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Regional Lung Derecruitment and Inflammation during 16 Hours of Mechanical Ventilation in Supine Healthy Sheep

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Abstract

Background—Lung derecruitment is common during general anesthesia. Mechanical ventilation with physiological tidal volumes could magnify derecruitment, and produce lung dysfunction and inflammation. We used Positron Emission Tomography to study the process of derecruitment in normal lungs ventilated for 16 h, and the corresponding changes in regional lung perfusion and inflammation.

Methods—Six anesthetized supine sheep were ventilated with $V_T = 8\text{ mL/kg}$ and positive end-expiratory pressure = 0. Transmission scans were obtained at 2-h intervals to assess regional aeration. Emission scans were acquired at baseline and after 16 h for the tracers: (a) ^{18}F -fluorodeoxyglucose to evaluate lung inflammation, and (b) ^{13}N to calculate regional perfusion and shunt fraction.

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Results—Gas fraction decreased from baseline to 16 h in dorsal (0.31 ± 0.13 to 0.14 ± 0.12 , $P < 0.01$) but not ventral regions (0.61 ± 0.03 to 0.63 ± 0.07 , $P = \text{NS}$), with time constants of 1.5–44.6 h. Whereas the vertical distribution of relative perfusion did not change from baseline to 16 h, shunt increased in dorsal regions (0.34 ± 0.23 to 0.63 ± 0.35 , $P < 0.01$). The average pulmonary net ^{18}F -fluorodeoxyglucose uptake rate in six regions of interest along the ventral-dorsal direction increased from $3.4 \pm 1.4 \cdot 10^{-3} \text{min}^{-1}$ at baseline to $4.1 \pm 1.5 \cdot 10^{-3} \text{min}^{-1}$ after 16 h ($P < 0.01$), and the corresponding average regions of interest ^{18}F -fluorodeoxyglucose phosphorylation rate increased from $2.0 \pm 0.2 \cdot 10^{-2} \text{min}^{-1}$ to $2.5 \pm 0.2 \cdot 10^{-2} \text{min}^{-1}$ ($P < 0.01$).

Conclusions—In normal lungs mechanically ventilated without positive end-expiratory pressure, loss of aeration occurs continuously for several hours and is preferentially localized to dorsal regions. Progressive lung derecruitment was associated with increased regional shunt, implying an insufficient hypoxic pulmonary vasoconstriction. The increased pulmonary net uptake and phosphorylation rates of ^{18}F -fluorodeoxyglucose suggest incipient inflammation in these initially normal lungs.

Introduction

In normal lungs, induction of anesthesia and muscle paralysis produce lung derecruitment with significant atelectasis.¹ These are associated with functional deterioration such as decreased lung compliance, impairment of oxygenation, increased pulmonary vascular resistance, and, potentially, the development of lung injury and pulmonary infections.^{2,3}

It is generally accepted that derecruitment develops within minutes after induction of general anesthesia with muscular relaxation.¹ However, it is unclear if and how fast derecruitment progresses after establishment of mechanical ventilation in normal lungs. Indeed, Cai *et al.* reported no change in non-aerated regions up to 7 h after mechanical ventilation with pure oxygen and zero positive end-expiratory pressure (PEEP) in patients with healthy lungs.⁴ In contrast, others reported a progressive increase in nonaerated lung after 40–90 min of mechanical ventilation with or without pure oxygen and zero PEEP in patients with normal lungs undergoing neuro or general surgery.^{3,5,6}

There is increasing awareness about the risks of lung injury associated with high tidal volumes, and physiological tidal volumes (6–8 ml/kg) have been proposed for mechanical ventilation in the operating room and intensive care unit.^{7–9} This practice, however, raises the concern that lung derecruitment could be further facilitated, inducing hypoxemia and inflammation, especially in the absence of an associated PEEP.^{2,10} Indeed, experimental studies suggested that mechanical ventilation of the normal lung with low tidal volumes and zero PEEP could lead to stress-related damage to endothelial and alveolar epithelial cells.¹¹ Consequently, it is important to determine whether lung derecruitment is a progressive process leading to activation of potentially injurious mechanisms in normal lungs ventilated with physiological tidal volumes. Because time is a key variable determining the magnitude of lung injury resulting from mechanical ventilation,¹² that process could be particularly relevant during periods corresponding to long surgeries.

Accordingly, we used multitracer Positron Emission Tomography (PET) imaging to investigate progressive derecruitment during prolonged (16 h) mechanical ventilation by

quantifying regional lung aeration and the corresponding changes in ^{18}F -fluorodeoxyglucose (^{18}F -FDG) uptake, a measure of inflammatory cell metabolic activation.¹³⁻¹⁵ We also measured regional perfusion because its topographical distribution could be affected by the development of atelectasis and also modulate the development of regional inflammation.¹³ Better understanding of the dynamics of regional lung derecruitment and its relationship to regional perfusion and inflammation could help to optimize the application of strategies such as PEEP and recruitment maneuvers aimed at minimizing lung injury and maximizing gas exchange during mechanical ventilation of normal lungs. In this study, we ventilated sheep for 16 h with $V_T = 8 \text{ mL/kg}$ and zero PEEP to reflect the use of physiological tidal volumes and the practice of ventilating anesthetized patients without PEEP, which remains prevalent.^{16,17}

Materials and Methods

All animal experiments were performed in compliance with institutional guidelines approved by the Subcommittee on Research Animal Care at the Massachusetts General Hospital and in accordance with the “Guide for the Care and Use of Laboratory Animals” published by the National Institutes of Health (publ. no. 86-23, revised 1996).

Experimental Protocol

Six sheep ($22.6 \pm 2.4 \text{ kg}$) age 3-4 months old were anesthetized, intubated and mechanically ventilated (Ohmeda 7800 ventilator, Datex-Ohmeda, Madison, WI). Anesthesia was maintained with a continuous infusion of ketamine and propofol titrated to heart rate and blood pressure. Paralysis was established with a bolus of pancuronium (0.1 mg/kg) at induction and repeated every 90 min ($0.02\text{-}0.04 \text{ mg/kg}$). For monitoring and collection of blood samples, we cannulated one femoral artery and introduced a pulmonary artery catheter *via* the right external jugular vein.

The animals were placed supine in the PET scanner (Scanditronix PC4096; General Electric, Milwaukee, WI) with the caudal end of the field of view just superior to the dome of the diaphragm. Final positioning was determined after a recruitment maneuver with airway pressure held at $40 \text{ cmH}_2\text{O}$ for 30 s. Sheep were mechanically ventilated for 16 h with a volume control mode, $V_T = 8 \text{ mL/kg}$, PEEP = 0, inspired oxygen fraction (FiO_2) 0.3 (adjusted to maintain an arterial oxygen saturation 90% measured by coximetry (OSM3, Radiometer, Copenhagen, Denmark), inspiratory-to-expiratory ratio = 1:2, initial respiratory rate of 8 breaths/min and adjusted to maintain the arterial carbon dioxide partial pressure (PaCO_2) between 32 and 45 mmHg. Physiological data were collected at baseline and at 4-h intervals. At the end of the study, the animals were euthanized by intravenous bolus injection of potassium chloride.

PET Imaging Protocol and Processing

The experimental system and methods of analysis have been previously presented in detail.^{15,18-20} The PET camera collected 15 transverse slices of 6.5-mm thickness providing 3-dimensional information over a 9.7-cm-long field of view, which we previously estimated to encompass ~70% of the total sheep lung volume. Resulting images consisted of an

interpolated matrix of $128 \times 128 \times 15$ voxels with a size of $2.0 \times 2.0 \times 6.5$ mm each. Three different types of PET images were acquired:

1. **Transmission scans:** using a rotating pin-source of ^{68}Ge for 10 min²⁰ were obtained at baseline and 2-h intervals, resulting in nine scans for each animal. From these, the following variables were calculated:

- a. Gas fraction (F_{gas})²⁰: calculated for the whole lung and for six coronal regions of interest (ROIs) of equal heights along the ventral-dorsal axis (ROI1 was the most ventral and ROI6 the most dorsal) for all nine scans. The squared coefficient of variation ($CV^2 = [SD/\text{mean}]^2$) was used to quantify voxel-by-voxel the heterogeneity of F_{gas} in the whole imaged lung.²¹
- b. Relative nonaerated lung volume defined as the percentage of the total lung volume with $F_{\text{gas}} < 0.1$.²² $\text{Nonaerated}_{\text{volume}}$ was calculated as:²³

$$\text{Nonaerated}_{\text{volume}} = \frac{\text{number nonaerated voxels}}{\text{total number of voxels}} \cdot 100$$

- c. Relative nonaerated lung mass as a percentage of total lung mass ($\text{Nonaerated}_{\text{mass}}$) was calculated as the sum of the densities ($D = 1 - F_{\text{gas}}$) of all nonaerated voxels divided by the sum of the densities of all voxels within the field of view:²³

$$\text{Nonaerated}_{\text{mass}} = \frac{\sum_{i=1, \text{non-aerated voxels}} D_i}{\sum_{i=1, \text{all voxels}} D_i} \cdot 100$$

- d. Regional time constant for regional loss of aeration: to quantify the rate of regional derecruitment, regional F_{gas} was fitted with an exponential function:

$$F_{\text{gas}}(t) = F_{\text{gas}}(\text{baseline}) \cdot e^{-t/\tau} + F_{\text{gas}}(\text{end})$$

where $F_{\text{gas}}(t)$ is the regional measurement at time t , $F_{\text{gas}}(\text{baseline})$ is the F_{gas} value at baseline (after recruitment maneuver), $F_{\text{gas}}(\text{end})$ is the asymptotic F_{gas} value, and τ is the time constant of F_{gas} decrease over time (*i.e.*, time required for approximately 63% decrease in F_{gas}). Visual inspection of the time course of F_{gas} revealed little temporal variation in coronal ROIs 1-4. We therefore calculated τ only for ROIs 5 and 6.

2. **Emission scans after intravenous ^{13}NN -saline:** performed at baseline and at the end of the 16-h mechanical ventilation period. Analysis of the lung tracer kinetics of ^{13}NN following an intravenous injection of ^{13}NN -saline during a 60-s apnea at

mean lung volume was used to compute voxel level perfusion²⁰ and allowed for the measurement of:

- a. Relative pulmonary perfusion: relative perfusion to each of the six ROIs was computed as the peak regional ^{13}NN activity (mean of all voxels of the ROI) measured after the ^{13}NN bolus injection divided by the sum of the peak activities of all ROIs.
 - b. Perfusion heterogeneity: measured on a voxel-by-voxel basis using squared coefficients of variation of perfusion ($\text{CV}^2_{\text{perf}}$) and of density-normalized perfusion ($\text{CV}^2_{\text{perf}/D}$) computed by voxel-by-voxel division of perfusion by the respective density.
 - c. Regional pulmonary shunt: shunt in each ROI was derived by modeling the kinetics of ^{13}NN .^{19,24}
3. **Emission scans after intravenous ^{18}F -FDG:** obtained for quantification of regional ^{18}F -FDG kinetics. After ^{13}NN clearance, ^{18}F -FDG (~ 5 mCi) was infused at a constant rate through the jugular catheter over 60 s. Starting at the beginning of ^{18}F -FDG infusion, sequential PET frames ($9 \times 10\text{s}$, $4 \times 15\text{s}$, $1 \times 30\text{s}$, $7 \times 60\text{s}$, $15 \times 120\text{s}$, $1 \times 300\text{s}$, $3 \times 600\text{s}$) were acquired over 75 min while pulmonary arterial blood was sampled at 5.5, 9.5, 25, 37, and 42.5 min.¹³ The ^{18}F -FDG kinetics in each ROI was fitted to a three-compartment model consisting of an intravascular compartment, a precursor compartment representing the concentration of ^{18}F -FDG available for phosphorylation (*i.e.*, the concentration of ^{18}F -FDG that can serve as a substrate for hexokinase), and a metabolized compartment accounting for the concentration of ^{18}F -FDG that has been phosphorylated by hexokinase.^{25,26} In this analysis, the ^{18}F -FDG net uptake rate (K_i), a measure of cellular metabolic activity, is expressed as: $K_i = F_e \cdot k_3$; the constant k_3 is the rate of ^{18}F -FDG phosphorylation, proportional to hexokinase activity, and F_e is the fraction of the ROI occupied by the precursor compartment.^{25,27} K_i , k_3 , and F_e were calculated for each of the six coronal ROIs.

Selection of Voxels for Analysis

For each animal, two lung masks were created: one at baseline and another at 16 h. The masks were created using a semiautomatic method. An initial mask was generated by automatic threshold selection combining aerated regions from transmission scans and nonaerated perfused regions from ^{13}NN perfusion scans. This mask was manually refined to exclude areas corresponding to main bronchi and large pulmonary vessels.

For the analysis of temporal changes in aeration, a single mask was created containing voxels present both at baseline and 16 h (*i.e.*, this mask represented the “intersection” of the baseline and 16 h masks).

Blood and lung neutrophil counts, and histological examination

After euthanasia, the lungs of four animals were excised and filled with Trump's fixative to a pressure of 25 cmH₂O. After fixation, a block of lung tissue was sampled from the ventral

and dorsal regions and embedded in paraffin. Sections of 5 μm were cut, mounted and stained with hematoxylin and eosin for light microscopy. Alveolar and septal neutrophils were counted in 10 randomly selected high-power (400x) fields per slide (one ventral and one dorsal slide per animal) by a reader blinded to the sampled tissue identification. In addition, alveolar edema, alveolar hemorrhage, septal thickening, and capillary congestion were evaluated semiquantitatively with a modified four-grade scale (absent = 0, mild = 1, moderate = 2, and marked = 3).²⁸ Leukocyte count was measured at the start and end of the protocol in five animals.

Statistical Analysis

Variables were tested for normality with the Shapiro-Wilk test. Data are expressed as median and interquartile interval (IQR 25, 75%) or as mean $\pm$ standard deviation. We compared physiological values before and after 16 h of mechanical ventilation with paired Student *t*-test when data were normally distributed or with Wilcoxon's signed-rank test when data were not normally distributed. PET-acquired data (F_{gas} , perfusion, shunt, and ^{18}F -FDG variables) were compared at two time points (baseline and 16 h) and among the six ROIs with a linear mixed-effect model. The categorical variables time and ROI and their interaction (when significant) were modeled as fixed effects, and the variation among individuals for each variable was modeled with random coefficients for the intercepts. Bonferroni *post-hoc* tests were performed when the overall *P* was less than 0.05. Multivariate regression for the six animals at the nine time points was used to study the dependence of relative nonaerated lung mass on FIO_2 and time points. Analyses were performed using SPSS version 13 (SPSS, Chicago, IL). All statistical tests were two-tailed, and significance was set at $P < 0.05$.

Results

Physiologic variables at baseline and after 16 h of mechanical ventilation

Peak airway pressure increased, while dynamic compliance, $\text{PaO}_2/\text{FIO}_2$ ratio, and pH decreased from baseline to 16 h (table 1). Respiratory rate, PaCO_2 , and cardiovascular variables were stable in the same period (table 1).

Regional lung aeration

At baseline, there was a heterogeneous vertical distribution of F_{gas} ($P < 0.001$, fig. 1). Mean F_{gas} in ventral ROIs were within the normal range ($0.5 < F_{\text{gas}} < 0.9$), whereas dorsal ROIs (ROIs 5-6) had mean F_{gas} below 0.5, compatible with poor aeration. There was a significant change in the regional distribution of F_{gas} following 16 h of mechanical ventilation ($P < 0.01$). Whereas ventral regions (ROIs 1-2) presented minimal changes in F_{gas} over time (0.61 ± 0.03 vs. 0.63 ± 0.07 , $P = \text{NS}$), dorsal regions (ROIs 5-6) showed significant reduction of F_{gas} (0.31 ± 0.13 vs. 0.14 ± 0.12 , $P < 0.01$) (figs. 1 and 2). The decrease in F_{gas} with time in ROIs 5 and 6 had time constants of 24.0 h (IQR 14.9, 35.7h) and 10.7 h (IQR 4.3, 11.3h), respectively (fig. 3). There was a significant increase in spatial heterogeneity of F_{gas} ($\text{CV}^2_{F_{\text{gas}}}$) from baseline to 16 h (table 2).

In line with the changes in F_{gas} , nonaerated lung volume increased after 16 h (0.8% IQR 0.2, 3.5 to 12.6% IQR 11.2, 17.8; $P = 0.03$, fig. 4A), as did nonaerated lung mass (1.5 IQR 0.3, 5.8 % to 19.2 IQR 18.1, 26.1%; $P = 0.03$, fig. 4B). In five of the six animals, nonaerated lung comprised >3% of the lung volume throughout the protocol (fig. 4). Multivariate regression showed a significant dependence of nonaerated lung mass on FIO_2 ($P < 0.05$).

Regional lung perfusion and regional shunt

At baseline and following 16 h of mechanical ventilation, relative perfusion was lowest in the most ventral regions and increased progressively along the ventral-dorsal axis to reach a plateau at ROIs 4 to 6 (fig. 5). Relative perfusion to each ROI was not significantly redistributed after 16 h of mechanical ventilation ($P = 0.99$, fig. 2C and 5), even though the spatial heterogeneity of perfusion tended to increase over time, irrespective of whether this heterogeneity was calculated with ($P = 0.09$) or without ($P = 0.10$) normalization of perfusion by voxel-by-voxel tissue density (table 2).

At baseline, regional shunt fractions were negligible in ventral regions (ROIs 1-2) but increased progressively towards dorsal regions (ROIs 5-6, fig. 6) (0.34 ± 0.23 vs. 0.63 ± 0.35 , $P < 0.01$). After 16 h, global shunt fraction was markedly higher than at baseline (table 2) due to a significant increase in shunt in dorsal regions (ROIs 5-6, fig. 6).

Regional ^{18}F -FDG kinetics and neutrophilic inflammation

At baseline and after 16 h of mechanical ventilation, K_i had a heterogeneous vertical distribution with dorsal regions (ROIs 5-6, fig. 2D and 7) showing higher values than ventral regions. The regional distribution of the volume of the precursor compartment (Fe) paralleled the distribution of K_i , while k_3 was homogeneously distributed along the vertical axis (fig. 7).

After 16 h of mechanical ventilation, K_i was significantly increased ($P < 0.01$, fig. 7). This increase was homogeneous in all ROIs as reflected by the nonsignificant interaction term (fig. 7) and was related to an increase in k_3 ($P < 0.01$), without change in Fe ($P = 0.65$, fig 7). Average ROI K_i increased from $3.4 \pm 1.4 \cdot 10^{-3} \text{min}^{-1}$ at baseline to $4.1 \pm 1.5 \cdot 10^{-3} \text{min}^{-1}$ after 16 h ($P < 0.01$), and the corresponding average ROI k_3 increased from $2.0 \pm 0.2 \cdot 10^{-2} \text{min}^{-1}$ to $2.5 \pm 0.2 \cdot 10^{-2} \text{min}^{-1}$ ($P < 0.01$).

Histology

Blood neutrophil counts more than doubled from baseline to the end of 16 h of mechanical ventilation (table 1). Lung neutrophil counts were low and not significantly different between ventral (4.2 neutrophils/field IQR 2.8, 6.7) and dorsal regions (6.7 IQR 5.3, 7.6) at the end of the protocol. Histological analysis revealed mild septal edema and capillary congestion in dorsal regions in a predominantly normal parenchyma with no alveolar edema or hemorrhage.

Discussion

The main findings of this study of prolonged mechanical ventilation with physiological tidal volumes and zero PEEP in supine healthy sheep were: 1) loss of lung aeration with corresponding increase in volume and mass of nonaerated lung occurred progressively along 16 h of mechanical ventilation. Time constants suggested the continuation of lung derecruitment beyond that period in some animals; 2) the spatial distribution of the loss of aeration was heterogeneous, with dorsal regions accounting for most of the aeration loss; 3) shunt in dorsal regions increased with time whereas regional relative perfusion did not change after 16 h despite ongoing atelectasis in dorsal regions. Thus, hypoxic pulmonary vasoconstriction was not effective enough to avoid the increase in shunt and deterioration of gas exchange; 4) there was an increase in the net ^{18}F -FDG uptake at 16 h, predominantly due to increase in the rate constant k_3 , which describes ^{18}F -FDG phosphorylation. Despite this increase, regional net ^{18}F -FDG uptake values at 16h were still within a range compatible with values measured in normal lung regions.^{13,15,29}

The chosen V_T was consistent with current recommendations for normal lungs.¹⁰ We used PEEP = 0 because use of no or minimal PEEP still represents a prevalent practice in the operating room^{16,17}, even though such approach differs from current recommendations.¹⁰

We observed marked topographical heterogeneity in the process of aeration loss. Atelectasis during anesthesia is commonly understood as due to a fall in functional residual capacity, associated airway closure, and reabsorption atelectasis.^{3,5,30} Anesthetized mechanically ventilated patients with normal lungs present a vertical gradient in lung aeration produced by the gradient in local transpulmonary pressures.³¹ However, it is unknown how this aeration gradient changes over long periods of mechanical ventilation. We showed progressive dorsal lung derecruitment, contrasting with no change in ventral aeration over 16 h. If those aeration gradients were to depend exclusively on vertical position, they would not be expected to change with time. Thus, our results indicate that time-dependent factors play a key role in determining the vertical distribution of aeration in normal lungs.

There is conflicting data on temporal changes in lung derecruitment following initiation of mechanical ventilation. While some reported no additional derecruitment once aeration loss was established,^{1,4,32} others indicated progressive derecruitment.^{3,5,6} Our measurements revealed large time constants for dorsal derecruitment (1.5-44.6 h), a finding that contrasts with previous results suggesting rapid stabilization.^{1,33} The observed increased peak airway pressure and decreased dynamic compliance and $\text{PaO}_2/\text{FIO}_2$ from baseline to 16 h reinforce the functional relevance of that progressive derecruitment. Previous results were mostly based on short periods (14-20 min)^{1,33}. A study on supine neurosurgical patients did not show significant derecruitment after 7 h.⁴ The differences between our findings and those studies may result from our longer period of observation. Our findings concur with the progressive loss of aeration observed in patients after 40-90 min of mechanical ventilation with PEEP = 0.^{3,5,6} The measured derecruitment time constants further indicate that derecruitment may continue for hours to days. This knowledge is of clinical importance because it shows that progressive lung derecruitment, rather than deterioration of the lung

condition, may explain day-to-day falls in oxygenation or changes in radiographic presentation.

At least three mechanisms could explain the observed regional lung derecruitment: compression atelectasis,² absorption atelectasis,² and airway-parenchymal interdependence.^{34,35} Although compression atelectasis would explain the initial loss of aeration, it cannot entirely account for the results since it develops within minutes.^{36,37} The fact that we found an association between FIO_2 and amount of nonaerated tissue is consistent with a contribution of absorption atelectasis to derecruitment. However, given the study design, derecruitment could have prompted the increases in FIO_2 to maintain the target oxygen saturation. Our measured derecruitment time constants are consistent with theoretical time constants during gas absorption due to complete or partial airway closure.^{30,38} Finally, airway-parenchymal interdependence could be an additional contributing factor as parenchymal tethering is reduced at low lung volumes,^{34,35} resulting in airway closure and lung derecruitment. Muscle relaxants³⁹ and propofol⁴⁰, used in this study, reduce functional residual capacity by facilitating some of the discussed mechanisms.

At baseline, perfusion was distributed predominantly to middle and dorsal lung, even after normalization by tissue density (fig. 2B and 2C). Following 16 h of mechanical ventilation, we did not observe a significant redistribution of perfusion, despite the loss of aeration in dorsal regions, even though spatial heterogeneity of perfusion tended to increase (table 2). Such results suggest a trend to increased nongravitational heterogeneity of perfusion. They also contradict the expectation that perfusion would substantially decrease in regions of reduced aeration due to intact hypoxic pulmonary vasoconstriction in the normal lung.⁴¹ Instead, we found increased regional shunt in dorsal regions (fig. 6) and deterioration in global gas exchange (increased global shunt, table 2). This finding confirms that dorsal regions contribute significantly to gas exchange dysfunction and that hypoxic pulmonary vasoconstriction was insufficient to avoid the increase in shunt.

Several factors could explain the absence of perfusion redistribution in the vertical direction: (1) overdistension of the aerated lung diverting blood flow to nonaerated regions,^{18,42} resulting from a fixed V_T applied in the presence of substantial nonaerated lung; (2) inhibition of hypoxic pulmonary vasoconstriction due to early inflammation;⁴³ (3) attenuation of hypoxic pulmonary vasoconstriction by high mixed venous oxygen tension.⁴⁴ Further investigation will be required to determine those mechanisms.

K_i and k_3 were significantly increased following 16 h of mechanical ventilation, compatible with neutrophil activation in the early stages of lung injury.¹³⁻¹⁵ Increases in K_i ¹⁵ and k_3 (representing the ^{18}F -FDG rate of phosphorylation)⁴⁵ have been used to measure increases in cell activity and inflammation. Thus, they could be indicative of a preinjury condition. The changes in k_3 , suggestive of increased metabolic activity per cell, explain most of the changes in K_i . The absence of histological signs of significant damage, compatible with the absence of temporal changes in F_e , reinforces the interpretation that the increase in K_i and k_3 are possible early events in the pathogenesis of ventilator-induced lung injury, suggestive of a mild and incipient inflammatory process. Indeed, such incipient inflammation is likely

reversible⁴⁶ but could progress if the lungs are ventilated for longer periods of time⁹ or in the presence of a second injurious stimulus.¹³

Values of K_i both at baseline and after 16h of mechanical ventilation were within the range found previously in control lungs of healthy sheep.^{13,15,29} Consequently, they conflict with previous estimates of concentration of stress in derecruited normal lung.⁴⁷ The absence of a regionalized increase in k_3 could suggest that the increase in K_i after 16h was either not associated to injurious factors – ventral hyperinflation and dorsal low-volume injury - or that these factors resulted in similar consequences to inflammation. Another possibility is that the increase in ^{18}F -FDG uptake rate represents a broader proinflammatory effect caused by mild cyclical stretching of macrophages and epithelial cells.^{48,49} It is tempting to hypothesize that such metabolic changes could be modified by interventions such as PEEP, recruitment maneuvers, and prone position. Indeed, a recent study showed improved lung function and reduced pulmonary infection scores when a protective ventilatory strategy predominantly based on PEEP = 10 *versus* 0 cmH₂O was used intraoperatively during abdominal surgery.⁵⁰ Such findings are compatible with the current study's suggestion of incipient inflammation during ventilation at low lung volumes, even when tidal volumes are not exaggerated, and with the PEEP-induced reduction of lung mechanical injury at all length scales⁵¹.

Limitations of the imaging techniques have been discussed in detail.^{13,15,20,52} The field of view of the PET scanner encompassed approximately 70% of the lung,²⁰ omitting portions of the apex and base. The large amount of nonaerated tissue at 16h could be explained partially by species differences: we studied young sheep, which are known to have reduced collateral ventilation potentially facilitating reabsorption atelectasis.⁵³ Because peak instead of plateau pressures were measured, changes in airway resistance in addition to alveolar collapse could have contributed to the observed increases in airway pressure. Although no gas humidifier was used during the study, no endotracheal tube obstruction was noticed after inspection at the end of the experiment, suggesting that this was unlikely to have affected the measured peak pressures. Given that all animals received a muscle relaxant, our results may not directly apply to conditions in which muscle relaxants are not used such as intensive care unit patients. Additionally, factors such as presence of acute respiratory distress syndrome, sepsis, prone position, and PEEP importantly influence lung derecruitment and our results cannot be directly extrapolated to those cases.

In summary, lung derecruitment occurred as a continuous process along 16 h in supine sheep with previously healthy lungs mechanically ventilated with physiological tidal volume and zero PEEP. The increase in the amount of nonaerated lung in dorsal regions did not affect the distribution of relative perfusion, and was associated with an increase in regional shunt indicating an insufficient hypoxic pulmonary vasoconstriction. There was an increase in the net ^{18}F -FDG uptake at 16 h associated with an increase in the rate of ^{18}F -FDG phosphorylation, suggestive of incipient inflammation.

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Final Boxed Summary Statement

What we already know about this topic

- In normal lungs, induction of anesthesia and muscle paralysis produce lung derecruitment associated with functional deterioration and potentially lung injury and pulmonary infection
- Dynamics of regional lung derecruitment and its relationship to regional perfusion and inflammation remains poorly understood

What this article tells us that is new

- In normal sheep lungs mechanically ventilated (over 16 h, 8 mL/kg, zero positive end-expiratory pressure) progressive derecruitment is associated with increased regional shunt, implying insufficient hypoxic pulmonary vasoconstriction, and incipient inflammation

Fig. 1.

Mean regional gas fraction (F_{gas}) for all animals ($n = 6$) at baseline and after 16 h of mechanical ventilation in six coronal regions of interest (ROIs) of equal height along the ventral-dorsal axis (ROI 1 is the most ventral, and ROI 6, the most dorsal). Note that a significant decrease in gas fraction occurred only in dorsal regions (ROIs 5-6). * $P < 0.05$ (baseline vs. 16 h); # $P < 0.01$ (vs. previous ROI at baseline); ‡ $P < 0.05$ (vs. previous ROI at 16 h).

Fig. 2.

Images of (A) lung aeration, (B) perfusion, (C) density normalized perfusion, and (D) ^{18}F -FDG net uptake rate at the beginning (baseline) and end of the study (16 h) for a typical case. In this example, there is a decrease in gas fraction in dorsal regions from baseline to 16 h (A), associated with an increase in perfusion in the same regions (B). After normalization by density (lung tissue) (C), such increase in perfusion in dorsal regions was no longer visualized. ^{18}F -FDG uptake (D) increased from baseline to 16 h.

Fig. 3.

Time course of gas fraction (F_{gas}) in six coronal regions of interest (ROIs) of equal height along the ventral-dorsal axis (ROI 1 is the most ventral, and ROI 6, the most dorsal) during 16 h of mechanical ventilation for two animals with different time constants of dorsal lung derecruitment. Temporal changes in F_{gas} were not significant in nondependent regions. In contrast, dorsal regions (ROIs 5 and 6) showed systematic loss of aeration, with variability in time constants (τ): (A) Case of equal and long time constants in ROI 5 and 6. (B) Case with different and shorter time constants for the same ROIs. The dotted lines indicate the exponential fitting.

Fig. 4.

Amount of nonaerated lung (gas fraction less than 0.1) for all animals ($n = 6$) at baseline and at 16 h shown as percentage of lung volume (A) and percentage of lung tissue mass (B). Median (solid line) and interquartile range (gray box) are represented.

Fig. 5.

Regional relative perfusion *versus* six coronal regions of interest (ROIs) of equal heights along the ventral-dorsal axis (ROI 1 is the most ventral and ROI 6 the most dorsal) at baseline and after 16 h of mechanical ventilation presented for all animals ($n = 6$) # $P < 0.01$ (vs. previous ROI at baseline); ‡ $P < 0.05$ (vs. previous ROI at 16 h).

Fig. 6.

Regional shunt fraction at baseline and after 16 h for the six coronal regions of interest (ROIs) along the ventral-dorsal axis measured in all animals ($n = 6$) (ROI 1 is the most ventral, and ROI 6, the most dorsal). * $p < 0.05$ (baseline vs. 16 h); # $P < 0.01$ (vs. previous ROI at baseline); ‡ $P < 0.05$ (vs. previous ROI at 16 h).

Fig. 7.

Regional net ^{18}F -fluorodeoxyglucose uptake rate (K_i) (A), rate of ^{18}F -FDG trapping, proportional to hexokinase activity (k_3) (B), and fraction of lung volume occupied by the extravascular substrate compartment (F_e) (C) at baseline and after 16 h for all animals ($n = 6$) in six coronal regions of interest (ROIs) of equal height along the ventral-dorsal axis (ROI 1 is the most ventral, and ROI 6, the most dorsal). # $P < 0.01$ (vs. previous ROI at baseline); ‡ $P < 0.05$ (vs. previous ROI at 16 h).

Table 1
Global Physiological Variables at Baseline and 16 Hour

	Baseline	16 h
Mean arterial pressure (mmHg)	95 ± 4	88 ± 13
Mean pulmonary arterial pressure (mmHg)	11 ± 5	14 ± 7
Cardiac output (L/min)	5.4 ± 1.1	4.7 ± 0.9
Heart rate (beats/min)	154 ± 24	143 ± 39
PvO ₂ (mmHg)	39 ± 3	46 ± 6
Tidal volume (mL)	204 ± 28	198 ± 19
Tidal volume (mL/kg)	9.0 ± 1.1	8.8 ± 0.7
Respiratory rate (breaths/min)	26 ± 3	24 ± 3
Peak airway pressure (cmH ₂ O)	16.8 ± 5.7	26.0 ± 7.6 *
Dynamic compliance (mL/cmH ₂ O)	12.5 ± 3.2	7.82 ± 2.0 *
pH	7.43 ± 0.05	7.32 ± 0.10 *
PaO ₂ (mmHg)	86 ± 34	66 ± 11
PaCO ₂ (mmHg)	33 ± 4.4	35 ± 2.3
FiO ₂	0.30 (0.30-0.35)	0.50 (0.30-0.60)
PaO ₂ /FiO ₂ (mmHg)	274 ± 129	152 ± 67 *
Blood neutrophil count (1/μl)	2094 ± 628	4721 ± 1481 *

FiO₂ = inspired fraction of oxygen; PaCO₂ = arterial partial pressure of carbon dioxide; PaO₂/FiO₂ = ratio between arterial partial pressure of oxygen and FiO₂; PvO₂ = mixed venous partial pressure of oxygen.

* $P < 0.05$ for the comparison with baseline; values are mean ± SD or median (interquartile range).

Table 2
Imaging Derived Variables at Baseline and 16 Hour

Variable	Baseline	16 h
Aeration		
F _{gas}	0.51 (0.44, 0.52)	0.42 (0.37, 0.52)
CV ² _{F_{gas}}	0.10 ± 0.05	0.26 ± 0.10 *
Perfusion		
CV ²	0.34 ± 0.09	0.52 ± 0.27
CV ² _D	0.28 ± 0.05	0.40 ± 0.17
Shunt fraction	0.13 (0.11, 0.21)	0.33 (0.14, 0.55) *

CV²_{F_{gas}} = squared coefficient of variation of F_{gas}; CV² = squared coefficient of variation of perfusion; CV² /D = squared coefficient of variation of perfusion normalized by density; F_{gas} = Gas fraction.

* $P < 0.05$. Values are mean ± SD or median (interquartile range). All variables are dimensionless.