivered. However, our study is centered on the analysis of the NDP, MID, CEN, and DEP in the same slice and at the same time and so a potential bias (if any) affects them simultaneously. Moreover, the “movement” of the lung, on the z-axis passing from FRC to IC, in our model is of 20 mm and the average step change between consecutive volumes is 1.6 mm. We considered this movement not affecting the results of the present study as previously reported by Zinserling et al. (47).

It may be hypothesized that the movement of diaphragm during respiration, creating a shift from cranial to caudal regions of “nonair” content, can locally overwhelm the effect of gas entering the ROI and confound the interpretation of data. In the Supplemental Data, we report the illustration of the
absolute contents of gas and tissue at the different postures and modalities of ventilation, at end inspiration and end expiration. In all conditions, it is possible to notice that the quantity of gas entering the slice is higher than the apparent loss of tissue. Moreover, in none of the slices included in the analysis of respiratory sequences the diaphragm muscle was present. In line with Appelberg et al. (1), we did not find any difference in behavior to inflation for short distances between the analyzed slice and the ones at a shorter distance to the diaphragm.

The definition of the ROIs has been based on anatomical landmarks. Considering that the lung modifies its dimensions also on the transverse plane, any calculation of volumes would have been affected by the increment of any parenchymal component of the slice. By this reason, we chose to weight each volume measurement for the number of voxel composing the ROIs.

The dynamic patterns to which the animal was exposed, have a timing conditioned by the rate of CT exposures available on our equipment. Therefore, we decided to have a longer inspiration than the baseline ventilation used during preparation to collect the highest number of samples per breath. It may be possible that a higher RR would alter the results and the differences in stress and strain between the ROIs.

The Ptp as it is measured by airway and esophageal pressure, is a sort of weighted mean of the transpulmonary pressures acting on the lungs. Our experimental setup did not allow to measure regional transpulmonary pressure directly. On the other hand we have used the ΔPtp: referencing Pes to its end-expiratory value would eliminate all the artifacts that equally affect pressure at end inspiration and end expiration. However, a limit to this approach is that it cannot compensate measurement errors by any change in the vertical pleural pressure gradient by the inflation of the lung or any change in mediastinal compression on the esophagus by such inflation. We have to assume that such errors are small.

The position of the oesophageal balloon was checked by CT to be in the closest proximity of the slice to be analyzed. However, the oesophagus is located (see Fig. 2) at the border between the MID and CEN with DEP. If we admit that pleural pressure has regional differences (21) along the vertical axis mainly for gravitational causes, we can reason that in the DEP the ΔPtp can be lower than measured (for a higher pleural pressure); for an analog reason in the NDP, the ΔPtp can be higher than measured. In a stress-to-strain plot, this vertical shift in opposite direction of the regional curves enlarges the gap between the curves and enhances the finding of inhomogeneity.

Conclusions

It can be concluded that strain is nonuniformly distributed in the mechanically ventilated, healthy lung and that the stress-to-strain relationship is well described by exponential models. Prone positioning attenuates the differences between dependent and nondependent lung regions. Ventilation in volume or pressure control mode showed no essential differences. The regional effects of body positioning and ventilatory mode should be further explored in patients with acute lung injury.

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DISCLOSURES

No conflicts of interest are declared by the authors.

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