Relation between Shunt, Aeration, and Perfusion in Experimental Acute Lung Injury

Guido Musch¹, Giacomo Bellani^{1,2}, Marcos F. Vidal Melo¹, R. Scott Harris³, Tilo Winkler¹, Tobias Schroeder¹, and Jose G. Venegas¹

¹Department of Anesthesia and Critical Care, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts;

²Department of Experimental Medicine, Ospedale San Gerardo and Università Milano-Bicocca, Monza, Italy; and ³Department of Medicine, Pulmonary and Critical Care Unit, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts

Rationale: In a pulmonary process characterized by spatially heterogeneous loss of aeration, the impairment of gas exchange is expected to depend on the regional distribution of perfusion relative to that of aeration.

Objectives: To investigate how regional aeration, shunt, and perfusion are interrelated at different levels of end-expiratory pressure and how their interplay relates to global shunt fraction in acute lung injury.

Methods: Regional shunt and perfusion were assessed by imaging with positron emission tomography the pulmonary kinetics of [¹³N]nitrogen infused in saline solution in five sheep after lung lavage. The lung field was divided in six horizontal regions.

Measurements and Main Results: Each animal showed an inverse relation between regional shunt (F_s) and gas (F_g) fractions: $F_s = -m \cdot F_g + F_{s0}$. This relation was similar among animals ($m = 1.25 \pm 0.14$, $F_{s0} = 0.75 \pm 0.15$) and invariant with end-expiratory pressure, despite lack of correlation between global shunt and gas fractions and large interanimal variability in global shunt fraction. When this relation was used to estimate global shunt fraction as a perfusion-weighted average of the estimates of regional shunt fraction derived from regional gas fraction, 72% of the interanimal variability in global shunt fraction could be explained.

Conclusions: Despite large interanimal variability in global shunt fraction, there was a consistent inverse relation between regional shunt and gas fractions, independent of end-expiratory pressure. Most of the interanimal variability in global shunt fraction could be explained by the combined effect of this relation and the distribution of perfusion on regional shunt, rather than by differences in global aeration.

Keywords: artificial respiration; adult respiratory distress syndrome; positron emission tomography; mechanical ventilators; X-ray computed tomography

A decrease in lung aeration and the ensuing impairment of oxygenation are defining features of acute lung injury (ALI) and the acute respiratory distress syndrome (1). Because in ALI the loss of aeration is spatially heterogeneous, with poorly aerated lung tissue being predominantly in dependent regions (2–4), the relation between reduction in aeration and impairment of gas exchange may not be straightforward. Indeed, computed tomographic (CT) studies reported conflicting data on this relation.

(Received in original form March 24, 2007; accepted in final form October 11, 2007)

Supported by National Institutes of Health grants HL076464 and HL086827, and by a research grant from the Foundation for Anesthesia Education and Research and the American Society of Critical Care Anesthesiologists.

Correspondence and requests for reprints should be addressed to Guido Musch, M.D., Department of Anesthesia and Critical Care, Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02114. E-mail: gmusch@partners.org

This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 177, pp 292–300, 2008

Originally Published in Press as DOI: 10.1164/rccm.200703-484OC on October 11, 2007

Internet address: www.atsjournals.org

AT A GLANCE COMMENTARY

Scientific knowledge on the subject

Previous studies have reported conflicting results when attempting to relate global loss of aeration to shunt fraction, or Pa_{O_2} , in acute lung injury.

What This Study Adds to the Field

In lavage-induced acute lung injury, we found an inverse relation between regional shunt and gas fractions, which was independent of positive end-expiratory pressure and consistent between animals despite lack of correlation between global shunt and gas fractions and large variability in global shunt.

Although some studies found that shunt fraction, or venous admixture, correlated positively with average lung density (5) and lung mass (6), and negatively with functional residual capacity (6), another study did not find a correlation with the extent of lung opacification (3). When the contribution of density-defined tissue “compartments” to gas exchange impairment was probed, results have been inconsistent. Pa_{O_2} , shunt fraction, or venous admixture, and their changes with positive end-expiratory pressure (PEEP), have been variably reported as strongly (7), moderately (5), or not correlated (6, 8) with the fraction of “nonaerated” lung, defined by CT attenuation greater than –100 Hounsfield units (HU). The fraction of poorly aerated lung tissue, defined by CT attenuation between –500 and –100 HU, was not correlated with Pa_{O_2} or shunt fraction in two studies (5, 7). In contrast, Crotti and coworkers (9) reported a stronger correlation of Pa_{O_2} with the fraction of poorly aerated tissue than with that of nonaerated tissue, and Malbouisson and colleagues (6) found a correlation between PEEP-induced alveolar recruitment and improvement of oxygenation only by accounting for the increase in aeration of both poorly aerated and nonaerated tissue.

A possible explanation for these discrepancies is that, whereas measurements of CT attenuation are usually performed at a fixed airway pressure, commonly at end inspiration or expiration, blood gases are sampled throughout the respiratory cycle. Because Pa_{O_2} varies during the respiratory cycle in the acutely injured lung (10, 11), lack of synchrony between the phases of the cycle at which lung density and blood gases are measured is expected to introduce spurious variability in the relation between shunt fraction and lung aeration. Another possible explanation is that measurements of regional density do not take into account the effect of the regional distribution of perfusion on gas exchange. This effect may be important, as suggested by the fact that impairment of hypoxic pulmonary vasoconstriction with shift of perfusion toward edematous, atelectatic, or consolidated

Figure 1. Protocol schema. *a* = animal number; PEEP = positive end-expiratory pressure, cm H₂O; PET = positron emission tomography. ¹³N₂ = [¹³N]nitrogen.

regions worsened oxygenation (12–14), whereas perfusion redistribution away from edematous regions ameliorated hypoxemia (13, 15). When the loss of aeration is spatially heterogeneous, dissecting the relation between tissue density and *global* shunt requires determination of both *regional* shunt fraction and perfusion. Indeed, previous studies that evaluated regional aeration by CT and shunt by inert gas elimination, which does not enable a spatial analysis of the distribution of shunt or perfusion, could neither ascertain the topographical relation between shunt and density nor isolate the mechanism responsible for the dissociation between PEEP-induced lung recruitment and PEEP's effect on global shunt fraction (16). Accordingly, the need to combine measurements of regional perfusion, shunt, and lung structure relationship in ALI (4) was recently stated.

In this study, we assessed the relation between aeration and shunt fraction in a lung lavage model of ALI. Each animal was studied at two levels of end-expiratory pressure to increase the spectrum of values of aeration and shunt fraction over which this relation was explored. Our aim was to investigate how regional aeration, shunt, and perfusion are interrelated in ALI and how the interplay among these variables is reflected in the

global shunt fraction. To pursue this aim, we used positron emission tomography (PET) imaging of the pulmonary kinetics of [¹³N]nitrogen (¹³N₂) infused intravenously in saline solution to measure regional perfusion and shunt fraction.

Some of the results of this study were previously reported in the form of an abstract (17).

METHODS

Methods used are further described in the online supplement.

Experimental Protocol

In five ventilated sheep (tidal volume, 13.7 ± 2.7 ml/kg; respiratory rate, 18 breaths/min; inspiratory-to-expiratory ratio, 1:1.5; F_{IO₂}, 1), lung injury was induced by lavage (18) with 500 ml (24 ± 2 ml/kg) of 0.2% polysorbate 80 in saline (19, 20). If PaO₂ was higher than 300 mm Hg (21) at 30 minutes after the first lavage, a second lavage (500 ml) was performed. If PaO₂ was lower than 50 mm Hg, PEEP was set at 5 cm H₂O, and increased further to 10 cm H₂O if PaO₂ remained lower than 50 mm Hg. According to this algorithm, animals 1 and 2 underwent two lavages and did not require PEEP, animal 3 underwent a single lavage

Figure 2. Shunt fraction measured from blood gases (F_{sO_2}) versus gas fraction of the imaged lung field (F_{G_L}) at lower (solid circles) and higher (open circles) positive end-expiratory pressure (PEEP). The increment in PEEP reduced F_{sO_2} and increased F_{G_L} in all animals. The animal number is next to each solid circle. There was no significant correlation between F_{sO_2} and F_{G_L} ($r = -0.11$, $P = 0.8$).

without PEEP, whereas animals 4 and 5 required PEEP of 10 and 5 cm H_2O , respectively, after a single lavage (Figure 1). After injury, the animals were placed in supine position in the PET scanner and ventilated at previous settings (Table E1 of the online supplement). Five minutes after a “recruitment maneuver” to 50 cm H_2O , physiologic data were collected and PET scans acquired to measure regional aeration, shunt, and perfusion at the baseline PEEP level (lower PEEP). PEEP was then increased to 15 cm H_2O in all animals (higher PEEP) and, 20 minutes later, physiologic data and PET scans were collected. At least 2 hours passed between the last lung lavage and measurements at lower PEEP.

PET Imaging

The PET scanner imaged 15 slices of thorax, corresponding to approximately 70% of the lung (22).

Transmission (density) scan. This scan was used to demarcate the lung field and to calculate gas fraction (23–26) of the lung (F_{G_L}) and of six horizontal regions of interest (ROIs) of equal height (F_{G_i} , $i = 1$ –6, in the ventral-to-dorsal direction). Relative lung volume was calculated as the ratio of the number of voxels within an ROI (n_i) to that in the lung field (n_L). Ventral-to-dorsal and cranial-to-caudal gas fraction gradients were calculated by linear regression (25).

$^{13}N_2$ -saline bolus infusion (perfusion) scan. $^{13}N_2$ dissolved in saline was infused intravenously over the initial 3 seconds of a 60-second apnea performed at mean airway pressure (20, 27, 28). Because of the low solubility of nitrogen, the pulmonary kinetics of $^{13}N_2$ during apnea, measured with sequential PET frames, differ between regions that are perfused and aerated, in which virtually all $^{13}N_2$ accumulates in the alveolar airspace in proportion to regional perfusion (25, 29), and regions with shunting alveolar units. In the latter, the concentration of $^{13}N_2$ reaches a peak in early apnea, related to regional perfusion, followed by a decrease during the remainder of apnea. This decrease is related to regional shunt, because flooded, atelectatic, or consolidated units do not retain $^{13}N_2$ (20, 22, 28). By fitting the time-activity curve of $^{13}N_2$ with a tracer kinetic model (30, 31), regional perfusion ($\dot{Q}_i$), expressed as a fraction of perfusion to the imaged lung, and shunt fraction (F_{s_i}) were estimated for each ROI in which the coefficient of determination of the model fit was higher than 0.975. To determine the contribution of an ROI to global shunt fraction, relative regional shunted perfusion was calculated as follows: $\dot{Q}_{s_i} = \dot{Q}_i \cdot F_{s_i}$, where $\dot{Q}_{s_i}$ represents the fraction of imaged perfusion shunted within an ROI.

Statistical Analysis

Comparisons between lower and higher PEEP were performed with the Sign test (32), comparisons between ROIs with the Friedman test (33).

TABLE 1. GLOBAL PHYSIOLOGIC VARIABLES

	Lower PEEP	Higher PEEP
Pa_{CO_2} , mm Hg	36 ± 3	38 ± 7
pHa	7.43 ± 0.07	7.40 ± 0.08
Pa_{O_2} , mm Hg	118 ± 74	176 ± 97
$P\bar{V}_{CO_2}$, mm Hg	41 ± 3	43 ± 6
pH $\bar{V}$	7.41 ± 0.06	7.37 ± 0.07
$P\bar{V}_{O_2}$, mm Hg	42 ± 11	48 ± 15
F_{sO_2}	0.41 ± 0.22	0.31 ± 0.14
F_{G_L}	0.38 ± 0.04	0.52 ± 0.05
CO, L/min	3.5 ± 1.2	3.0 ± 1.1
HR, beats/min	93 ± 17	98 ± 23
MAP, mm Hg	88 ± 11	83 ± 20
MPAP, mm Hg	16 ± 8	20 ± 8
PCWP, mm Hg	5 ± 4	7 ± 3
T, °C	36.1 ± 1.7	36.0 ± 1.6

Definition of abbreviations: CO = cardiac output; F_{sO_2} = global shunt fraction (Berggren equation); F_{G_L} = global gas fraction; HR = heart rate; MAP = mean arterial pressure; MPAP = mean pulmonary arterial pressure; PCWP = pulmonary capillary wedge pressure; PEEP = positive end-expiratory pressure; pHa = arterial pH; pH $\bar{V}$ = mixed venous pH; T = body temperature.

Data presented are mean $\pm$ SD.

* $P < 0.05$, lower versus higher PEEP (Sign test).

RESULTS

Global Physiologic Variables

At lower PEEP, there was large interanimal variability in Pa_{O_2} and shunt fraction (F_{sO_2}), whereas the range of variation of gas fraction was narrow (Figure 2). Consequently, the coefficient of variation of F_{sO_2} was fivefold greater than that of F_{G_L} (0.54 vs. 0.11). As expected, in all animals the increment in PEEP improved Pa_{O_2} (Table 1), reduced shunt fraction, and increased gas fraction (Figure 2). The coefficient of variation of F_{sO_2} remained fivefold greater than that of F_{G_L} at higher PEEP (0.45 vs. 0.09). F_{sO_2} was not correlated with F_{G_L} ($r = -0.11$, $P = 0.8$). Shunt fraction of the imaged lung measured by PET (F_{s_L}) was correlated with F_{sO_2} ($F_{sO_2} = 0.97 \cdot F_{s_L} + 0.05$; $r = 0.93$, $P < 0.001$).

Relation between Regional Aeration, Shunt, and Perfusion

All animals had a vertical gradient of gas fraction favoring nondependent (ventral) regions (Table E2). The gradient was steeper at lower PEEP than at higher PEEP (0.039 ± 0.013 vs. 0.029 ± 0.017 cm $^{-1}$, respectively; $P < 0.05$). This ventral-to-dorsal gradient was significantly greater than the absolute value of the cranial-to-caudal gradient (0.034 ± 0.015 vs. 0.008 ± 0.003 cm $^{-1}$, respectively; $P < 0.01$). Regional $^{13}N_2$ time-activity curves (Figure 3) revealed a vertical dependency of gas exchange impairment: From ROI 4 to ROI 6, there was a progressively greater drop of tracer activity during the latter part of apnea, consistent with worsening shunt.

For each animal, there was an inverse relation between regional shunt (F_s) and gas (F_G) fractions (Figure 4):

$$F_s = -m \cdot F_G + F_{s_0} \quad (1)$$

From less dependent (ventral) to more dependent (dorsal), the ROIs showed progressively higher shunt and lower gas fraction. The interanimal variability in the regression parameters was low, as coefficients of variation for m and F_{s_0} were, respectively, 0.11 and 0.19. Consequently, a single relation could fit all data points ($F_s = -1.27 \cdot F_G + 0.77$; $r = -0.90$, $P < 0.0001$). This relation remained essentially unchanged when only the data at lower PEEP were fitted ($F_s = -1.37 \cdot F_G + 0.79$; $r = -0.90$, $P < 0.0001$). Thus, the effect of increasing PEEP was to shift data

Figure 3. $[^{13}\text{N}]$ nitrogen ($^{13}\text{N}_2$) pulmonary kinetics and positron emission tomography images from animal 5 at lower (*upper panel*) and higher (*lower panel*) positive end-expiratory pressure (PEEP). For the tracer kinetics, the imaged lung field was divided in six horizontal regions of interest (ROIs) of equal height. Each symbol shape corresponds to the same ROI at lower (*solid symbols*) and higher (*open symbols*) PEEP. The ROI symbol shape succession in the dependent (dorsal) to nondependent (ventral) direction is as follows: ROI 6, *diamond*; ROI 5, *inverted triangle*; ROI 4, *square*; ROI 3, *triangle*; ROI 2, *hexagon*; ROI 1, *X*. Images of one cross-sectional slice of lung are shown for illustration. A bolus of $^{13}\text{N}_2$ in saline solution was infused intravenously over 3 s at the beginning of a 60-s apnea (*left of vertical dotted line*). The distribution of $^{13}\text{N}_2$ during early apnea (between 5 and 10 s) reflects regional perfusion (peak apnea image). The distribution of $^{13}\text{N}_2$ at the end of apnea (between 40 and 60 s) is proportional to perfusion only to aerated alveolar units, which retain $^{13}\text{N}_2$ during apnea (end-apnea image). A drop in tracer activity from a peak in early apnea to a plateau in late apnea reflects the presence of shunting units, which do not retain $^{13}\text{N}_2$. Note that this drop became progressively greater from ROI 4 to ROI 6, reflecting the progressive increase of regional shunt fraction along dependent ROIs (*see Figure 4*). In contrast, tracer kinetics in nondependent ROIs resembled those of a nonshunting lung, in which tracer activity rises rapidly toward a plateau proportional to regional perfusion. The PEEP increment reduced the magnitude of the drop in tracer activity from peak to plateau in ROIs 4 through 6, reflecting the reduction of shunt fraction in dependent ROIs (*see Figure 4*). The reduction of regional shunt can be visually appreciated from the images: whereas at lower PEEP there was a substantial drop of tracer activity between peak and end-apnea images in the dorsal lung (*arrowheads*), this drop was considerably smaller at higher PEEP, indicating that aeration and gas exchange had been partly restored by the PEEP increment.

points corresponding to dependent ROIs (i.e., ROIs 4–6) down and to the right along the F_s versus F_G relation (Figure 4). The improvement of regional shunt due to the PEEP increment could be visualized from the PET images (Figure 3): Compared with those at lower PEEP, images at higher PEEP showed increased $^{13}\text{N}_2$ retention at the end of apnea in the dorsal lung, indicating restored aeration and gas exchange.

To assess whether global shunt fraction could be recovered from regional aeration if the distribution of perfusion (Figure 5) and the relation between regional shunt and gas fractions (Equation 1) were taken into account, we calculated for each animal at each PEEP level:

$$F_{s\text{EST}} = \sum_i [\dot{Q}_i \cdot (-m \cdot F_{Gi} + F_{s0})]$$

where the summation was performed over the ROIs for which, in a given animal at a given PEEP, $\dot{Q}_i$ could be estimated from the tracer kinetic model (Figure 5). $F_{s\text{EST}}$ thus represents the perfusion-weighted average of the estimates of regional shunt fraction derived from measurements of regional gas fraction through the relation expressed by Equation 1. $F_{s\text{EST}}$ was correlated with $F_{s\text{O}_2}$ ($F_{s\text{O}_2} = 0.87 \cdot F_{s\text{EST}} + 0.07$; $r = 0.85$, $P < 0.01$), and most of the interanimal variance in $F_{s\text{O}_2}$ ($r^2 = 0.72$) was explained by this relation (Figure 6).

In contrast to the vertical distribution of perfusion, which depended on the size of the ROIs and reflected the shape of the lung (Figure 5), perfusion relative to lung volume [$\dot{Q}_i \cdot (n_L/n_i)$] increased from nondependent (ventral) to dependent (dorsal) ROIs (Figure 7).

Figure 4. Regional shunt (F_s) and gas (F_G) fractions in horizontal regions of interest (ROIs) at lower (solid symbols) and higher (open symbols) positive end-expiratory pressure (PEEP). The ROI symbol shape succession in the dependent (dorsal) to nondependent (ventral) direction is as follows: ROI 6, diamond; ROI 5, inverted triangle; ROI 4, square; ROI 3, triangle; ROI 2, hexagon. For each animal, there was an inverse relation between regional shunt and gas fractions: $F_s = -m \cdot F_G + F_{s0}$ (Equation 1 in RESULTS). When data from all animals were pooled (bottom right panel: ALL), a single equation could describe this relation: $F_s = -1.27 \cdot F_G + 0.77$; $r = -0.90$, $P < 0.0001$ (solid line). This equation remained essentially unchanged when only the data at lower PEEP (solid circles) were fitted: $F_s = -1.37 \cdot F_G + 0.79$; $r = -0.90$, $P < 0.0001$ (dotted line).

Regional Shunted Perfusion

Relative shunted perfusion varied significantly between ROIs both at lower ($P < 0.01$) and higher ($P < 0.05$) PEEP (Figure 5). Although gas fraction was systematically lower in ROI 6 compared with ROI 5 both at lower ($P < 0.05$) and higher ($P < 0.05$) PEEP (Figure 4), shunted perfusion was highest, on average, in ROI 5.

The net effect of the PEEP increment on shunted perfusion of the most dependent (dorsal) ROI (i.e., $\dot{Q}_{s6} = \dot{Q}_6 \cdot F_{s6}$) was the result of two processes with competing effects: a systematic increase in aeration with an ensuing reduction in regional shunt fraction (Figure 4), which tended to decrease shunted perfusion, and a shift of perfusion toward the most dependent ROI (Figure 5), which tended to increase shunted perfusion. When the shift of perfusion outweighed the beneficial effect of the increase in aeration, as in ROI 6 of animal 1, the net effect was an increase, rather than a decrease, of regional shunted perfusion at the higher PEEP.

DISCUSSION

The main result of this study was the finding of an inverse relation between regional shunt and gas fractions that was systematic and consistent both within and between animals, and unaffected by PEEP, despite lack of relation between global shunt and gas fractions and large interanimal variability in global shunt fraction. A secondary result was the experimental

demonstration that a shift of perfusion can act as a mechanism of dissociation between the PEEP-induced gain in aeration of a nonaerated region (i.e., “anatomical” recruitment) and the ensuing contribution of that region to global shunt fraction (i.e., “functional” recruitment).

Rationale and Critique of the Experiment

We chose the lung lavage model because it presents the vertical distribution of lung density characteristic of ALI (34, 35) and maintains significant recruitment with sufficient PEEP (36–39). Therefore, this model appeared likely to allow investigation of the relation between shunt and gas fractions over a wide spectrum of lung inflation, especially with varying levels of PEEP. Because lower PEEP was set according to oxygenation and could hence be expected to differ between animals, we chose a single higher PEEP to assess whether any interanimal variability present at lower PEEP was maintained at a uniform PEEP. Although the lavage model does not encompass the pathophysiologic complexity of human ALI, it is likely to reflect several of its features, such as surfactant dysfunction (40, 41), atelectasis (18), and edema (36, 38). Addition of a detergent (19, 21, 39) allows the target hypoxemia to be reached with a lower volume of fluid than pure saline (34, 35, 41). More important, the detergent inactivates any surfactant produced after injury, affording the model to remain stable over time (19). Although the percentage of lavage fluid recovered ($60.1 \pm 0.3\%$) was lower than in other studies (34, 41), the amount of fluid retained (289 ± 104 ml) was similar.

Figure 5. Perfusion fraction in ventral-to-dorsal regions of interest (ROIs 1–6) for each animal and their average (bottom right panel). For each ROI, the *solid bars* represent measurements at lower positive end-expiratory pressure (PEEP) and the *open bars* the measurements at higher PEEP. The total length of the *bar* represents the fraction of perfusion going to the corresponding ROI (i.e., relative perfusion, $\dot{Q}_i$). The length of the *hatched* portion of the *bar* corresponds to the fraction of imaged perfusion being shunted in the ROI (i.e., relative shunted perfusion, $\dot{Q}_{si} = \dot{Q}_i \cdot F_{si}$), which represents the ROI's contribution to global shunt fraction. The remaining length of the *bar* corresponds to the fraction of perfusion participating in gas exchange within the ROI. Note that shunted perfusion increased in ROI 6 of animal 1 at higher compared with lower PEEP. $\dot{Q}_i$ was significantly ($^{\dagger}P < 0.01$) different between ROIs within each PEEP level (Friedman test). $\dot{Q}_{si}$ was also significantly different between ROIs at lower ($^{\dagger}P < 0.01$) and higher ($^{\S}P < 0.05$) PEEP. Perfusion is not reported for ROIs with a coefficient of determination of the tracer kinetic model fit lower than 0.975, which corresponded to the most ventral ROIs with very low blood flow.

To prevent alveolar derecruitment throughout the apnea (10, 35) and to match lung volume during the $^{13}\text{N}_2$ scan, at which regional shunt was measured, with mean lung volume during the transmission scan, at which aeration was measured, apnea was performed at an airway pressure equal to mean airway pressure during breathing. Blood gas samples were drawn over two respiratory cycles to minimize variability due to sample timing (11). Accordingly, measurements of shunt and aeration should reflect the physiologic condition of the lung at approximately mean lung volume and be comparable to measurements of lung density obtained by averaging dynamic CT frames over consecutive respiratory cycles (42, 43). This approach should minimize the spurious variability that could be introduced in the relation between shunt and aeration by lack of temporal registration between gas exchange and lung density measurements.

We used an FiO_2 of 1 to be consistent with the majority of previous studies that probed the relation between shunt fraction, or PaO_2 , and lung density (6, 7, 34, 38, 42, 43). At high FiO_2 , the contribution of alveolar units with low ventilation-to-perfusion ratio to shunt fraction measured with the Berggren equation is expected to decrease. If poorly aerated regions represented low ventilation-to-perfusion units, it could be argued that high FiO_2 would lead to underestimating their contribution to hypoxemia. Although discrepancies in the literature could be

partly related to differences in FiO_2 , inconsistencies regarding the effect of poorly aerated tissue, as defined by CT, on shunt or PaO_2 persist among studies performed at similar FiO_2 , whether it is 1 (6, 7, 42, 43) or less (5, 9), indicating that differences in FiO_2 alone can not explain those discrepancies. It is possible that, had we used lower FiO_2 , shunt would have been less, because an FiO_2 of 1 is expected to promote resorption atelectasis (44) and blunt hypoxic pulmonary vasoconstriction in shunting regions through its effect on mixed venous PO_2 (45).

The rationale for the analysis by horizontal ROIs was the large and systematic variation of gas fraction in the ventral-to-dorsal direction (Table E2). Despite the fact that the ROIs were large, the variation of aeration and perfusion across these ROIs was sufficient to capture the major source of interanimal variability in shunt fraction. However, variation of aeration in the cranial-to-caudal direction, which occurs in ALI (2), may also be important, as suggested by the fact that animals 4 and 5, with the most severe hypoxemia, had lower gas fraction in caudal compared with cranial lung, whereas the other animals had higher gas fraction in caudal lung (Table E2).

Relation between Shunt Fraction, Aeration, and Perfusion

Despite absence of any significant relation between global shunt and gas fractions, the inverse relation between regional shunt

Figure 6. (A) Shunt fraction measured from blood gases (FS_{O_2}) versus that estimated (FS_{EST}) from regional gas fraction (FG_i ; Figure 4), perfusion fraction (Q_i ; Figure 5), and the regional relation between shunt and gas fractions ($FS = -m \cdot FG + FS_0$; Equation 1 in RESULTS and Figure 4) as follows: $FS_{EST} = \sum_i [Q_i \cdot (-m \cdot FG_i + FS_0)]$. The numbers next to each circle denote the corresponding animal at lower (solid circles) and higher (open circles) positive end-expiratory pressure. (B) Bland-Altman plot of the relation between FS_{EST} and FS_{O_2} . Bias was 0.023 (solid line) and limits of agreement (i.e., bias $\pm 1.96 \cdot SD$) were 0.218 and -0.172 (dotted lines).

and gas fractions was consistent among animals, as shown by the low coefficient of variation of the regression parameters. Furthermore, this relation was not affected by the level of PEEP, because a single equation could fit all data at lower PEEP, even though lower PEEP varied from 0 to 10 cm H_2O , and this equation remained essentially unchanged when data obtained at PEEP of 15 cm H_2O were added. These results differ from previous ones in several respects. Tokics and coworkers (16) reported a linear relation between fraction of atelectatic lung and global shunt fraction in anesthetized healthy subjects. In contrast, we could not confirm a similar relation in this model of ALI. Although the marked variability in global shunt fraction was unrelated to global gas fraction, most of this variability could be recovered from an estimate of global shunt fraction that accounted for the effects of the inverse relation between regional shunt and gas fractions and of regional perfusion on regional shunted perfusion (Figure 6). The disconnect between regional and global behavior is potentially clinically relevant because it suggests that, whereas regional shunt fraction is tightly linked to regional aeration, global indices of hypoxemia may not reflect the severity of loss of aeration in ALI. Accordingly, a high shunt fraction or low $Pa_{O_2}/F_{I_{O_2}}$ may not

be sensitive indicators of loss of aeration and hence may not be suited, for example, to assess lung derecruitment or stratify patients for their risk of ventilator-associated lung injury. Mechanisms such as hypoxic pulmonary vasoconstriction (12–15) or vascular compression and obstruction (46) may alter the distribution of perfusion and thus be responsible for the global to regional disconnect in ALI.

Another difference with the findings of Tokics and colleagues (16) is that, whereas they showed that the slope of the relation between fraction of atelectatic lung and global shunt fraction changed with PEEP, we found that the regional relation between shunt and gas fractions was invariant with PEEP. In the absence of regional measurements of perfusion and shunt, Tokics and coworkers (16) were admittedly unable to isolate the mechanism responsible for that change of slope. Combination of topographical measurements of perfusion and shunt allowed us to demonstrate that PEEP-induced shift of perfusion toward dependent regions, consistent with previous results (47–49), can dampen or even outweigh the beneficial effect of improved aeration on regional shunt. This observation could offer one explanation for the dissociation that can occur between PEEP-induced lung recruitment and variation in gas exchange in ALI (50). It also lends experimental evidence to the speculation that a shift of perfusion toward atelectatic areas was the most likely mechanism for the PEEP-induced change in slope of the relation between fraction of atelectatic lung and global shunt fraction observed in healthy anesthetized subjects (16).

In line with previous results (27), ROIs showed a progressive increase in shunt fraction as gas fraction decreased below approximately 0.5. This observation, combined with the notion that “true” shunt is the main mechanism of gas exchange impairment in ALI at high $F_{I_{O_2}}$ (51, 52), suggests that the inverse relation between regional shunt and gas fractions is due to a “quantal” loss of gas-exchanging units, so that the decrease in aeration of a region is not apportioned uniformly among its units, but is absorbed only by some of them, which become atelectatic, while the remaining units preserve their aeration and gas exchange. The progressive increase in regional shunt fraction could thus be the effect of an increase in the ratio of shunting to normally aerated alveoli. This could provide an explanation, in addition to the lack of temporal registration between gas exchange and CT measurements, for variable findings on the relation between global shunt fraction, or Pa_{O_2} , and CT-defined lung compartments (3, 5, 7–9, 42, 43). Indeed, what is defined as poorly aerated lung over the resolution element, be it a voxel or an ROI, could comprise nonaerated shunting units and units with normal ventilation-to-perfusion ratio. The contribution to global shunt fraction, however, will be a function of the partitioning of perfusion between these two types of units and of total perfusion, both of which may vary depending on several factors (12–15, 46). The systematic relation between regional shunt and gas fractions (Figure 4) is also compatible with the strong correlation reported between fraction of lung with CT attenuation greater than -300 HU and global shunt fraction (42, 43). Because the increase in regional shunt fraction is progressive as gas fraction decreases below 0.5, a CT threshold close to -500 HU will tend to overestimate global shunt fraction, whereas a threshold close to -100 HU will underestimate it. An intermediate threshold, around -300 HU, would be expected to yield the best correlation. As Figure 4 shows, however, this should not be interpreted as indicating that a single threshold can separate the shunting and nonshunting compartments, at least as long as spatial resolution remains lower than the alveolar scale.

The observation that nondependent ROIs were hypoperfused relative to their lung volume (Figure 7), but had normal gas fraction (Figure 4), likely represents the regional basis of

Figure 7. Perfusion fraction ($\dot{Q}_i$) normalized by relative lung volume (n_i/n_L) in ventral-to-dorsal regions of interest (ROIs 1–6) at lower (solid bars) and higher (open bars) positive end-expiratory pressure (PEEP). $\dot{Q}_i \cdot (n_L/n_i)$ is expected to be unity when perfusion is distributed proportionally to lung volume. $\dot{Q}_i \cdot (n_L/n_i)$ increased from lower than unity in nondependent (ventral) ROIs to greater than unity in the most dependent (dorsal) ROIs. This vertical increase was significant at both lower and higher PEEP. $^{\dagger}P < 0.01$ (Friedman test).

our previous finding that the aerated alveolar volume of distribution of infused $^{13}\text{N}_2$ is lower than intrapulmonary gas volume in supine sheep that underwent lung lavage (31). Thus, in ALI, not only functional residual capacity is reduced but the volume of gas participating in gas exchange may be further decreased because of the disparity in the distribution of gas content relative to that of perfusion.

In summary, in an experimental model of ALI with spatially heterogeneous loss of aeration, we found a systematic inverse relation between regional, but not global, shunt and gas fractions. This relation was not affected by PEEP. This relation appears to be relevant to understand the gas exchange effects of changes in regional aeration because most of the interanimal variability in global shunt fraction could be explained by the combined effect of this relation and of the distribution of perfusion on regional shunted perfusion, rather than by differences in global aeration.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

Acknowledgment: The authors thank Kevin R. O'Neill, M.D., for tracer kinetic modeling; Steven B. Weise for technical assistance with image acquisition; John A. Correia, Ph.D., William M. Buceliewicz, and David F. Lee, B.S., for preparation of the radioisotope; O. Syrkina, M.D., for assistance with animal preparation; and Warren M. Zapol, M.D., for continued support of this project.

References

1. Falke KJ, Pontoppidan H, Kumar A, Leith DE, Geffin B, Laver MB. Ventilation with end-expiratory pressure in acute lung disease. *J Clin Invest* 1972;51:2315–2323.
2. Puybasset L, Cluzel P, Chao N, Slutsky AS, Coriat P, Rouby JJ. A computed tomography scan assessment of regional lung volume in acute lung injury. *Am J Respir Crit Care Med* 1998;158:1644–1655.
3. Goodman LR, Fumagalli R, Tagliabue P, Tagliabue M, Ferrario M, Gattinoni L, Pesenti A. Adult respiratory distress syndrome due to pulmonary and extrapulmonary causes: CT, clinical, and functional correlations. *Radiology* 1999;213:545–552.
4. Gattinoni L, Caironi P, Pelosi P, Goodman LR. What has computed tomography taught us about the acute respiratory distress syndrome? *Am J Respir Crit Care Med* 2001;164:1701–1711.
5. Gattinoni L, Pesenti A, Bombino M, Baglioni S, Rivolta M, Rossi F, Rossi G, Fumagalli R, Marcolin R, Mascheroni D, *et al.* Relationships between lung computed tomographic density, gas exchange, and PEEP in acute respiratory failure. *Anesthesiology* 1988;69:824–832.
6. Malbouisson LM, Muller JC, Constantin JM, Lu Q, Puybasset L, Rouby JJ. Computed tomography assessment of positive end-expiratory pressure-induced alveolar recruitment in patients with acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2001;163:1444–1450.
7. Borges JB, Okamoto VN, Matos GFJ, Caramaz MPR, Arantes PR, Barros F, Souza CE, Victorino JA, Kacmarek RM, Barbas CSV, *et al.* Reversibility of lung collapse and hypoxemia in early acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2006;174:268–278.

8. Brunet F, Jeanbourquin D, Monchi M, Mira JP, Fierobe L, Armaganidis A, Renaud B, Belghith M, Nouira S, Dhainaut JF, *et al.* Should mechanical ventilation be optimized to blood gases, lung mechanics, or thoracic CT scan? *Am J Respir Crit Care Med* 1995;152:524–530.
9. Crotti S, Mascheroni D, Caironi P, Pelosi P, Ronzoni G, Mondino M, Marini JJ, Gattinoni L. Recruitment and derecruitment during acute respiratory failure: a clinical study. *Am J Respir Crit Care Med* 2001;164:131–140.
10. Baumgardner JE, Markstaller K, Pfeiffer B, Doebrich M, Otto CM. Effects of respiratory rate, plateau pressure, and positive end-expiratory pressure on PaO_2 oscillations after saline lavage. *Am J Respir Crit Care Med* 2002;166:1556–1562.
11. Pfeiffer B, Syring RS, Markstaller K, Otto CM, Baumgardner JE. The implications of arterial PO_2 oscillations for conventional arterial blood gas analysis. *Anesth Analg* 2006;102:1758–1764.
12. Gust R, Kozlowski J, Stephenson AH, Schuster DP. Synergistic hemodynamic effects of low-dose endotoxin and acute lung injury. *Am J Respir Crit Care Med* 1998;157:1919–1926.
13. Brimiouille S, Julien V, Gust R, Kozlowski JK, Naeije R, Schuster DP. Importance of hypoxic vasoconstriction in maintaining oxygenation during acute lung injury. *Crit Care Med* 2002;30:874–880.
14. Easley RB, Fuld MK, Fernandez-Bustamante A, Hoffman EA, Simon BA. Mechanism of hypoxemia in acute lung injury evaluated by multidetector-row CT. *Acad Radiol* 2006;13:916–921.
15. Schuster DP, Anderson C, Kozlowski J, Lange N. Regional pulmonary perfusion in patients with acute pulmonary edema. *J Nucl Med* 2002;43:863–870.
16. Tokics L, Hedenstierna G, Strandberg A, Brismar B, Lundquist H. Lung collapse and gas exchange during general anesthesia: effects of spontaneous breathing, muscle paralysis, and positive end-expiratory pressure. *Anesthesiology* 1987;66:157–167.
17. Musch G, Bellani G, O'Neill KR, Harris RS, Vidal Melo MF, Winkler T, Venegas JG. Functional PET imaging of the effect of PEEP on regional gas exchange in acute lung injury [abstract]. *Am J Respir Crit Care Med* 2004;169:A545.
18. Lachmann B, Robertson B, Vogel J. In vivo lung lavage as an experimental model of the respiratory distress syndrome. *Acta Anaesthesiol Scand* 1980;24:231–236.
19. Tsuzaki K, Hales CA, Strieder DJ, Venegas JG. Regional lung mechanics and gas transport in lungs with inhomogeneous compliance. *J Appl Physiol* 1993;75:206–216.
20. Musch G, Harris RS, Vidal Melo MF, O'Neill KR, Layfield JDH, Winkler T, Venegas JG. Mechanism by which a sustained inflation can worsen oxygenation in acute lung injury. *Anesthesiology* 2004;100:323–330.
21. DiRocco JD, Pavone LA, Carney DE, Lutz CJ, Gatto LA, Landas SK, Nieman GF. Dynamic alveolar mechanics in four models of lung injury. *Intensive Care Med* 2006;32:140–148.
22. Vidal Melo MF, Layfield D, Harris RS, O'Neill K, Musch G, Richter T, Winkler T, Fischman AJ, Venegas JG. Quantification of regional ventilation-perfusion ratios with PET. *J Nucl Med* 2003;44:1982–1991.
23. Rhodes CG, Valind SO, Brudin LH, Wollmer PE, Jones T, Buckingham PD, Hughes JMB. Quantification of regional V/Q ratios in humans by use of PET. II. Procedure and normal values. *J Appl Physiol* 1989;66:1905–1913.
24. Brudin LH, Rhodes CG, Valind SO, Jones T, Jonson B, Hughes JMB. Relationships between regional ventilation and vascular and extravascular volume in supine humans. *J Appl Physiol* 1994;76:1195–1204.
25. Musch G, Layfield JDH, Harris RS, Vidal Melo MF, Winkler T, Callahan RJ, Fischman AJ, Venegas JG. Topographical distribution of pulmonary perfusion and ventilation, assessed by PET in supine and prone humans. *J Appl Physiol* 2002;93:1841–1851.
26. Vidal Melo MF, Harris RS, Layfield D, Musch G, Venegas JG. Changes in regional ventilation after autologous blood clot pulmonary embolism. *Anesthesiology* 2002;97:671–681.
27. Richter T, Bellani G, Harris RS, Vidal Melo MF, Winkler T, Venegas JG, Musch G. Effect of prone position on regional shunt, aeration, and perfusion in experimental acute lung injury. *Am J Respir Crit Care Med* 2005;172:480–487.
28. Musch G, Venegas JG, Bellani G, Winkler T, Schroeder T, Petersen B, Harris RS, Vidal Melo MF. Regional gas exchange and cellular metabolic activity in ventilator-induced lung injury. *Anesthesiology* 2007;106:723–735.
29. Mijailovich SM, Treppo S, Venegas JG. Effects of lung motion and tracer kinetics corrections on PET imaging of pulmonary function. *J Appl Physiol* 1997;82:1154–1162.
30. Galletti GG, Venegas JG. Tracer kinetic model of regional pulmonary function using positron emission tomography. *J Appl Physiol* 2002;93:1104–1114.
31. O'Neill K, Venegas JG, Richter T, Harris RS, Layfield JDH, Musch G, Winkler T, Vidal Melo MF. Modeling kinetics of infused ^{13}N -saline in acute lung injury. *J Appl Physiol* 2003;95:2471–2484.
32. Armitage P, Berry G. Statistical methods in medical research, 2nd ed. Oxford, UK: Blackwell Scientific Publications; 1987.
33. Glantz SA. Primer of biostatistics, 6th ed. New York: McGraw-Hill; 2005.
34. Luecke T, Roth H, Herrmann P, Joachim A, Weisser G, Pelosi P, Quintel M. PEEP decreases atelectasis and extravascular lung water but not lung tissue volume in surfactant-washout lung injury. *Intensive Care Med* 2003;29:2026–2033.
35. Neumann P, Berglund JE, Fernandez Mondejar E, Magnusson A, Hedenstierna G. Dynamics of lung collapse and recruitment during prolonged breathing in porcine lung injury. *J Appl Physiol* 1998;85:1533–1543.
36. Downie JM, Nam AJ, Simon BA. Pressure-volume curve does not predict steady-state lung volume in canine lavage lung injury. *Am J Respir Crit Care Med* 2004;169:957–962.
37. Van der Kloot TE, Blanch L, Youngblood AM, Weinert C, Adams AB, Marini JJ, Shapiro RS, Nahum A. Recruitment maneuvers in three experimental models of acute lung injury. *Am J Respir Crit Care Med* 2000;161:1485–1494.
38. Luecke T, Meinhardt JP, Herrmann P, Weiss A, Quintel M, Pelosi P. Oleic acid vs saline solution lung lavage-induced acute lung injury. *Chest* 2006;130:392–401.
39. Halter JM, Steinberg JM, Schiller HJ, DaSilva M, Gatto LA, Landas S, Nieman GF. Positive end-expiratory pressure after a recruitment maneuver prevents both alveolar collapse and recruitment/derecruitment. *Am J Respir Crit Care Med* 2003;167:1620–1626.
40. Spragg RG, Lewis JF. Pathology of the surfactant system of the mature lung. *Am J Respir Crit Care Med* 2001;163:280–282.
41. Lewis J, McCaig L, Hafner D, Spragg R, Veldhuizen R, Kerr C. Dosing and delivery of a recombinant surfactant in lung-injured adult sheep. *Am J Respir Crit Care Med* 1999;159:741–747.
42. Markstaller K, Kauczor HU, Weiler N, Karmrodt J, Doebrich M, Ferrante M, Thelen M, Eberle B. Lung density distribution in dynamic CT correlates with oxygenation in ventilated pigs with lavage ARDS. *Br J Anaesth* 2003;91:699–708.
43. David M, Karmrodt J, Bletz C, David S, Herweling A, Kauczor HU, Markstaller K. Analysis of atelectasis, ventilated, and hyperinflated lung during mechanical ventilation by dynamic CT. *Chest* 2005;128:3757–3770.
44. Rothen HU, Sporre B, Engberg G, Wegenius G, Reber A, Hedenstierna G. Prevention of atelectasis during general anaesthesia. *Lancet* 1995;345:1387–1391.
45. Marshall C, Marshall B. Site and sensitivity for stimulation of hypoxic pulmonary vasoconstriction. *J Appl Physiol* 1983;55:711–716.
46. Greene R, Zapol WM, Snider MT, Reid L, Snow R, O'Connell RS, Novelline RA. Early bedside detection of pulmonary vascular occlusion during acute respiratory failure. *Am Rev Respir Dis* 1981;124:593–601.
47. Schuster DP, Howard DK. The effect of positive end-expiratory pressure on regional pulmonary perfusion during acute lung injury. *J Crit Care* 1994;9:100–110.
48. Kallas HJ, Domino KB, Glenn RW, Anderson EA, Hlastala MP. Pulmonary blood flow redistribution with low levels of positive end-expiratory pressure. *Anesthesiology* 1998;88:1291–1299.
49. Richard JC, Decailliot F, Janier M, Annat G, Guerin C. Effects of positive end-expiratory pressure and body position on pulmonary blood flow redistribution in mechanically ventilated normal pigs. *Chest* 2002;122:998–1005.
50. Gattinoni L, Caironi P, Cressoni M, Chiumello D, Ranieri VM, Quintel M, Russo S, Patroniti N, Cornejo R, Bugedo G. Lung recruitment in patients with the acute respiratory distress syndrome. *N Engl J Med* 2006;354:1775–1786.
51. Dantzker DR, Brook CJ, Dehart P, Lynch JP, Weg JG. Ventilation-perfusion distributions in the adult respiratory distress syndrome. *Am Rev Respir Dis* 1979;120:1039–1052.
52. Matamis D, Lemaire F, Harf A, Teisseire B, Brun-Buisson C. Redistribution of pulmonary blood flow induced by positive end-expiratory pressure and dopamine infusion in acute respiratory failure. *Am Rev Respir Dis* 1984;129:39–44.