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Mild Endotoxemia during Mechanical Ventilation Produces Spatially Heterogeneous Pulmonary Neutrophilic Inflammation in Sheep

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ABSTRACT

Background: There is limited information on the regional inflammatory effects of mechanical ventilation and endotoxemia on the production of acute lung injury. Measurement of ^{18}F-fluorodeoxyglucose (^{18}F-FDG) uptake with positron emission tomography allows for the regional, *in vivo* and noninvasive, assessment of neutrophilic inflammation. The authors tested whether mild endotoxemia combined with large tidal volume mechanical ventilation bounded by pressures within clinically acceptable limits could yield measurable and anatomically localized neutrophilic inflammation.

Methods: Sheep were mechanically ventilated with plateau pressures = 30–32 cm H_2O and positive end-expiratory pressure = 0 for 2 h. Six sheep received intravenous endotoxin (10 ng · kg^{-1} · min^{-1}), whereas six did not (controls), in sequentially performed studies. The authors imaged with positron emission tomography the intrapulmonary kinetics of infused ^{13}N-nitrogen and ^{18}F-FDG to compute regional perfusion and ^{18}F-FDG uptake. Transmission scans were used to assess aeration.

Results: Mean gas fraction and perfusion distribution were similar between groups. In contrast, a significant increase in ^{18}F-FDG uptake was observed in all lung regions of the endotoxin group. In this group, ^{18}F-FDG uptake in the middle and dorsal regions was significantly larger than that in the ventral regions. Multivariate analysis showed that the ^{18}F-FDG uptake was associated with regional aeration ($P < 0.01$) and perfusion ($P < 0.01$).

Conclusions: Mild short-term endotoxemia in the presence of heterogeneous lung aeration and mechanical ventilation with pressures within clinically acceptable limits produces marked spatially heterogeneous increases in pulmonary neutrophilic inflammation. The dependence of inflammation on aeration and perfusion suggests a multifactorial basis for that finding. ^{18}F-FDG uptake may be a sensitive marker of pulmonary neutrophilic inflammation in the studied conditions.

What We Already Know about This Topic

- Endotoxemia causes pulmonary inflammation, but whether this is homogeneous throughout the lungs during large tidal volume ventilation is not known

What This Article Tells Us That Is New

- In sheep with mild endotoxemia and large tidal volume ventilation within ranges used clinically, neutrophil inflammation occurred heterogeneously, related to local aeration and perfusion
- In this situation, regional pulmonary injury might occur by increased mechanical stress and localized inflammation

ENDOTOXEMIA and mechanical ventilation are frequently associated in clinical practice.[1–3] Acute lung injury (ALI) due to endotoxemia has been characterized as a generalized process of lung inflammation.[4–6] In contrast, a key element in ALI is the heterogeneous spatial distribution of lung aeration.[7–10] Studies to date indicate that endotoxin exposure either preceding or accompanying injurious mechanical ventilation augments lung inflammation and injury.[11–13] Indeed, isolated cell, *ex vivo* and *in vivo* small animal studies showed that exposure to high tidal volumes and endotoxin leads to increased production of neutrophil-attracting cytokines,[11–16] increased neutrophil counts in the bronchoalveolar lavage,[13,16] and worsening of gas exchange and respiratory mechanics.[12,13,17] Although these investigations illustrate basic inflammatory processes induced by the combination of excessive lung strain and endotoxin, they do not allow for a straightforward translation of those findings to specific regions of the heterogeneously aerated and perfused

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large animal lung. Specifically, it is not known whether, in the setting of endotoxemia occurring in a large animal whose lungs present mechanical heterogeneity similar to that in humans, inflammation during mechanical ventilation would develop homogeneously or heterogeneously either in nondependent areas potentially subjected to overdistension[18,19] or in dependent regions subjected to cyclic recruitment and/or atelectrauma.[9,20] Also unknown is whether there is a dependence of inflammation on regional lung aeration or perfusion. Furthermore, it is unclear whether any inflammatory changes can be detected at less injurious levels of endotoxemia and mechanical ventilation than the exaggerated conditions used in most studies.

A study of these regional effects is relevant because aeration heterogeneity is regarded as a key factor to regionally amplify mechanical forces during mechanical ventilation[21] and produce lung injury.[7–9] Understanding such heterogeneities could also assist clinical decisions on the optimal use of strategies aimed at minimizing lung injury by reducing heterogeneity of aeration, such as the open lung approach[22] and high-frequency ventilation,[23] and redistributing perfusion, such as inhaled nitric oxide[24] and noisy ventilation,[25,26] according to the degree of lung injury severity, which is itself highly correlated with the percentage of recruitable lung.[27]

Positron emission tomography (PET) imaging after intravenous injection of ^{18}F-fluorodeoxyglucose (^{18}F-FDG) has been used to quantify pulmonary inflammation *in vivo* and noninvasively in the nontumoral lung.[6,28–30] Neutrophils are an essential component of ALI due to either endotoxin or mechanical ventilation. The increases in both neutrophil numbers and activity contribute to increased ^{18}F-FDG uptake during lung inflammation.[31,32] Because ^{18}F-FDG-PET imaging can detect changes in lung neutrophil kinetics before their migration into the alveolar space,[6] it has been proposed as a potentially powerful tool to study the early phases of neutrophil trafficking and state of activation during ALI. In line with such arguments, we showed that whole-lung ^{18}F-FDG uptake is increased after 90 min of injurious mechanical ventilation, correlates with neutrophil infiltration, and potentially precedes lung dysfunction.[29]

In this study, we used a sheep model of large tidal volume mechanical ventilation bounded by clinically accepted pressure limits, designed to promote lung derecruitment (positive end-expiratory pressure [PEEP] = 0) and maximal inflation within accepted plateau pressures (P_{plat} = 30–32 cm H_2O) for 2 h. This strategy was chosen to deliberately promote, in a healthy lung, regional heterogeneity of aeration within clinically observed alveolar pressures ranges and not to test specific ventilatory settings applicable to a clinical condition. By using this model and methods of regional pulmonary ^{18}F-FDG kinetics modeling, we sought to test whether combining mechanical ventilation of a heterogeneously expanded lung with mild endotoxemia could yield measurable and anatomically localized levels of neutrophilic inflammation.

Materials and Methods

The experimental protocols were approved by the Massachusetts General Hospital Subcommittee on Research Animal Care (Boston, Massachusetts). Twelve sheep (22.3 ± 5.9 kg, approximately 3 months old) were fasted overnight and premedicated with intramuscular ketamine (4 mg/kg) and midazolam (2 mg/kg). After intravenous induction of anesthesia with ketamine (4 mg/kg), an endotracheal tube was inserted. General anesthesia was maintained with a continuous infusion of propofol and fentanyl titrated to heart rate and blood pressure. Pancuronium 0.1 mg/kg at induction and repeated every 90 min (0.02–0.04 mg/kg) was used for muscle paralysis. Each sheep was 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. After a recruitment maneuver, they were mechanically ventilated with P_{plat} = 30–32 cm H_2O, PEEP = 0, inspired O_2 fraction = 0.3 (adjusted to an arterial O_2 saturation >0.88), inspiratory-to-expiratory time ratio = 1:2, respiratory rate = 18 breaths/min or higher to maintain the arterial carbon dioxide pressure ($Paco_2$) between 32 and 45 mmHg. If $Paco_2$ were less than 32 mmHg when respiratory rate = 18 breaths/min, a variable dead space was added to the breathing circuit aiming at that $Paco_2$ range. Physiologic data were collected, and PET scans were acquired both at the start of the protocol and after 2 h of mechanical ventilation, except for the FDG scan performed at the end of the study. After the initial set of scans, six sheep (endotoxin group) received a continuous 10 ng · kg^{-1} · min^{-1} intravenous infusion of endotoxin (*Escherichia coli* O55:B5, List Biologic Laboratories Inc., Campbell, CA), whereas six did not (controls). Studies were performed sequentially in each group.

PET Imaging Protocol and Processing

The experimental system and methods of analysis have been described in detail.[29,30,33–35] Scans consisted of 15 cross-sectional slices of 6.5-mm thickness over a 9.7-cm-long axis field, providing three-dimensional data for an estimated 70% of the total lung volume.[35] For each slice, resulting images, consisting of 128 × 128 voxels of 6 × 6 × 6.5-mm size, were low-pass filtered to 12 × 12 mm to a final volumetric resolution of approximately 0.9 cm^3.

1. Transmission scans: used to correct for attenuation and to calculate regional gas fraction (F_{gas}). We categorized pulmonary parenchyma as nonaerated (F_{gas}<0.1), poorly aerated (0.1 ≤ F_{gas} <0.5), normally aerated (0.5 ≤ F_{gas} < 0.85), and hyperinflated (F_{gas} ≥ 0.85).[36,37]
2. Emission scans
   a. Intravenous ^{13}N-nitrogen (^{13}NN)-saline: used to measure regional pulmonary perfusion and shunt from the lung tracer kinetics after a bolus injection of ^{13}NN-saline during a 60-s apnea at mean lung volume.[35,38] Because of the low solubility of nitrogen in blood and tissues (partition coefficient water-to-air is 0.015 at 37°C), in perfused and aerated regions, vir-

tually all ^{13}NN diffuses into the alveolar airspace at first pass, accumulating in proportion to regional perfusion. In regions with shunting alveolar units, there is a peak of tracer concentration in the early PET frames, corresponding to arrival of the bolus of tracer with pulmonary blood flow, followed by a decrease toward a plateau due to lack of retention of ^{13}NN in nonaerated units. The magnitude of this decrease is related to regional shunt. Perfusion and shunt fraction were calculated with a tracer kinetics model.[39]

b. Intravenous ^{18}F-FDG: used for quantification of regional neutrophilic inflammation.[29,30] After ^{13}NN clearance, ^{18}F-FDG (5–10 mCi) was infused at a constant rate through the jugular catheter over 60 s, and, starting at the beginning of ^{18}F-FDG infusion, sequential PET frames (9 × 10 s, 4 × 15 s, 1 × 30 s, 7 × 60 s, 15 × 120 s, 1 × 300 s, and 3 × 600 s) were acquired while pulmonary arterial blood was sampled at 5.5, 9.5, 25, 37, and 42.5 min. Blood samples (1 ml) were spun down, and the activity of plasma was measured in a gamma counter cross-calibrated with the PET scanner. The plasmatic activities of those samples were used to calibrate the blood-pool region of interest (ROI) (see Selection of Voxels for Analysis section) and obtain an image-derived input function taking into account partial-volume and spillover effects.[33] ^{18}F-FDG PET scans were acquired only after injury because of the 110-min half-life of ^{18}F-FDG.

Inside cells, ^{18}F-FDG is phosphorylated by hexokinase to ^{18}F-FDG-6-phosphate, which accumulates in proportion to cellular metabolic rate. ^{18}F-FDG net uptake rate (K_i), a measure of cellular metabolic activity, was calculated at the ROI level by fitting the ^{18}F-FDG kinetics with a three-compartment model of Sokoloff *et al.*[30,40] To account for potential effects of lung inflation and blood volume on regional K_i, we standardized K_i by lung tissue, thus computing a specific K_i as $K_{is} = K_i/F_{tissue}$, where $F_{tissue} = (1\text{-}F_{gas}\text{-}F_{blood})$ and F_{blood} is the fractional volume of the blood compartment obtained from the model of Sokoloff *et al.* K_{is} is proportional to ^{18}F-FDG uptake per gram of lung tissue.

Patlak analysis[41] was used to compute K_i at the voxel level and construct parametric images (fig. 1) and illustrations of regional kinetics (fig. 2).

Selection of Voxels for Analysis

Identification of the aerated lung fields was done by thresholding the transmission scans. Early frames of the ^{13}NN-saline infusion emissions scans were used to identify nonaerated perfused lung fields. The combination of those fields resulted in the final lung mask for each animal. We manually excluded areas corresponding to main bronchi and large pulmonary vessels.

Three ROIs of same vertical height (ventral, middle, and dorsal) were defined by dividing the three-dimensional lung mask with two horizontal planes and used for quantification of regional F_{gas}, and ^{13}NN and ^{18}F-FDG kinetics.

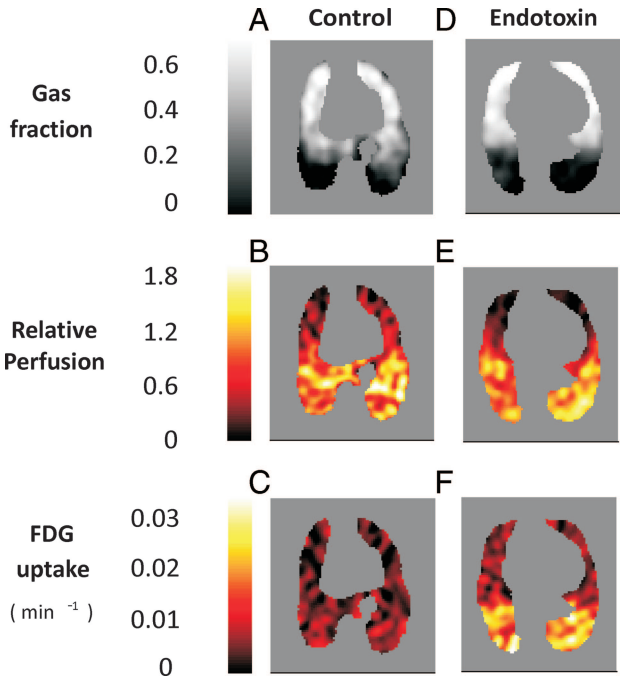

Fig. 1. Representative images of gas fraction, perfusion, and net ^{18}F-fluorodeoxyglucose uptake rate (K_i) in supine sheep after 2 h of mechanical ventilation with positive end-expiratory pressure = 0 cm H_2O and plateau pressure of 30–32 cm H_2O in the control (*A–C*) and after mild endotoxemia (*D–F*). There is a clear vertical dependence of aeration and perfusion in both groups. No significant change in mean gas fraction or perfusion redistribution was observed with endotoxemia. In contrast, the increase in magnitude and degree of heterogeneity of the net ^{18}F-fluorodeoxyglucose uptake is apparent in the endotoxin group when compared with the control group. FDG = fluorodeoxyglucose.

A blood-pool ROI was defined by thresholding the regional activity of ^{13}NN during the first 5 s after ^{13}NN-saline injection. During this time, ^{13}NN is confined mostly to the right heart cavities and pulmonary arteries, and only a minor amount has diffused into the alveolar gas volume.[33]

Histologic Analysis

Lungs from four animals of the control group and five animals of the endotoxin group were excised at the end of the experiment and fixed with Trump's fixative (4% formaldehyde and 1% glutaraldehyde in phosphate-buffered saline) at a pressure of 25 cm H_2O. A block of lung tissue was sampled from ventral and dorsal regions and embedded in paraffin. Sections of 5-μm thickness were cut, mounted, and stained with hematoxylin-eosin for light microscopy. Lung neutrophils were counted in 40 randomly selected high-power (400×) fields per animal (10 per region) by two investigators, who were blinded to the group assignment. In addition, perivascular and alveolar edema, alveolar hemorrhage, septal thickening, and capillary congestion were evaluated semiquantitatively with a four-grade scale (absent = 0, mild = 1, moderate = 2, and marked = 3).

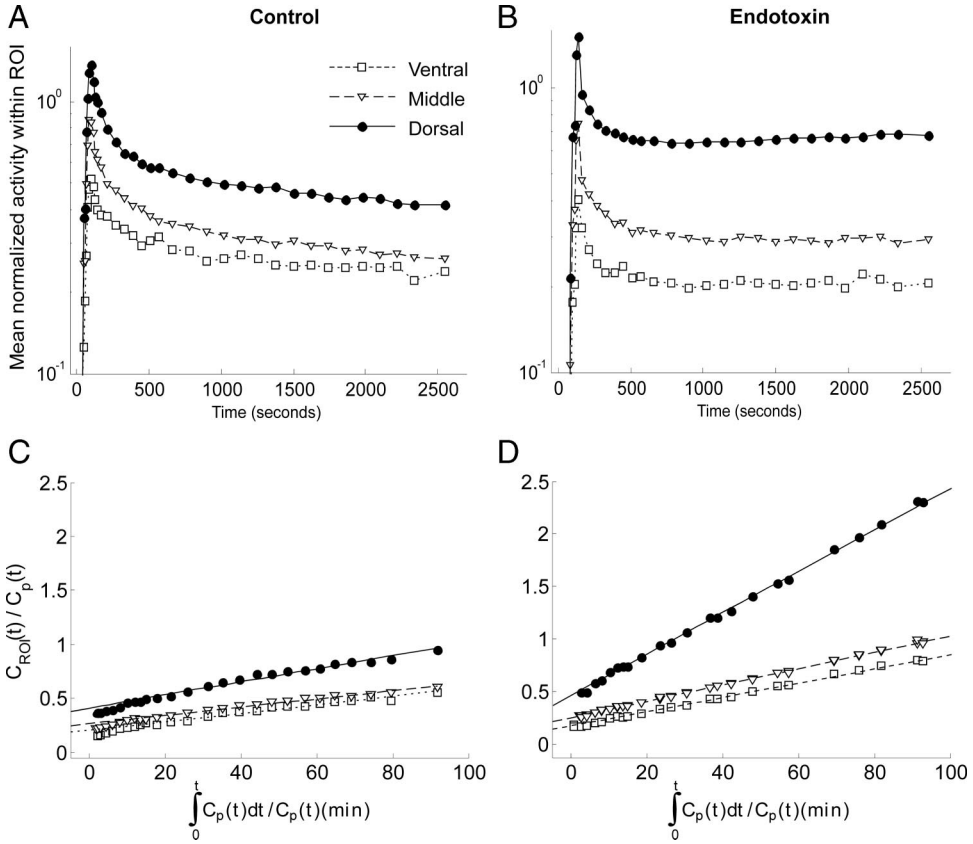

Fig. 2. Pulmonary ^{18}F-fluorodeoxyglucose (^{18}F-FDG) kinetics and corresponding Patlak plots in three vertical regions of interest (ROI) in a representative animal from the control and another from the endotoxin group. (*A*, *B*) ^{18}F-FDG activity is expressed as the average ^{18}F-FDG activity within the ROI normalized to the peak activity for the imaged lung. Plots were generated for the same animals shown in figure 1. Tracer kinetics in the dorsal region of the animal receiving endotoxin (*B*, *closed circles*) shows the inflow of ^{18}F-FDG in the lung with an early peak followed by a drop to a slightly positive slope, representing increased ^{18}F-FDG uptake. Total activity in that dorsal region is also larger than that in the ventral and middle regions. In contrast, the kinetics of the ventral regions of the control animal shows a decreasing slope following the peak, compatible with small ^{18}F-FDG uptake (*A*, *open squares*). This negative slope is also present in the middle and dorsal regions of the control group, whereas a plateau is observed in the same regions of the endotoxin group, consistent with higher ^{18}F-FDG uptake in the endotoxin than in the control group. (*C*, *D*) Quantification of the pulmonary ^{18}F-FDG kinetics as Patlak plots, in which the regional ^{18}F-FDG activity ($C_{ROI}(t)$) normalized to the plasmatic ^{18}F-FDG acitivity ($C_p(t)$) is plotted against the integral of plasma activity normalized to plasma activity. In these plots, the slope of the linear regression represents ^{18}F-FDG uptake. Note the different regional ^{18}F-FDG uptakes.

Statistical Analysis

Variables were tested for normality with the Shapiro-Wilk test. We expressed values as means and standard deviations for normally distributed variables and median and interquartile ranges (25–75%) otherwise. For normally distributed variables, we used the independent samples Student *t* test for comparisons between groups and paired *t* tests for comparisons between time points in the same group. For not normally distributed variables, we used the Wilcoxon rank sum test for comparisons between groups and the Wilcoxon signed rank test for comparisons between time points in the same group. Regional F_{gas}, perfusion, and shunt in ventral, middle, and dorsal regions within and between groups and before and after 2 h of mechanical ventilation were compared with a linear mixed-effect model.[42] This model was chosen because the analysis involved observations in the same individual at different time points and topographic lung regions. The categorical variables group, region, and time were modeled as fixed effects, and the variation among individuals was modeled by assuming random coefficients for the intercept (lme4 package, R statistical environment, R 2.6.2, Vienna, Austria). To study the dependence of K_{is} on F_{gas} and perfusion, plots of K_{is} *versus* perfusion and F_{gas} were built as follows: in each animal, F_{gas} and perfusion were computed voxel-by-voxel; functional compartments of aeration and perfusion were created by grouping together voxels belonging to tertiles of low, intermediate, and high $F_{gas,}$ or of low, intermediate, and high perfusion; and K_{is} computed for the ROIs defined as the set of voxels in each tertile of F_{gas} or perfusion. Furthermore, we sought to identify the best predictors of K_{is} in the endotoxin group. For this, perfusion, F_{gas}, squared F_{gas}, and lung volume in each one of the aeration categories were computed in the ventral, middle, and dorsal ROIs and univariately regressed against K_{is}. Variables significantly associated with K_{is} in univariate analyses ($P < 0.1$) were included in a backward stepwise multivariate mixed-effect model using

Table 1. Cardiovascular and Respiratory Variables and Neutrophil Counts in Peripheral Blood at Baseline and after 2 h of Mechanical Ventilation

	Control (n = 6)		Endotoxin (n = 6)	
	Baseline	2 h	Baseline	2 h
Mean systemic arterial pressure (mmHg)	74 ± 14	81 ± 13	92 ± 12	78 ± 13
Mean pulmonary artery pressure (mmHg)	14 ± 8	15 ± 9	16 ± 9	21 ± 5
Cardiac output (l/min)	4.9 ± 2.7	5.2 ± 2.8	4.3 ± 1.2	4.5 ± 0.8
PvO_2 (mmHg)	47 ± 7	48 ± 7	50 ± 10	46 ± 12
FiO_2 (%)	0.32 ± 0.04	0.35 ± 0.05	0.33 ± 0.08	0.43 ± 0.18
Tidal volume (ml/kg)	17.8 (17.0–23.5)	14.9 (13.9–19.0)	18.8 (15.6–20.9)	13.9 (11.7–17.9)*
Respiratory rate (bpm)	19 ± 1	19 ± 3	19 ± 2	21 ± 3
pH	7.44 ± 0.07	7.41 ± 0.09	7.39 ± 0.14	7.21 ± 0.15
PaO_2/FiO_2 (mmHg)	243 ± 66	228 ± 88	255 ± 74	162 ± 67*
$PaCO_2$ (mmHg)	34 (30–41)	36 (32–51)	33 (30–40)	43 (41–46)
Neutrophil count ($\times 10^3/\mu$l)	1.78 (1.63–2.43)	3.89 (3.21–5.72)	3.09 (1.32–3.96)	0.23 (0.14–0.25)*

Variables are expressed as mean ± SD for normally distributed variables and median and interquartile range (25–75%) otherwise.
*$P < 0.01$ *vs.* baseline.
FiO_2 = inspired fraction of oxygen; $PaCO_2$ = arterial partial pressure of carbon dioxide; PaO_2/FiO_2 = ratio between arterial partial pressure of oxygen and FiO_2; PvO_2 = mixed venous partial pressure of oxygen.

similar considerations as those described earlier. The multivariate regression analysis was applied. All statistical tests were two-tailed, and the significance was set at $P < 0.05$.

Results

Global Physiologic Variables

By experimental design, PEEP and P_{plat} were kept at 0 and 30 cm H_2O, respectively. Along 2 h of mechanical ventilation, mean tidal volume decreased in both groups, significantly in the endotoxin group (table 1). In this group, respiratory rate was increased to maintain the $PaCO_2$ within the predefined acceptable range. The shift in $PaCO_2$ toward the upper limit of the accepted range was associated with a nonsignificant difference in pH (table 1). Oxygenation was reduced in the endotoxin group during the 2 h of the study ($P = 0.02$). Hemodynamics was stable during the experiment and comparable between groups. Circulating neutrophil counts decreased significantly in the endotoxin group ($P = 0.02$).

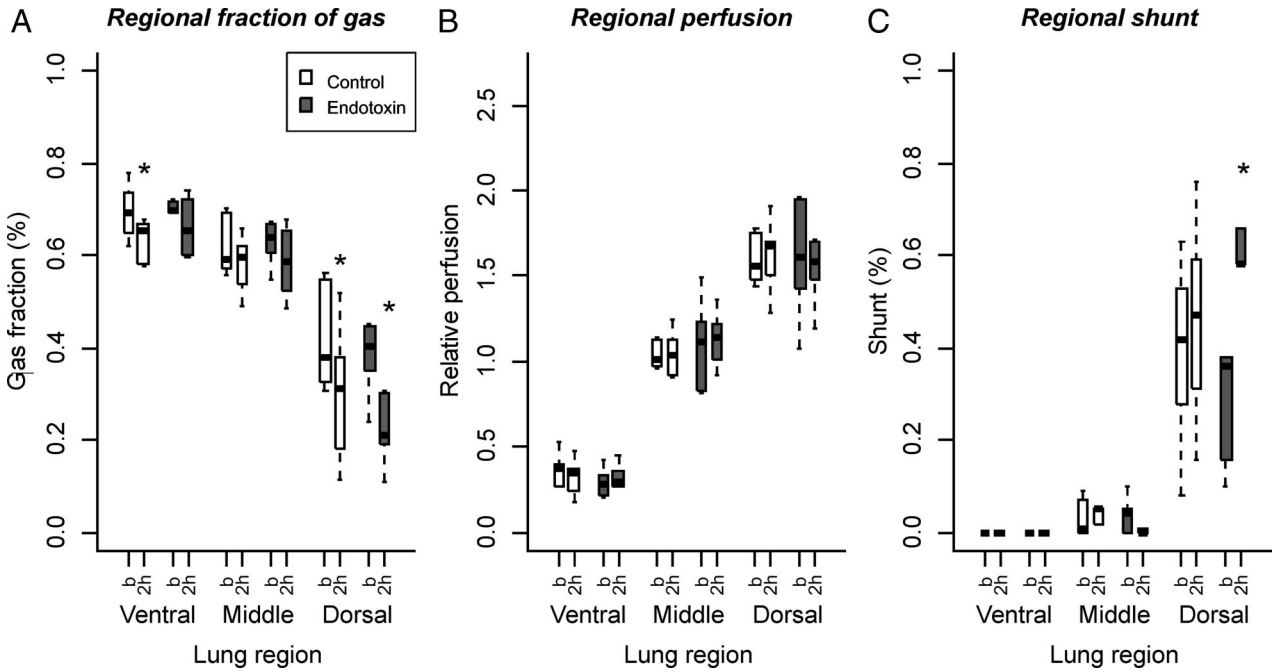

Fig. 3. Gas fraction (*A*), mean normalized perfusion (*B*), and shunt fraction (*C*) in the ventral, middle, and dorsal regions in the control (n = 6) and endotoxin (n = 6) groups of supine sheep at baseline and after 2 h of mechanical ventilation with positive end-expiratory pressure = 0 cm H_2O and plateau pressure of 30–32 cm H_2O. Data are expressed as *box* (median, twenty-fifth, and seventy-fifth percentile) and *whiskers* (range) for each region, group, and time point. On the x axis, "b" indicates measurements at baseline, and "2h" indicates measurements after 2 h of mechanical ventilation. *$P < 0.05$ *versus* baseline.

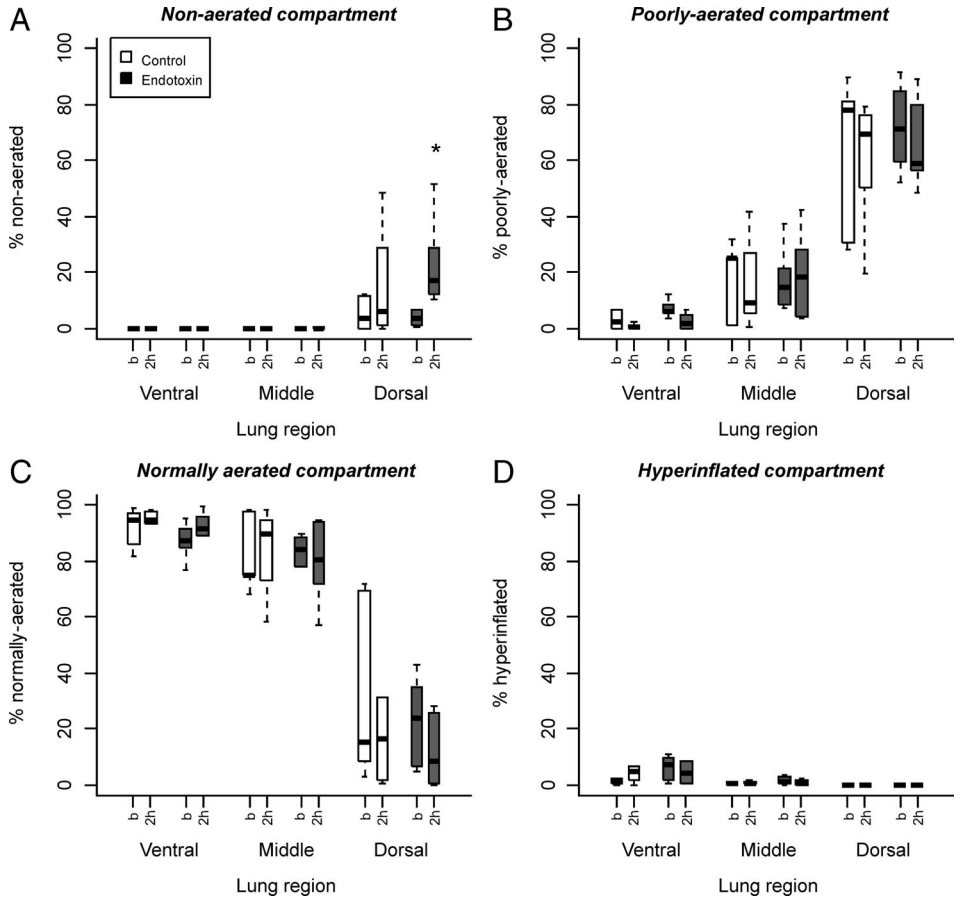

Fig. 4. Fraction of voxels within each of the regions of interest (ventral, middle, and dorsal) classified as nonaerated (*A*) (gas fraction <0.1), poorly aerated (*B*) (0.1 ≤ gas fraction < 0.5), normally aerated (*C*) (0.5 ≤ gas fraction <0.85) and hyperinflated (*D*) (gas fraction ≥0.85) in supine sheep of control (n = 6) and endotoxin (n = 6) groups at baseline and after 2 h of mechanical ventilation with positive end-expiratory pressure = 0 cm H_2O and plateau pressure of 30–32 cm H_2O. Data are expressed as *box* (median, twenty-fifth, and seventy-fifth percentile) and *whiskers* (range) for each region, group, and time point. On the x axis, "b" indicates measurements at baseline, and "2h" indicates measurements after 2 h of mechanical ventilation. *$P < 0.05$ *versus* baseline.

Regional Aeration at Baseline and after Mechanical Ventilation

Mean F_{gas} of the imaged lung was similar in both groups at baseline (controls = 0.57 ± 0.08 and endotoxin group = 0.58 ± 0.04, NS) and after 2 h of mechanical ventilation (controls = 0.56 ± 0.08, endotoxin group = 0.53 ± 0.06, NS; figs. 1 and 3). The regional distribution of F_{gas} at baseline and after 2 h of mechanical ventilation was not statistically different between the two groups (fig. 3). F_{gas} decreased significantly after 2 h in the dorsal regions in both groups and in the ventral regions of the control group (fig. 3).

There was no statistical difference in aeration between groups at baseline and after 2 h of mechanical ventilation (fig. 4). Most voxels in the ventral and middle regions were normally aerated, whereas voxels in the dorsal regions were either poorly aerated or nonaerated (fig. 4). The proportion of hyperinflated voxels was small in all regions. After 2 h of mechanical ventilation, the size of the nonaerated compartment increased significantly only in the dorsal ROIs of the endotoxin group ($P = 0.02$).

Perfusion and Shunt Fraction

The regional distribution of perfusion before and at the end of the 2-h period was comparable between groups (figs. 1 and 3). The distribution of perfusion was inversely related with that of aeration along the vertical ROIs. In the ventral and middle regions of both groups, shunt fraction was small and remained unchanged after 2 h of mechanical ventilation (fig. 3). Shunt increased significantly in the dorsal regions of the endotoxin group.

Regional ^{18}F-FDG Kinetics and Regional Neutrophilic Inflammation

PET assessment of ^{18}F-FDG kinetics yielded regional plots that were suitable for analysis in all cases of both groups (figs. 2A and B). These plots were characterized by an early peak followed by either a continuously decreasing curve in controls or a plateau or slightly ascending slope in the endotoxin group. Quantification of the kinetics with Patlak plots (figs. 2C and D) or computations derived from the model of *Sokoloff et al.* (table 2) evidenced the differences in regional ^{18}F-FDG uptake, particularly high in dorsal ROIs.

Table 2. ^{18}F-Fluorodeoxyglucose Uptake Rates for the Control and Endotoxin Groups in Ventral, Middle, and Dorsal Lung Regions and in the Whole Lung

	Ventral		Middle		Dorsal		Whole Lung	
	Control	Endotoxin	Control	Endotoxin	Control	Endotoxin	Control	Endotoxin
K_i (10^{-3}/min)	4.1 ± 0.4	6.0 ± 2.3	3.7 ± 0.6	7.6 ± 3.7*	6.5 ± 1.6	16.2 ± 7.5*	4.7 ± 0.7	10.2 ± 4.4*
K_{is} (10^{-2}/min)	1.4 ± 0.1	2.2 ± 0.7	1.4 ± 0.2	2.9 ± 1.6*	1.4 ± 0.2	3.1 ± 1.1*	1.6 ± 0.2	3.4 ± 1.5*

Variables are expressed as mean ± SD.

* $P < 0.05$ *versus* control group.

K_i = net ^{18}F-FDG uptake rate; K_{is} = specific net ^{18}F-FDG uptake rate.

In the control group, regional K_i was low, showed a small interanimal variability in all ROIs, and was not significantly different among the ROIs of the same animal (table 2; fig. 5A). Likewise, specific K_i (K_{is}), representing the standardization of K_i by the amount of lung tissue in the ROI, was not significantly different for the different ROIs (table 2; fig. 5B), despite the differences in regional perfusion and aeration. In contrast, global and regional K_i were larger in the endotoxin group, with mean values of global K_i and K_{is} more than twofold greater than those measured in the control group (table 2; fig. 5C). Furthermore, there were large differences in K_i and K_{is} among ROIs in the endotoxin group, in addition to larger interanimal variability in these parameters. On average, K_i and K_{is} increased progressively from ventral to dorsal ROIs (figs. 5C and D). As a result, K_i in the endotoxin group was 168% and K_{is} was 46%, larger in dorsal than in ventral ROIs.

K_{is} increased monotonically with perfusion in the endotoxin group, but not in the controls (figs. 6A and B). A different relationship was observed between K_{is} and F_{gas} in the endotoxin group (figs. 6C and D). K_{is} values in the two extremes of aeration were larger than those at the intermediate F_{gas} tertile. No changes in K_{is} were observed in the control

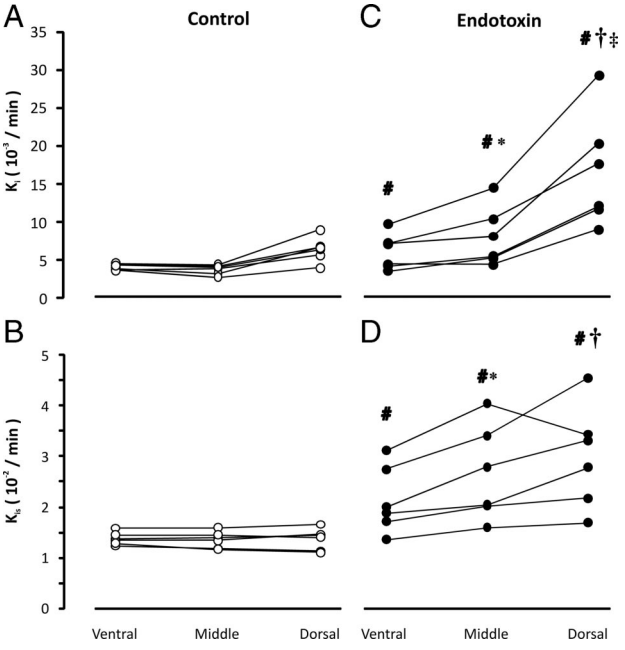

Fig. 5. Regional net ^{18}F-fluorodeoxyglucose uptake rate (K_i) and specific K_i (K_{is}) in supine sheep after 2 h of mechanical ventilation with positive end-expiratory pressure = 0 cm H_2O and plateau pressure of 30–32 cm H_2O, without (control, n = 6, *A*, *B*) and with mild endotoxemia (endotoxin, n = 6, *C*, *D*). Control values of K_i and K_{is} (*A*, *B*) are quite homogeneous within and between animals. Mild endotoxemia produced an increase in the average whole-lung values of K_i and K_{is} with significant regional heterogeneity (*C*, *D*), larger in dorsal regions. *$P < 0.05$ between middle and ventral regions; †$P < 0.05$ dorsal *versus* ventral regions; ‡$P < 0.05$ dorsal *versus* middle regions; #$P < 0.05$ endotoxin *versus* control group. *Circles* represent individual data points.

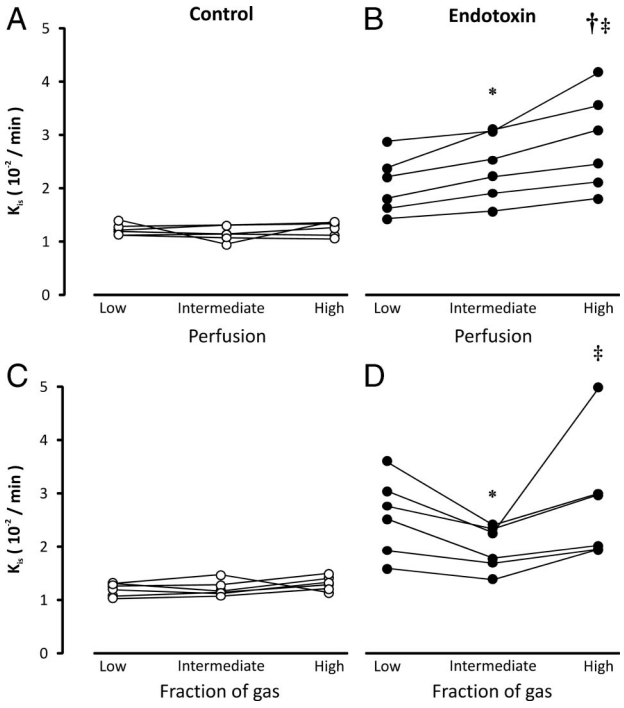

Fig. 6. Specific regional net ^{18}F-fluorodeoxyglucose uptake rate (K_{is}) in supine sheep after 2 h of mechanical ventilation with positive end-expiratory pressure = 0 cm H_2O and plateau pressure of 30–32 cm H_2O, without (control, n = 6) and with mild endotoxemia (endotoxin, n = 6). Each lung region corresponds to one third of the lungs segmented according to the amount of perfusion (*A*, *B*) or gas fraction (*C*, *D*). Low, intermediate, and high in the horizontal axes correspond to the first, second, and third tertiles, respectively. Note the linear relationship between perfusion and K_{is} as opposed to a relationship with a minimum at intermediate values between gas fraction and K_{is}. *$P < 0.05$ middle *versus* ventral regions; †$P < 0.05$ dorsal *versus* ventral regions; ‡$P < 0.05$ dorsal *versus* middle regions. *Circles* represent individual data points.

Table 3. Neutrophil Counts per High Power Field (×400) and Indices of Parenchymal Injury for the Control and Endotoxin Groups in Ventral and Dorsal Lung Regions

	Ventral		Dorsal	
	Control	Endotoxin	Control	Endotoxin
Neutrophil count	2.9 ± 1.3	3.9 ± 1.7	5.9 ± 1.5	12.5 ± 7.1*
Perivascular and alveolar edema	0 (0–0)	1 (0–1)	1 (0–1)	1 (1–1)
Alveolar hemorrhage	0 (0–0)	0 (0–1)	1 (1–1)	1 (1–1)*
Septal thickening	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
Congestion	1 (1–1)	1 (1–1)	1 (1–2)	2 (2–2)

Semiquantitative scale for indices: absent = 0, mild = 1, moderate = 2, and marked = 3. Variables are expressed as mean ± SD for normally distributed variables and median and interquartile range (25–75%) otherwise.

* $P < 0.05$ *vs.* control group.

group for the different F_{gas} tertiles. We approximated the relationship between K_{is} and F_{gas} with a quadratic function. Univariate analyses showed that the amount of lung in the nonaerated ($P = 0.08$), poorly aerated ($P = 0.03$), and normally aerated compartments ($P = 0.03$), and squared-F_{gas} ($P = 0.05$), F_{blood} ($P = 0.02$), and perfusion ($P = 0.01$), were significantly associated with K_{is}. Multivariate regression showed that only perfusion (slope = $0.5 \times 10^{-2} \cdot min^{-1}$, n = 18 observations, $P < 0.01$) and squared-F_{gas} (slope = $4.7 \times 10^{-2} \cdot min^{-1}$, n = 18 observations, $P < 0.01$) remained significantly associated with K_{is} among the ROIs in the endotoxin group.

Histologic Findings

Neutrophil counts in the dorsal regions of the endotoxin group were more than double the counts in the same regions of the control group ($P < 0.05$), whereas no significant difference was present in the ventral regions (table 3). Moderate or severe alveolar hemorrhage was focal and present in only 11% of the high-power fields studied. On average, hemorrhage was mild and more intense in the dorsal regions of the control group (table 3). Perivascular and alveolar edema, septal thickening, and capillary congestion were very mild in both groups, without statistically significant differences.

Discussion

The most important findings of the current study were as follows: (1) mild short-term endotoxemia in the presence of heterogeneous lung aeration and perfusion and large tidal volume mechanical ventilation bounded by clinically acceptable pressure limits produced spatially heterogeneous increases in pulmonary neutrophilic inflammation; (2) these regional increases of neutrophilic inflammation were significantly related to both regional aeration and perfusion and were detectable with PET within 2 h of injury; and (3) in the absence of endotoxemia, pulmonary ^{18}F-FDG uptake was low and uniformly distributed at those settings of mechanical ventilation.

Pulmonary ^{18}F-FDG uptake has been shown to be a marker of the concentration and degree of activation of neutrophils in the nontumoral lung.[6,28] We used the model of *Sokoloff et al.* [40] to derive regional parameters from ^{18}F-FDG kinetics. This choice was based on the observation of "Sokoloff-type" tracer kinetics in both control and endotoxin groups (fig. 2), according to a previously described strategy for model selection.[30] Based on that analysis, Sokoloff-type kinetics is suggestive of a minimal degree of lung edema.[30]

The ventilator settings chosen for the study produced in both groups the intended heterogeneity in lung expansion with significant fraction of nonaerated and poorly aerated units in dependent regions (PEEP = 0) and a predominance of normal aeration in nondependent regions (limitation of P_{plat} to 30–32 cm H_2O). In fact, nondependent areas of the control group presented values of K_i and K_{is} that matched those observed in uninjured lungs of prone sheep in two previous studies[29,30] and did not show any detectable histopathologic injury. PEEP = 0 was the likely cause for the decrease in F_{gas} in both groups after 2 h of mechanical ventilation. As intended, the used dose of endotoxin did not lead to changes in perfusion distribution to ventral, middle, and dorsal regions (fig. 3B). This result emphasizes the small dose used in our study, which was either equivalent to or lower than doses considered mild and devoid of lung injurious effects in previous investigations.[6,43] The nonaerated lung compartment increased in the most dependent regions with endotoxemia. At least three factors could account for this observation: surfactant dysfunction, increase in regional blood volume, and regional edema. All three are compatible with the increased regional shunt found in dependent regions. Given the very mild degrees of edema apparent on histologic samples, the two first factors are the most likely.

Endotoxin has been shown to induce systemic inflammation and to increase sequestration of neutrophils in the lungs yielding increased whole-lung ^{18}F-FDG uptake.[4–6] Previous *ex vivo* and *in vivo* small animal models described an increase in the release of inflammatory mediators and a modification in inflammatory cell infiltration due to endotoxin, which depended on tidal volume.[4,11,12,14] However, no previous study has investigated whether and how those results can be extrapolated to the heterogeneously aerated and perfused large animal lung. Such extrapolation is complex, given that mechanical strain is heterogeneously distributed in the me-

chanically ventilated heterogeneously aerated lung and that there is still controversy over the contribution of overdistension and low volume ventilation to inflammation during ventilator-induced lung injury. Thus, it is difficult to predict how mild endotoxemia in the presence of heterogeneous pulmonary perfusion would interact with that heterogeneous distribution of lung inflation to affect lung inflammation, a condition related to clinical situations such as intraoperative mechanical ventilation during surgical interventions involving subclinical endotoxemia. A previous large animal study showed that endotoxemia produced increased whole lung neutrophil activation.[6] However, no data were provided on regional inflammation or on the combined effect of endotoxemia and mechanical ventilation. Understanding regional heterogeneities is essential to optimize the use of strategies to reduce lung injury by reducing heterogeneity of aeration[22,23] and perfusion,[26,44] according to the degree of lung injury severity.

Our results indicate that the combination of mild endotoxin doses with ventilatory settings, which did not by themselves cause injury, resulted in a substantial increase in pulmonary neutrophilic inflammation. This increase showed a heterogeneous spatial distribution, characterized by a vertical dependence of neutrophilic inflammation (K_i and K_{is}), more intense in dependent regions. The fact that the observed increase in ^{18}F-FDG uptake was still significant after correction for regional lung tissue (K_{is}), including correction for regional lung collapse and blood volume, supports the conclusion that it was not a mere consequence of the increase in the amount of pulmonary tissue per unit volume of lung or in regional blood volume in derecruited regions because it was still significant after correction for regional lung tissue (K_{is}). The increase in neutrophil counts in dependent regions of the endotoxin group reinforces the imaging findings.

Remarkably, changes in global and regional ^{18}F-FDG uptake in the endotoxemia group were markedly larger than those in regional aeration and perfusion. The average K_i and K_{is} in the endotoxin group were more than twofold compared with those in the control group, whereas average F_{gas} and pulmonary perfusion distributions were similar in the two groups. Such similarity supports the inference that perfusion distribution *per se* could not explain the changes in global and regional ^{18}F-FDG uptake during endotoxemia. Furthermore, although deterioration of regional aeration and shunt in the endotoxin group was limited to the dorsal regions, and distributions of F_{gas} and perfusion were similar in the two groups, ^{18}F-FDG uptake (K_i) in the endotoxin group was larger than that in the control group in all lung regions: 47% larger in ventral regions, 103% in middle regions, and 150% in dorsal regions. These results suggest that ^{18}F-FDG may be an early and sensitive marker of regional pulmonary inflammation induced by the combination of endotoxemia and ventilator-induced lung injury.

Endotoxemia could contribute to the development of regional inflammation during mechanical ventilation by activation of neutrophils, resulting in their augmented response to the inflammatory stimuli produced by localized mechanical strain, which could be excessive in heterogeneous lungs. Our findings are, therefore, in line with the two-hit theory,[11] although we used both injurious stimuli simultaneously and not sequentially as in typical two-hit studies. The changes in circulating neutrophil counts suggest another form of interaction between mechanical ventilation and endotoxemia. The nonsignificant increase of circulating neutrophils in the control group, a trend noted in previous studies,[6,10] might be explained by release from the marginated pool due to mechanical ventilation. The lack of migration of these cells to the lungs or the lack of activation of these cells in the lungs implies that an additional hit would have a greater than normal supply of neutrophils to call on. The observed fall in circulating neutrophils in the endotoxin group shows that this occurred and that neutrophils have either marginated in the capillaries or migrated into the lungs. The increase in K_i and K_{is} in the endotoxin group indicates that these neutrophils became activated.

The multivariate regression analysis showed that both perfusion and regional aeration were important to explain the observed regional values of K_{is}. The linear relationship between K_{is} and regional perfusion (fig. 6) could represent the increase in regional inflammation with the increase in regional load of endotoxin, inflammatory cells, including neutrophils, and mediators of inflammation to the more perfused regions predominant in the dependent lung. This association between regional lung perfusion and K_{is} is important because therapeutic interventions can modify perfusion distribution, for example, as recently shown with noisy ventilation.[26,44] In contrast to the direct relationship between K_{is} and regional perfusion, the relationship between K_{is} and F_{gas} was biphasic with large K_{is} in the low and high extremes of aeration and low K_{is} for F_{gas} values in the normal range (fig. 6). The large K_{is} for low F_{gas} may represent the contribution of low volume lung injury associated with nonaerated and poorly aerated predominantly in dependent regions,[9,20] whereas the high K_{is} for larger F_{gas} values suggest the contribution of lung overdistension. Although we did not find a significant fraction of hyperinflated areas, our measurements performed at mean lung volume underestimate hyperinflation, as discussed below. Thus, we speculate that overdistension could have occurred at end inspiration in regions of high F_{gas}.

Despite the substantial heterogeneity in lung aeration, we found a low and uniform distribution of neutrophilic inflammation in the control group. Such regional findings contrast with commonly invoked interdependence mechanisms,[21] which predict that the used P_{plat} would generate high local forces in regions lying in the interface between aerated and derecruited lung. These forces would be expected to result in inflammation and increased ^{18}F-FDG uptake. At least two factors could account for our finding. First, ^{18}F-FDG uptake could be an insensitive marker of regional lung mechanical injury. However, we showed that peak pressures of 50 cm H_2O applied for 90 min increased lung ^{18}F-FDG uptake in homogeneously expanded sheep lungs, supporting the sensi-

tivity of the technique.[29] Given that interdependence mechanisms would predict local pressures higher than 130 cm H_2O for P_{plat} = 30–32 cm H_2O,[21] if those local values were present, they should be detectable with our technique. Indeed, our histologic findings support the absence of parenchymal inflammation in the control group. Consequently, the second possibility is that the assumptions of that previously theorized interdependence model[21] may not accurately represent the expansion of the dorsal regions of a normal lung.

Advantages and limitations of the used imaging techniques have been discussed in detail previously.[29,30,33,38,39] Specifically to this research, the regional aeration of the lung was computed from PET transmission scans. Because these scans are collected for 10 min during uninterrupted mechanical ventilation, the calculated F_{gas} of a region represents its average aeration over the breathing cycle. F_{gas} is not only affected by motion but also by filtering during image reconstruction and processing, and partial volume effects.[45] The consequent limited spatial resolution (13 mm) could result in underestimation of the degree of regional hyperinflation compared with that measured from computed tomography images acquired during end-inspiratory breath holds at much higher spatial resolution. Additional limitations include (1) species differences: young sheep as studied in our work are known to have reduced collateral ventilation, which might make them more prone than adult humans to reabsorption atelectasis and lung collapse.[46] Also, presence of pulmonary intravascular macrophages in sheep may make them differently sensitive to endotoxemia-induced lung injury when compared with humans[47,48]; (2) ventilatory settings: we chose settings aimed at maximizing aeration heterogeneity. Settings used clinically might be associated with regional inflammation of different intensity and distribution from those found in the current work. For example, lung inflammation may be reduced by PEEP and lower tidal volumes,[49] despite the presence of a proinflammatory response even during mild mechanical ventilation.[50] Also, in contrast to controlled ventilation, noisy pressure support ventilation could modify inflammation by changing lung mechanics and gas exchange[25,26]; (3) effects of mechanical ventilation in normal and injured lungs: mechanical ventilation with low and high tidal volumes affect lungs differently, depending on their previous degree of injury.[51,52] Consequently, regional inflammation magnitude and distribution may differ in the case of previously injured lungs or lungs affected by other injurious mechanisms[51]; (4) interference with anesthesia: because both inhaled[53] and intravenous[54,55] anesthetic agents can modulate inflammation, use of different anesthetic regimens could modify the observed inflammatory pattern.

In summary, marked spatially heterogeneous increases in pulmonary neutrophilic inflammation result from mild short-term endotoxemia in the presence of heterogeneous lung aeration and perfusion, and large tidal volume mechanical ventilation bounded by clinically acceptable pressure limits. ^{18}F-FDG uptake may be a sensitive early marker of pulmonary neutrophilic inflammation in the studied conditions. The increase in spatial heterogeneity of inflammation was dependent both on regional perfusion and aeration, suggesting that factors beyond gas distribution can contribute to regional neutrophilic inflammation during ALI due to endotoxemia and mechanical ventilation.

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ANESTHESIOLOGY REFLECTIONS

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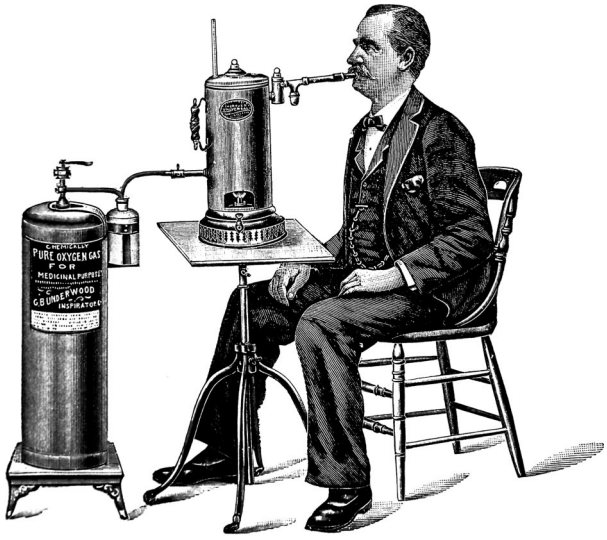

Before extending short-lived corporate branches to Canada and England, New York's G.B. Underwood Inspirator and Oxygen Company had popularized heating and medicating oxygen for treating an astonishing array of afflictions: anemia, asphyxia, asthma, blood poisoning, bronchitis, catarrh, cholera, consumption, croup, diabetes, diphtheria, hay fever, laryngitis, otitis media, pneumonia, tinnitus, typhoid fever, and even tuberculosis (both pulmonary and laryngeal). Advertisements assured that "oxygen heated by the Underwood 20th Century Pulmonary Inspirator is assimilated three times more readily and plentifully than when given cold." Noting that "one cylinder heated will do the work of three cold," G.B. Underwood underscored the economy of the Inspirator for rural physicians, "who have to pay a heavy freight on the oxygen they use in their cases." (Copyright © the American Society of Anesthesiologists, Inc. This image appears in color in the *Anesthesiology Reflections* online collection available at www.anesthesiology.org.)

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