

# Changes in Pulmonary Blood Flow during Gaseous and Partial Liquid Ventilation in Experimental Acute Lung Injury

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**Background:** It has been proposed that partial liquid ventilation (PLV) causes a compression of the pulmonary vasculature by the dense perfluorocarbons and a subsequent redistribution of pulmonary blood flow from dorsal to better-ventilated middle and ventral lung regions, thereby improving arterial oxygenation in situations of acute lung injury.

**Methods:** After induction of acute lung injury by repeated lung lavage with saline, 20 pigs were randomly assigned to partial liquid ventilation with two sequential doses of 15 ml/kg perfluorocarbon (PLV group, n = 10) or to continued gaseous ventilation (GV group, n = 10). Single-photon emission computed tomography was used to study regional pulmonary blood flow. Gas exchange, hemodynamics, and pulmonary blood flow were determined in both groups before and after the induction of acute lung injury and at corresponding time points 1 and 2 h after each instillation of perfluorocarbon in the PLV group.

**Results:** During partial liquid ventilation, there were no changes in pulmonary blood flow distribution when compared with values obtained after induction of acute lung injury in the PLV group or to the animals submitted to gaseous ventilation. Arterial oxygenation improved significantly in the PLV group after instillation of the second dose of perfluorocarbon.

**Conclusions:** In the surfactant washout animal model of acute lung injury, redistribution of pulmonary blood flow does not seem to be a major factor for the observed increase of arterial oxygen tension during partial liquid ventilation. (Key words: Acute respiratory distress syndrome; blood flow distribution; perfluorocarbon.)

PARTIAL liquid ventilation (PLV), combining the intrapulmonary instillation of perfluorocarbons in volumes up to the lung's functional residual capacity with conventional mechanical gaseous ventilation (GV),<sup>1</sup> is a new therapeutic strategy to improve gas exchange and ventilation-perfusion distribution in patients with acute respiratory distress syndrome. Numerous studies in animals with and without acute lung injury (ALI) and recent clinical investigations in adult patients and infants with

severe respiratory failure revealed the beneficial effect of this technique on lung mechanics and arterial oxygenation.<sup>1-4</sup>

Perfluorocarbons are dense, biologically inert, radiopaque, hydrophobic compounds with the capacity to dissolve large amounts of oxygen and carbon dioxide. At least two mechanisms are suggested by which the intrapulmonary administration of the liquid can improve gas exchange during PLV. First, the low surface tension of the compound may facilitate the recruitment of atelectatic, consolidated lung segments as indicated by the increase of pulmonary compliance<sup>4</sup> and functional residual capacity<sup>5</sup> observed during PLV. Second, the pooling of the dense perfluorocarbons in the dorsal lung regions might result in a compression of the pulmonary vasculature and cause a redistribution of pulmonary blood flow from the dorsal lung segments to the better-ventilated ventral areas. This has been shown to occur during both total liquid ventilation in an isolated lung preparation<sup>7</sup> and PLV in healthy animals.<sup>8</sup> However, certain forms of ALI can cause alterations of the pulmonary hemodynamics and blood flow as a result of different factors such as microembolism, hypoxic vasoconstriction, and a secondary response to mediator release, which may cause changes in the dispersion of pulmonary blood flow and interfere with the effect of intrapulmonary-instilled perfluorocarbons.

This study was performed to investigate changes in the distribution of pulmonary blood flow in a well-defined surfactant depletion animal model of ALI during PLV and controlled mechanical GV.

## Materials and Methods

The experimental protocol was approved by the appropriate governmental institution (Bezirksregierung Koeln, Koeln, Germany, 23.203.2 AC 38, 21/97), and the study was performed according to the Helsinki convention for the use and care of animals.

### Animal Preparation

After premedication with 5 mg/kg azaperone, 10 mg/kg ketamine, and 0.01 mg/kg atropine intramuscularly, anesthesia was induced in 25 healthy female pigs with a mean body weight of  $29.4 \pm 2.6$  kg (PLV group,  $29.9 \pm 3.6$  kg; GV group,  $29.1 \pm 2.3$  kg; attenuation

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control group,  $29.5 \pm 1.6$  kg) with a bolus of 2–3 mg/kg thiopental intravenously and maintained with the continuous infusion of  $6\text{--}10 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  thiopental and  $0.1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  fentanyl. Muscle relaxation was achieved with  $3 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  pancuronium bromide. The animals were positioned supine and intubated with a 8.0- to 9.0-mm internal diameter endotracheal tube. Volume-controlled ventilation was instituted in all animals using a respirator (Servo 300; Siemens Elema, Lund, Sweden) with a fraction of inspired oxygen ( $\text{Fio}_2$ ) of 1.0, a respiratory rate of 20 breaths/min, a mean tidal volume of 8 ml/kg, a positive end-expiratory pressure of 5 cmH<sub>2</sub>O, and an inspiration-expiration time ratio of 1:2 without pause time. The ventilator setting remained unchanged throughout the entire study protocol.

Arterial access was achieved by introducing a 18-gauge catheter (Vygon, Ecouen, France) into a femoral artery. A pulmonary artery catheter (model 93A-431-7.5 F; Baxter Healthcare Corporation, Irvine, CA) was advanced into a pulmonary artery under transduced pressure guidance through a 8.5-French venous sheath (Arrow Deutschland GmbH, Erding, Germany) positioned in a femoral vein.

The blood temperature, determined by means of the pulmonary artery catheter, was maintained at  $37.5 \pm 1.0^\circ\text{C}$  in all animals during the investigation by use of an infrared warming lamp and a warming pad. A balanced electrolyte solution was administered continuously at  $4\text{--}5 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  to maintain adequate hydration.

#### Data Acquisition

**Hemodynamics.** All hemodynamic measurements were taken with the animal in the supine position. Pressure transducers were calibrated to the center of the lateral chest, and central venous pressure, mean arterial pressure, mean pulmonary arterial pressure, and pulmonary artery occlusion pressure of all animals were transduced (PVP, Kirschseeon/Eglharting, Germany) and recorded (Model CS/3 Compact; Datex, Achim, Germany). Cardiac output was determined using standard thermodilution techniques (Baxter Deutschland GmbH, Unterschleißheim, Germany) and expressed as the mean of three measurements at end-expiration of different respiratory cycles. Heart rate was obtained from the blood pressure curve.

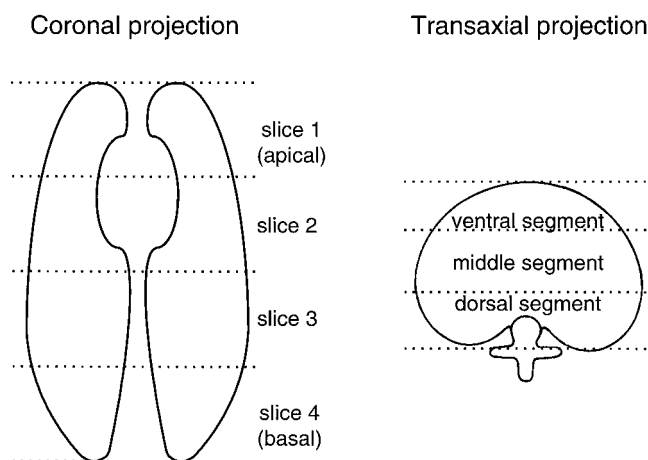
**Gas Exchange.** Arterial and mixed venous blood samples were collected anaerobically and immediately analyzed for partial pressure of oxygen ( $\text{Po}_2$ ), partial pressure of carbon dioxide ( $\text{Pco}_2$ ), and pH using standard blood gas electrodes (ABL 520; Radiometer Copenhagen, Copenhagen, Denmark). Species-specific spectrophotometry was performed to obtain arterial and mixed venous oxygen saturation and total hemoglobin concentration (OSM 3 Hemoximeter; Radiometer Copenhagen, Copenhagen, Denmark).

The oxygen content (ml/dl) of arterial oxygen concentration ( $\text{CaO}_2$ ) and mixed venous oxygen concentration

( $\text{CvO}_2$ ) samples was calculated using the following formula: Content of oxygen = (hemoglobin concentration  $\times 1.34 \times \% \text{O}_2$  – saturation/100) + ( $\text{Po}_2 \times 0.0031$ ). To calculate the pulmonary capillary oxygen content ( $\text{Cco}_2$ ), the pulmonary capillary oxygen tension was assumed to be equivalent to the alveolar  $\text{Po}_2$  which was estimated as follows: Barometric pressure –  $\text{Paco}_2$ /respiratory quotient (assuming that the respiratory quotient = 0.8)<sup>10</sup> – water vapor pressure (47 mmHg) – perfluorocarbon vapor pressure (76 mmHg at  $37.5^\circ\text{C}$  for the perfluorocarbon used in this study, only when PLV was performed).<sup>11</sup> The venous admixture ( $\text{Q}_s/\text{Q}_t$ ) was derived from the standard shunt equation:  $(\text{Cco}_2 - \text{CaO}_2)/(\text{Cco}_2 - \text{CvO}_2)$ . Some reservations have to be made regarding the calculation of shunt during PLV with this formula. As previously outlined by Mates *et al.*,<sup>11</sup> calculated shunt during PLV may not only be attributed to the perfusion of unventilated lung areas but also to some diffusion limitation for oxygen through perfluorocarbon in the ventilated but liquid-filled alveoli. The quantitative effect of this diffusion limitation as well as the correlation between the increase of intrapulmonary perfluorocarbon volume and the increase of calculated shunt cannot be estimated as yet. The impact of ventilation-perfusion heterogeneity on arterial oxygenation seems minimal as long as a  $\text{Fio}_2$  of 1.0 is used.

**Pulmonary Blood Flow.** Macroaggregates of human serum albumin were labeled with  $^{99\text{m}}\text{Tc}$  according to the protocol of the manufacturer ( $^{99\text{m}}\text{Tc}$ -Lyo-MAA, Mallinckrodt Medical B.V., Petten, The Netherlands) who guarantees a size between 10 and  $100 \mu\text{m}$  for 95% of the particles. After the intravenous administration of the radioactive-labeled macroaggregated human serum albumin particles in the supine animals, regional pulmonary blood flow distribution expressed as percentage of total activity measured in both lungs was determined using single-photon emission computed tomography (SPECT) with a triple-head gamma camera (Multispect 3; Siemens Erlangen, Germany) fitted with low-energy high-resolution collimators. SPECT previously has been validated for this purpose by other investigators.<sup>10,12–14</sup> Acquisition parameters were 360-degree rotation with 120 steps of 3 degrees each in a  $128 \times 128$  matrix, with acquisition time depending on the injected activity at each study point and an acquisition zoom of 1.23. Reconstruction was done by filtered back-projection using a Butterworth Filter of order 3.0 and a cutoff frequency of 0.5 cycles/pixel. This resulted in transaxial slices of 3.51 mm. Spatial resolution (full-width, half-maximum) measured with a 0.6-mm line source was 11.7 mm.

Using the ICON 7.1 software (Siemens, Erlangen, Germany), the lungs were divided into four transaxial slices numbered from one for the most apical to four for the most basal (fig. 1). Changes in blood flow distribution between dependent and nondependent lung regions were analyzed by an automatic algorithm; in each trans-



**Fig. 1.** To analyze blood flow distribution, the lung was divided into four equal transaxial slices from apical to basal. Changes in blood flow distribution between dorsal and ventral lung regions were analyzed by subdividing each slice into three coronal segments (ventral, middle, and dorsal) along the vertical axis.

axial slice, a rectangular region of interest, subsequently divided into three geometrically equal segments along the vertical axis (ventral, middle, and dorsal), was drawn around the lung shape using four points representing 13% of the maximum activity. This isocontour-based calculation guarantees an exact reproducibility of region of interest definition.<sup>15,16</sup> In this manner, a total of 12 lung segments was generated for each animal (fig. 1). For each study point, pulmonary blood flow to a particular lung segment was calculated as percentage of the total activity in both lungs using the following formula: Segmental pulmonary blood flow [%] = counts per segment/total counts in both lungs.

#### Experimental Procedure

**Induction of Lung Injury.** Lung injury was induced in all animals in supine position by surfactant depletion caused by repeated lung lavage as previously described and evaluated by Lachmann *et al.*<sup>17,18</sup> Briefly, after disconnecting the animals from the ventilatory circuit, the lungs were filled with 30 ml/kg of prewarmed saline (38°C, 0.15 M) through the endotracheal tube. Removal of the lavage fluid was achieved by tilting the animals head down at an angle of about 45 degrees using the gravitational gradient. ALI was defined as a  $P_{aO_2}$  consistently lower than 100 mmHg for 1 h at a  $F_{iO_2}$  of 1.0 and a positive end-expiratory pressure of 5 cmH<sub>2</sub>O after the last lung lavage.

**Partial Liquid Ventilation.** Partial liquid ventilation was initiated in the supine animal with the administration of two sequential doses of 15 ml/kg of perfluorocarbon (C<sub>8</sub>F<sub>18</sub>, "FC 3280"; 3M Chemical Products, Neuss, Germany) through the endotracheal tube during inspiration, using a swivel connector (Portex, Kent, United Kingdom). Each dose was administered into the airway

during a 10-min time interval resulting in a volume of approximately 2–2.5 ml of perfluorocarbon per respiratory cycle. The total volume of 30 ml/kg perfluorocarbon is estimated to be equivalent to the normal functional residual capacity of pigs. The perfluorocarbon used in our investigation is a highly purified industrial perfluorocarbon with a density of 1.75 g/cm<sup>3</sup>, a surface tension of 12 mN/m, and a vapor pressure of 61 mmHg at 25°C (76 mmHg at 37.5°C). Up to 40 ml O<sub>2</sub> and 192 ml CO<sub>2</sub> can be dissolved in 100 ml of this liquid (data from 3M Chemical Products, Neuss, Germany).

**Study Protocol.** Gas exchange, hemodynamic, and pulmonary blood flow data were assessed in supine position in all animals before ( $T_{Baseline}$ ) and after the induction of acute lung injury ( $T_{ALI}$ ). Subsequently, 20 animals were randomly assigned to one of two groups.

One group was submitted to PLV ( $n = 10$ , PLV group) by administering 15 ml/kg of perfluorocarbon through the endotracheal tube. As evaluated in previous studies losses of perfluorocarbon as a result of evaporation were continuously replaced during the rest of the study at volume of 4 ml · kg<sup>-1</sup> · h<sup>-1</sup> of perfluorocarbon.<sup>19,20</sup> Measurements of hemodynamic, gas exchange, and pulmonary blood flow distribution data were performed 1 h after instillation of this first dose of perfluorocarbon ( $T_{1h}$ ). Subsequently, a second dose of 15 ml/kg of perfluorocarbon was applied, and again, data of all parameters tested were determined 1 h after achieving the final intrapulmonary perfluorocarbon volume of 30 ml/kg ( $T_{2h}$ ).

In the second group of animals ( $n = 10$ , GV group) controlled mechanical ventilation was continued with no changes in the respirator setting, as outlined previously. Measurements of hemodynamic, gas exchange, and pulmonary blood flow distribution data was performed 1 h ( $T_{1h}$ ) and 2 h ( $T_{2h}$ ) after the assessment of ALI, corresponding to the study points of the PLV group.

To allow relative quantification of pulmonary blood flow by SPECT imaging at the four serial study points, appropriate fourfold-increasing amounts of radioactivity were injected at these points of time: 20 MBq <sup>99m</sup>Tc-Lyo-MAA at  $T_{Baseline}$ , 80 MBq at  $T_{ALI}$ , 320 MBq at  $T_{1h}$ , and 1280 MBq at  $T_{2h}$ . Adjusted to these increasing amount of radioactivity, the acquisition times dropped from 80 s per step at  $T_{Baseline}$  to 40 s per step at  $T_{ALI}$ , 10 s per step at  $T_{1h}$ , and 5 s per step at  $T_{2h}$ . A similar procedure to perform repeated measurements has already been used by other groups and is in accordance with the clinical routine.<sup>10,13</sup> The remaining acquisition parameters were kept constant. The overall amount of <sup>99m</sup>Tc-Lyo-MAA injected was about 1.5 mg. To avoid a possible error as a result of residual activity from previous injections of the radiotracer, measurements at  $T_{ALI}$ ,  $T_{1h}$ , and  $T_{2h}$  were corrected by subtracting the counts obtained during the previous SPECT scan corrected for different acquisition time and decay.<sup>10,13</sup>

Attenuation correction for the effects of the chest wall,



**Table 1. Gas Exchange and Metabolic Data**

	Group	T <sub>baseline</sub>	T <sub>ALI</sub>	T <sub>60</sub>	T <sub>120</sub>
PaO <sub>2</sub> (mmHg)	PLV	541 ± 35	57 ± 17	87 ± 59	180 ± 88*†
	GV	573 ± 36	59 ± 5	66 ± 9	66 ± 12
Paco <sub>2</sub> (mmHg)	PLV	30 ± 5	63 ± 15	62 ± 21	61 ± 21
	GV	34 ± 3	51 ± 8	55 ± 8	57 ± 12
Pvo <sub>2</sub> (mmHg)	PLV	51 ± 8	32 ± 10	39 ± 7	41 ± 6*
	GV	55 ± 7	38 ± 4	39 ± 4	39 ± 5
Q <sub>S</sub> /Q <sub>T</sub> (%)	PLV	15 ± 5	54 ± 15	52 ± 19	32 ± 16*†
	GV	11 ± 5	53 ± 7	50 ± 8	49 ± 11
pH	PLV	7.57 ± 0.07	7.29 ± 0.05	7.29 ± 0.07	7.31 ± 0.07
	GV	7.52 ± 0.04	7.32 ± 0.05	7.31 ± 0.06	7.31 ± 0.09

All data are presented as mean ± SD.

\*  $P < 0.05$  versus ALI, †  $P < 0.05$  versus GV group at the same study point.

PaO<sub>2</sub> = arterial oxygen pressure; PLV = partial liquid ventilation; GV = gaseous ventilation; Paco<sub>2</sub> = arterial carbon dioxide pressure; Pvo<sub>2</sub> = mixed venous oxygen pressure; Q<sub>S</sub>/Q<sub>T</sub> = venous admixture.

heart, and lung tissue was not performed in the animals submitted to PLV and GV because of the lack of a reliable transmission source. However, although the importance of attenuation in intact lungs is not universally accepted,<sup>21-23</sup> it seems possible that measurements of regional blood flow distribution in the group randomized for PLV were influenced by attenuation of the photon energy as a result of the dense perfluorocarbon, especially in the dorsal lung segments. Therefore, a third group of animals ( $n = 5$ , attenuation control group) was investigated. After induction of anesthesia and ALI as described previously, a single dose of 400 MBq <sup>99m</sup>Tc-Lyo-MAA was injected, and a first SPECT scan with an acquisition time of 10 s per step was performed (SPECT 1). Subsequently, perfluorocarbon was instilled in two sequential doses of 15 ml/kg each, and a SPECT scan with an acquisition time of 10 s per step was performed after each instillation (SPECT 2 and 3), with no further injection of <sup>99m</sup>Tc-Lyo-MAA.

### Statistics

All data are expressed as mean ± standard deviation. Data analyses were conducted using the NCSS 2000 software package (NCSS, Kaysville, UT). All data regarding the PLV group and the GV group were analyzed by two-way repeated-measures analysis of variance (ANOVA) for comparisons between and within groups. The statistical design for the ANOVA procedure was based on two within-group factors: group having two levels (GV group, PLV group) and study point having four levels (T<sub>Baseline</sub>, T<sub>ALI</sub>, T<sub>1h</sub>, T<sub>2h</sub>). For significant ANOVA results, *post hoc* correction for multiple comparisons was performed using Bonferroni test for all pairwise comparison. Values of  $P < 0.05$  were considered significant. Data analyses of the third group of animals designated to study the attenuation of photon energy by perfluorocarbon were performed using two-way repeated-measures ANOVA followed by Bonferroni

test for all-pairwise comparison when ANOVA revealed significant results.

### Results

All pigs ( $n = 25$ ) survived the entire study period. A total of 11 ± 3 lung lavages (PLV group 11 ± 3 lung lavages, GV group 12 ± 3 lung lavages) were necessary to achieve a persistent decrease in PaO<sub>2</sub> from 557 ± 38 mmHg to 58 ± 12 mmHg with an increase of Q<sub>S</sub>/Q<sub>T</sub> from 13 ± 1% to 54 ± 11% in the animals randomized to GV or PLV. Concomitantly, Paco<sub>2</sub> increased from 32 ± 4 mmHg to 57 ± 13 mmHg after the induction of ALI in these animals. Analyses of hemodynamic, gas exchange, and pulmonary blood flow data at T<sub>Baseline</sub> and T<sub>ALI</sub> revealed no differences between the PLV and the GV groups for all parameters tested. Total hemoglobin concentration and calculated capillary oxygen content remained unchanged in both groups throughout the study.

### Gas Exchange and Hemodynamics

Gas exchange and metabolic data for the PLV and GV groups are summarized in table 1. PLV resulted in a significant improvement of PaO<sub>2</sub> and Q<sub>S</sub>/Q<sub>T</sub> when compared with GV only when the higher of the two doses of perfluorocarbon was applied ( $P < 0.05$ ). No alteration in arterial oxygenation or venous admixture were observed in the GV group during the entire study period. Paco<sub>2</sub> and pH remained constant in both groups of animals after the initial changes following the induction of ALI.

Hemodynamic data for the PLV and GV groups are presented in table 2. Statistical analyses revealed no changes in any of the hemodynamic parameters tested within or between the two groups during the investigation.

### Pulmonary Blood Flow

To investigate regional changes in pulmonary blood flow distribution, the lungs were divided in four trans-

Table 2. Hemodynamic and Airway Pressure Data

	Group	T <sub>baseline</sub>	T <sub>ALI</sub>	T <sub>60</sub>	T <sub>120</sub>
PIP (cm H <sub>2</sub> O)	PLV	16 ± 1	27 ± 3	30 ± 3	32 ± 2
	GV	15 ± 1	26 ± 4	24 ± 3	25 ± 4
HR (beats/min)	PLV	82 ± 17	83 ± 10	79 ± 14	79 ± 13
	GV	85 ± 11	89 ± 6	83 ± 12	84 ± 13
MAP (mmHg)	PLV	86 ± 16	88 ± 10	87 ± 11	94 ± 6
	GV	94 ± 21	95 ± 9	93 ± 9	96 ± 6
MPAP (mmHg)	PLV	17 ± 4	27 ± 6	30 ± 6	30 ± 7
	GV	17 ± 3	29 ± 5	31 ± 4	32 ± 5
CVP (mmHg)	PLV	6 ± 3	7 ± 3	6 ± 2	6 ± 2
	GV	7 ± 3	8 ± 3	7 ± 3	7 ± 1
PAOP (mmHg)	PLV	9 ± 4	6 ± 3	8 ± 6	9 ± 4
	GV	7 ± 3	8 ± 3	7 ± 3	7 ± 3
CO (l/min)	PLV	4.6 ± 1.1	4.6 ± 1.3	4.0 ± 1.2	4.0 ± 0.8
	GV	4.5 ± 0.8	4.8 ± 1.0	4.5 ± 1.2	4.5 ± 1.1

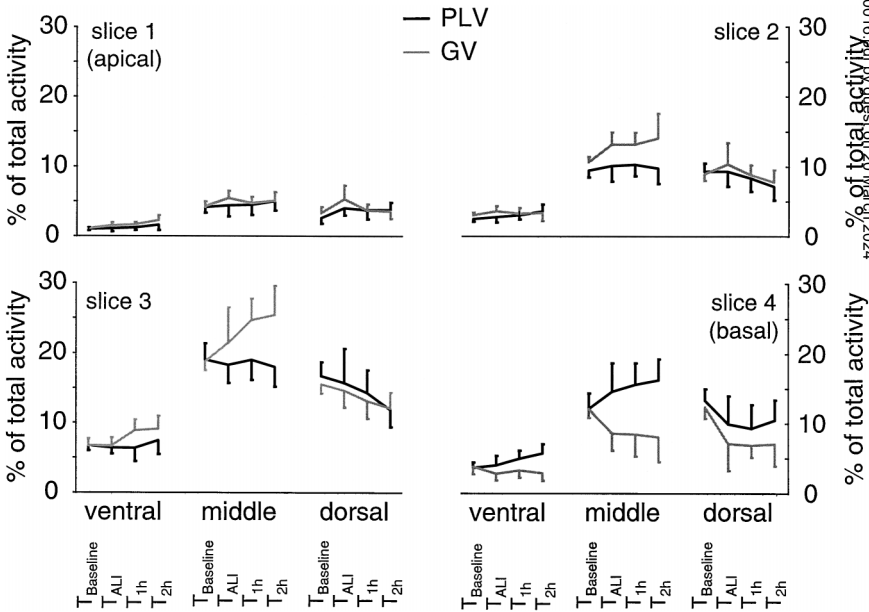
All data are presented as mean ± SD.  
PIP = peak inspiratory pressure; PLV = partial liquid ventilation; GV = gaseous ventilation; HR = heart rate; MAP = mean arterial pressure; MPAP = mean pulmonary arterial pressure; CVP = central venous pressure; PAOP = pulmonary artery occlusion pressure; CO = cardiac output.

axial slices which consisted of three segments each (ventral, middle, dorsal). Analyses were performed separately for each of the 12 segments for each animal, as well as for each of the four transaxial slices and the combined ventral, middle, and dorsal segments, respectively (fig. 1). When all animals randomized for either GV or PLV were analyzed together (n = 20), induction of ALI was found to cause an increase in pulmonary blood flow to the ventral segment of slice 2 and the middle segments of slices 1 and 2 (*P* < 0.05). Additionally, an increase in blood flow was observed in the combined middle segments of all slices, whereas it decreased in the dorsal slices (*P* < 0.05). In contrast, no effect of ALI on the pulmonary blood flow distribution was observed compared with T<sub>Baseline</sub> when the GV group (n = 10) and the PLV group (n = 10) were analyzed separately.

Neither PLV nor GV resulted in a redistribution of pulmonary blood flow regarding any of the 12 segments when values at T<sub>1h</sub> and T<sub>2h</sub> were compared with data obtained at T<sub>ALI</sub> (fig. 2). The trend toward a decrease in the combined dorsal segments and a concomitant increase in the ventral and middle segments did not reach statistical significance in either group (fig. 3). Representative SPECT images of slice 3 at all study points are presented in figure 4. Analyses in each of the four transaxial slices revealed no influence of PLV or time on regional blood flow distribution within the PLV or GV groups (fig. 5). Comparison of corresponding time points between the two groups of animals did not show any statistically significant difference.

**Attenuation by Perfluorocarbon.** In the attenuation control group, 11 ± 2 lung lavages with saline were required to induce ALI with a Pao<sub>2</sub> of 52 ± 14 mmHg.

Fig. 2. Pulmonary blood flow expressed as percentage of total activity measured in both lungs in each ventral, middle, and dorsal segment of transaxial slices one through four during gaseous ventilation (GV, n = 10) and partial liquid ventilation (PLV, n = 10). All data are presented as mean ± SD. Data from each segment are presented at four study points: T<sub>Baseline</sub> denotes prestudy conditions, T<sub>ALI</sub> denotes values after induction of acute lung injury (ALI), T<sub>1h</sub> denotes values 1 h after initiation of PLV with 15 ml/kg in the PLV group and 1 h after induction of ALI in the GV group, and T<sub>2h</sub> denotes values 1 h after initiation of PLV with 30 ml/kg in the PLV group and 2 h after induction of ALI in the GV group.



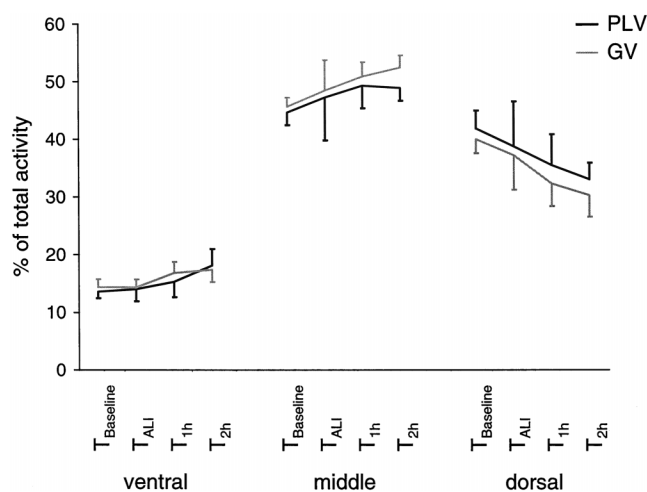


Fig. 3. Pulmonary blood flow expressed as percentage of total activity measured in both lungs along the vertical axis for the combined ventral, middle, and dorsal segments of slices one through four during gaseous ventilation (GV) and partial liquid ventilation (PLV). All data are presented as mean  $\pm$  SD. Data from each segment are presented at four study points: T-Baseline denotes prestudy conditions, T-ALI denotes values after induction of acute lung injury (ALI), T-1h denotes values 1 h after initiation of PLV with 15 ml/kg in the PLV group and 1 h after induction of ALI in the GV group, and T-2h denotes values 1 h after initiation of PLV with 30 ml/kg in the PLV group and 2 h after induction of ALI in the GV group.

Analyses of the segmental counts obtained during ventilation without perfluorocarbon and PLV with 15 and 30 ml/kg revealed no differences among the three study points (fig. 6).

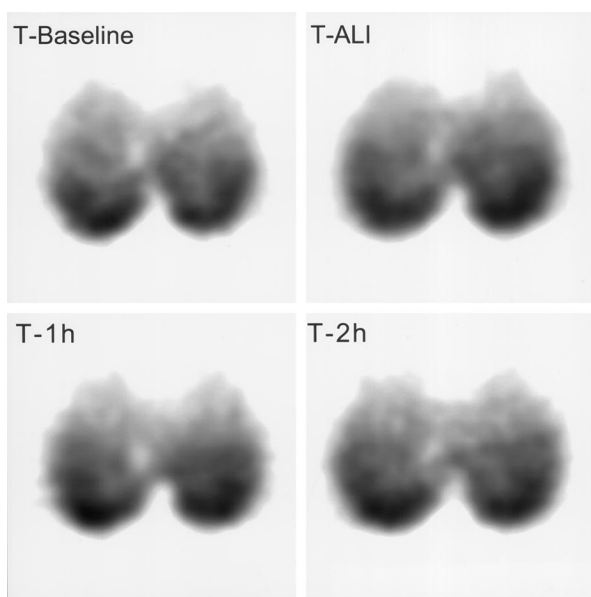


Fig. 4. Four representative single-photon emission computed tomography (SPECT) images of slice three from one animal from the PLV group. T-Baseline denotes prestudy conditions, T-ALI denotes values after induction of acute lung injury (ALI), T-1h denotes values 1 h after initiation of PLV with 15 ml/kg, and T-2h denotes values 1 h after initiation of PLV with 30 ml/kg.

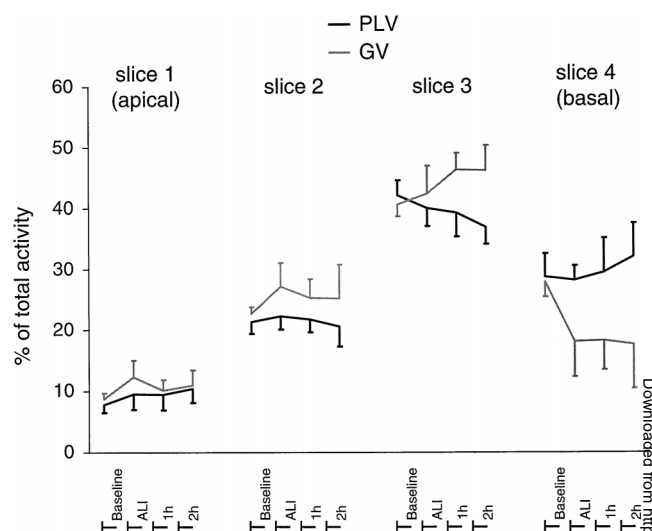


Fig. 5. Pulmonary blood flow expressed as percentage of total activity measured in both lungs in four transaxial lung slices during gaseous ventilation (GV,  $n = 10$ ) and partial liquid ventilation (PLV,  $n = 10$ ). All data are presented as mean  $\pm$  SD. Data from each slice are presented at four study points: T-Baseline denotes prestudy conditions, T-ALI denotes values after induction of acute lung injury (ALI), T-1h denotes values 1 h after initiation of PLV with 15 ml/kg in the PLV group and 1 h after induction of ALI in the GV group, and T-2h denotes values 1 h after initiation of PLV with 30 ml/kg in the PLV group and 2 h after induction of ALI in the GV group.

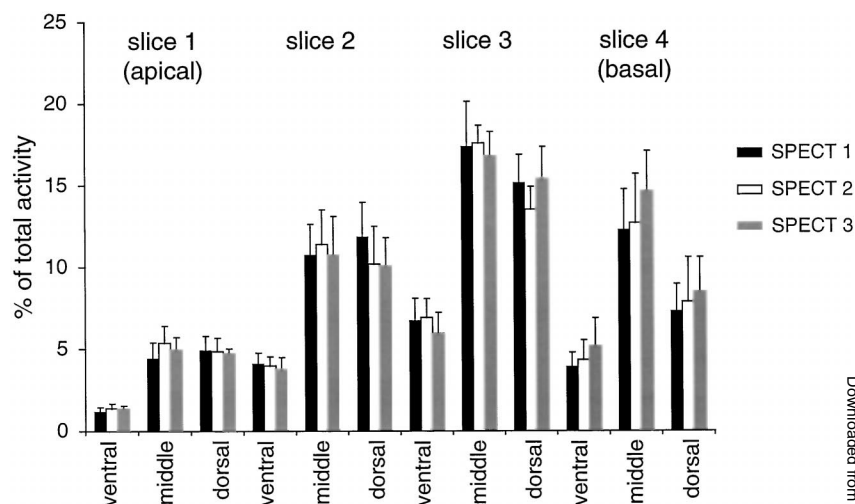
## Discussion

The purpose of this study was to investigate changes in pulmonary blood flow distribution during PLV in an experimental model of ALI. We found that PLV did not cause changes in pulmonary blood flow distribution compared with values obtained after induction of ALI in the PLV group or with animals submitted to GV. Arterial oxygenation improved significantly after the instillation of 30 ml/kg of perfluorocarbon in the PLV group but not in the GV group.

Compression of the pulmonary vasculature in the posterior lung and subsequent redistribution of pulmonary blood flow to nondependent, better-ventilated lung areas is one mechanism that has been proposed to account for the beneficial effect of PLV on arterial oxygenation in clinical and experimental ALI.<sup>6</sup> To investigate this hypothesis, we employed a surfactant washout animal model of ALI which demonstrates histologic findings similar to those in patients with acute respiratory distress syndrome<sup>17</sup> and has been extensively used by other investigators. As in acute respiratory distress syndrome, pulmonary shunt and oxygen-refractory hypoxemia caused by atelectasis and increased radiologic density in the dorsal pulmonary regions are typical features of the lung lavage model.<sup>24,25</sup>

Pulmonary blood flow distribution was determined by SPECT, a technique which is routinely used in clinical practice for this purpose and has been validated. Tow *et al.*<sup>14</sup> investigated whether the distribution of I<sup>125</sup>-labeled

Fig. 6. To study the attenuation caused by intrapulmonary instilled perfluorocarbon, pulmonary blood flow expressed as percentage of total activity measured in both lungs was determined in the ventral, middle, and dorsal segments of transaxial slices one through four in five animals. All data are presented as mean  $\pm$  SD. Data for each segment are presented at three study points: single-photon emission computed tomography (SPECT) 1 denotes gaseous ventilation, SPECT 2 denotes partial liquid ventilation with 15 ml/kg perfluorocarbon, and SPECT 3 denotes partial liquid ventilation with 30 ml/kg perfluorocarbon.



macroaggregates of human serum albumin (MAA) in the lung parallels that of  $\text{Cr}^{51}$ -labeled red blood cells. They found a high linear correlation between the regional distribution of the particles and the cells ( $r = 0.97$ ), indicating that the number of radiolabeled MAA particles trapped in a particular lung region is actually proportional to the pulmonary arterial blood flow to that region. Further evidence for the validity of the technique found in their study were the optimal size of the MAA particles ( $85\% \geq 9.2 \mu\text{m}$ , majority  $50 \mu\text{m}$ ) regarding the average internal diameter of the pulmonary capillaries ( $8 \mu\text{m}$ ),<sup>26</sup> the high extraction efficiency of 80% of the administered dose of particles in the first passage through the lung, and the uniform mixing of the particles prior to their arrival in the pulmonary arterial vessels. Using the injection of radiolabeled MAA to examine changes in regional pulmonary blood flow caused by changes in posture in six subjects, they were able to demonstrate the well-known effects of the erect and supine position on the pattern of pulmonary arterial blood flow. Lamm *et al.*<sup>10</sup> compared relative regional pulmonary blood flow (Qr) measured by SPECT-scanning with  $^{99\text{m}}\text{Tc}$ -MAA and by radiolabeled microspheres in three animals and found no statistical difference for the median Qr (SPECT  $0.90 \pm 0.1$ , microspheres  $1.00 \pm 0.15$ ,  $P = 0.07$ ) and the coefficient of variation (SPECT  $48.7 \pm 13.3$ , microspheres  $32.1 \pm 1.1$ ,  $P > 0.1$ ) comparing the two methods. Using the SPECT technique, they measured the frequency distribution of relative regional ventilation-perfusion ratios (V/Q) as well as the regional redistribution of relative V/Q depending on posture. A good correlation between SPECT in an intact canine thorax and scintillation camera counts of frozen slices of the thorax was reported by Osborne *et al.*,<sup>12</sup> indicating that a reasonable accuracy is possible when imaging an extended source of  $^{99\text{m}}\text{Tc}$ -labeled microspheres in the lung and therefore allowing the regional quantification of a physiologic function such as blood flow. Based on these results, SPECT with  $^{99\text{m}}\text{Tc}$ -labeled

MAA has been recently used to study the ventilation-perfusion distribution and correlation to atelectasis during anesthesia<sup>23</sup> and to make detailed quantitative estimates of regional pulmonary blood flow following changes in posture and breathing with or without continuous positive airway pressure.<sup>13</sup>

Attenuation correction is considered to be an important factor when performing SPECT with respect to gamma photons being attenuated during their way through the body. Because the gamma camera system used in our study was not equipped with an attenuation correction device, we had to rule out the possibility that changes in pulmonary blood flow during PLV were influenced or even simulated by attenuation artifacts caused by perfluorocarbon. We therefore performed SPECT acquisitions in five pigs with ALI using a single injection of 400 MBq  $^{99\text{m}}\text{Tc}$ -Lyo-MAA and acquisition of three sequential SPECT scans during GV and PLV with 15 or 30 ml/kg of perfluorocarbon. The rationale of this procedure was based on the finding that approximately 80% of the radiolabeled macroaggregates of albumin are extracted and fixated in the pulmonary capillaries during the first passage through the lung.<sup>14</sup> Therefore, only attenuation of the radioactive signal by perfluorocarbon and not the redistribution of the  $^{99\text{m}}\text{Tc}$ -Lyo-MAA-labeled particles following the onset of PLV, were likely to cause changes in the regional activity measured with SPECT. Using the surfactant washout animal model for this validation seemed appropriate to avoid differences in the experimental setting that could have influenced the results. When comparing the percentage of total activity measured in each pulmonary segment, we could not detect any significant differences indicating increased attenuation following the administration of 15 or 30 ml/kg perfluorocarbon. Therefore, we conclude that the administration of perfluorocarbon does not interfere with the determination of pulmonary blood flow distribution performed with SPECT itself. In addition, measured attenuation correction with the use of a transmis-



sion scan has been shown to reduce performance of SPECT by introducing noise into the corrected emission scan.<sup>27</sup> Especially in sequentially performed SPECT scans including the comparison of low-dose and high-dose studies as performed in our study, downscatter may result in serious errors of transmitted attenuation correction.<sup>22</sup>

Residual radioactivity in the mediastinal structure also could have influenced the measurements of regional pulmonary activity. However, because of the high first-pass extraction of <sup>99m</sup>Tc-Lyo-MAA, the size and number of the circulating aggregates of albumin after the first passage are small. They are metabolized in the liver and spleen and do not introduce an appreciable error in the measurements.<sup>14</sup>

Changes of pulmonary blood flow distribution along the vertical axis of the lung following the intrapulmonary instillation of perfluorocarbons have been shown in healthy animals.<sup>8</sup> However, ALI can interfere with the regional distribution of pulmonary blood flow, and the effect of PLV in such settings may be different from results obtained in subjects without respiratory failure. The severe ventilation-perfusion mismatch in the posterior lung segments caused by ALI is likely to cause hypoxic pulmonary vasoconstriction in these regions, which itself can result in a redirection of pulmonary blood flow to more anterior lung areas. This has been shown in animals with oleic acid-induced acute respiratory failure that maintained almost normal ventilation-perfusion ratios and arterial oxygenation as a result of a redistribution of regional pulmonary blood flow from dorsal to middle and ventral zones of the lungs after initiation of lung injury.<sup>28</sup> Elimination of hypoxic pulmonary vasoconstriction caused by the application of low doses of endotoxin prior to the induction of lung injury with oleic acid reversed this effect, and the animals demonstrated a severe deterioration of arterial oxygenation that was not observed when oleic acid was administered alone. A decrease in pulmonary blood flow directed to dependent lung regions in animals with oleic acid-induced ALI submitted to conventional mechanical ventilation has also been reported by Enrione *et al.*<sup>29</sup> They explained this observation by vascular closure following lung edema, which is most pronounced in the dependent lung regions when oleic acid is injected in the supine position.<sup>30</sup> Whereas the injection of oleic acid causes preferentially a severe endothelial damage with a sometimes excessive increase of mean pulmonary arterial pressure and subsequent deterioration of gas exchange,<sup>31</sup> lung lavage with saline results in atelectasis, alveolar epithelial lesions, and inflammatory reactions, thereby impairing arterial oxygenation.<sup>18</sup> Although suggested by our data from the GV group, it remains speculative whether these differences between the lung injury models, *i.e.*, differences in the homogeneity of the lung injury, exert different effects on pulmonary blood flow distribution. A high experimental variability in the

degree of lung injury will increase the difficulties in finding a significant effect of PLV on the pulmonary blood flow distribution when the total changes in regional activity are small.

In contrast to the GV animals in their study and previous investigations by other groups, Enrione *et al.*<sup>29</sup> found a preservation of pulmonary blood flow distribution in animals treated with PLV compared with data prior to and after the induction of ALI. They suggested that the preserved pattern of regional pulmonary blood flow was the result of the inhibition of hypoxic pulmonary vasoconstriction and a combined effect of liquid positive end-expiratory pressure and improved alveolar ventilation. Similar effects are likely to be the reason for the improvement of arterial oxygenation during PLV in our investigation. However, whereas injection of oleic acid caused a clear decrease of pulmonary blood flow to the most dependent lung segments due to its preference for the dorsal regions in the study by Enrione *et al.*,<sup>29</sup> saline lung lavage failed to produce such a clear effect on pulmonary blood flow distribution. Therefore, the changes following the instillation of perfluorocarbon in our investigation may have been less pronounced as well.

There are no studies investigating changes in pulmonary blood flow between apical and basal slices of the lung during PLV in animals with ALI. Doctor *et al.*<sup>28</sup> studied the effect of PLV on regional blood flow in healthy lambs and found a flow pattern favoring dependent over nondependent lung segments and diaphragmatic over apical transverse planes prior to the onset of PLV. We did not observe such a change in blood flow to apical or basal lung segments in animals with ALI submitted to PLV. This finding may have been due to the same reasons as the differences in pulmonary blood flow distribution between dorsal and ventral lung segments during PLV in animals with and without lung injury as discussed earlier.<sup>29</sup>

We found no redistribution of pulmonary blood flow during PLV, although arterial oxygenation improved significantly following the intrapulmonary instillation of perfluorocarbon. Improvement of Pao<sub>2</sub> in ALI is usually the result of an improvement in ventilation-perfusion ratios ( $V_A/Q$ ). Possible mechanisms by which PLV may affect  $V_A/Q$  include the improvement of alveolar ventilation as a result of the recruitment of previously atelectatic lung regions as well as an increase in perfusion of ventilated regions, probably as a result of the inhibition of hypoxic vasoconstriction in liquid-filled and thereby stabilized alveoli. Each of these mechanisms may be differentially important and expressed depending on the type of lung injury. In this regard, the improvement of  $V_A/Q$ , and not only the redistribution of pulmonary blood flow, may be important for the increase of arterial oxygenation during PLV.



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