Shunt and ventilation-perfusion distribution during partial liquid ventilation in healthy piglets

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Mates, Elisabeth A., Jacob Hildebrandt, J. Craig Jackson, Peter Tarcy-Hornoeh, and Michael P. Hlastala. Shunt and ventilation-perfusion distribution during partial liquid ventilation in healthy piglets. J. Appl. Physiol. 82(3): 933–942, 1997.—Replacing gas in the lung with perfluorocarbon fluids (PFC) and periodically ventilating with a gas [partial liquid ventilation (PLV)] has been shown to improve oxygenation in models of respiratory distress syndrome. We hypothesized that the addition of PFC to healthy lungs would result in shunt, diffusion impairment, and increased ventilation-perfusion (VA/Q) heterogeneity. Previously, Mates et al. showed that O2 shunt and alveolar-arterial CO2 difference increased linearly with dose in piglets given graded intratracheal doses of PFC (10, 20, and 30 ml/kg followed by mechanical ventilation with 100% O2) (E. A. Mates, J. C. Jackson, J. Hildebrandt, W. E. Truog, T. A. Standaert, and M. P. Hlastala. In: Oxygen Transport to Tissue XVI, 1994, p. 427–435). Here we report VA/Q distribution in the same animals, showing a 50% increase in VA/Q heterogeneity during PLV independent of PFC dose. Ventilation heterogeneity was the major factor in this increase, and there was no significant change in dead space ventilation. We also report on five animals given a single 20 ml/kg dose of PFC and followed for 3 h. They showed an increase in shunt during PLV but no change in alveolar-arterial CO2 difference. Widespread interest in the clinical application of PLV has led to a variety of studies exploring the effects of PLV on lung mechanics and gas exchange in animal models of RDS (2, 13, 14, 30). Relatively few investigators have addressed the physiology of gas exchange in healthy animals during PLV (4, 6, 17).

The majority of LV studies in the literature offer modest information about the changes that occur in gas exchange when PFC are instilled in the lung (1, 2, 4–7, 12, 20, 27, 30, 35). Arterial blood-gas values are often the only clues provided to interpret this complex issue. Studies of gas exchange during FTLV offer some insight into the physiology of gas exchange through a liquid medium; however, these results cannot be extrapolated to PLV where a mixture of gas and PFC resides in alveoli (12, 16, 19, 24). Fuhrman et al. (4) provided the most comprehensive data on gas exchange in healthy animals, reporting an alveolar-arterial O2 difference ([A-a]DO2) and arterial blood gases that indicated a mild increase in O2 shunt during PLV. Hernandez et al. (6) looked at cardiovascular and respiratory changes in healthy piglets during PLV with varying inspired PO2, showing relative desaturation as the inspired O2 fraction (FiO2) drops <0.3. Again, in their study, gas-exchange analysis was limited to arterial blood gases and O2 saturation. Considering the fundamental changes that must occur in the transport of gases in a liquid-filled lung, a quantitative evaluation of gas-exchange efficiency during LV would be valuable.

Given adequate ventilation and inspired PO2, the causes of gas-exchange inefficiency are traditionally enumerated as shunt, diffusion limitation, and ventilation-perfusion (VA/Q) mismatch. An increase in any one of these factors may result in increased arterial-alveolar differences in PO2 and PCO2. All contribute to venous admixture, the addition of mixed venous blood to arterial blood, lowering arterial PO2 (PaO2) and raising arterial PCO2 (PaCO2). Shunt, or right-to-left blood flow that does not participate in gas exchange in the lung, results in a lower PaO2 for a given inspired PO2. Shunt does not typically affect PaCO2, unless the shunt fraction is very high because CO2 removal is adequately controlled by increased ventilation. Increased VA/Q heterogeneity is the main cause of a decreased PaO2 in disease. Its effect on PaO2 is variable depending on the degree of ventilatory compensation present. In the face of increased VA/Q heterogeneity with little or no respiratory compensation, PaCO2 will increase. Diffusion limitation affects both gases, increasing PaCO2, decreasing PaO2, and widening the capillary-alveolar partial pressure differences. During PLV, where...
fluid resides in alveoli as well as in small and possibly large airways, any or all of the above mechanisms may be responsible for widened arterial-alveolar differences [(a-Å)D]. Understanding the mechanisms responsible for decrements in gas-exchange efficiency during PLV is important in determining the trade-off between improved lung inflation and impaired gas exchange. It also provides insight into gas-exchange physiology as patients are being weaned from PLV in the ICU.

In this paper, we continue an investigation of gas-exchange characteristics of the PFC-filled lung during PLV (17). The healthy newborn piglet model was chosen to elucidate the basic physiology of gas exchange through a liquid medium uncomplicated by the effects of disease. In a previous paper, Mates et al. (17) documented increased O2 shunt and arterial-alveolar CO2 difference [(a-Å)DCO2] during PLV and showed these changes were linearly proportional to the volume of PFC in the lung. We also attempted to measure Va/Q heterogeneity with the multiple inert gas elimination technique (MIGET) but found it was limited due to the high solubility of sulfur hexafluoride (SF6) in PFC. We concluded that the presence of PFC in the lung slightly impairs O2 and CO2 exchange in healthy lungs, but we were unable to delineate the mechanisms (i.e., diffusion limitation vs. Va/Q heterogeneity vs. shunt) responsible for these changes. In this publication, we reiterate the previous results (17), adding data for a total of nine animals given graded doses of PFC. We revisit our Va/Q analysis of PLV using a modified form of MIGET with SF6 eliminated, providing information on Va/Q heterogeneity during PLV with varying doses of PFC in the lung. In addition, we present a gas-exchange analysis of five control animals given a single dose of PFC and followed over time.

METHODS

Animal preparation. Fourteen piglets 7–14 days old and of either sex were sedated with ketamine and xylazine (24 and 2.75 mg/kg im, respectively). Anesthesia was maintained with pentobarbital sodium (6 mg·kg\(^{-1}\)·h\(^{-1}\) iv, supplemented with 6–13 mg/kg hourly as needed) or pentobarbital sodium and ketamine (3 and 5 mg·kg\(^{-1}\)·h\(^{-1}\) iv, respectively). Pancuronium bromide was administered in 0.2 mg/kg intravenous doses as needed to prevent respiratory efforts, followed by 3–5 mg/kg iv of pentobarbital sodium to ensure a surgical plane of anesthesia. Intravenous access was established via the left jugular vein, and the right carotid artery was cannulated for blood-gas and arterial pressure measurements (Fig. 1). A pulmonary arterial thermodilution catheter was placed under fluoroscopy via the right jugular vein for measurement of cardiac output (CO), central venous and wedge pressures, and mixed venous blood gases. The animals were tracheotomized with a metal Y-shaped cannula with a side port for the administration of PFC.

Fig. 1. Schematic of animal setup and instrumentation. VT, tidal volume; f, breathing frequency; FIO2, inspired O2 fraction; PFC, perfluorocarbon fluid; MIGET, multiple inert gas elimination technique; PEEP, positive end-expiratory pressure.
A Harvard single-piston animal ventilator was used to deliver tidal breaths of 100% O2 to animals in the supine position. Tidal volume and frequency were set to maintain PPaCO2 < 40 Torr during gas ventilation at the start of an experiment and were unchanged thereafter. Airway pressure was continuously monitored, and expiratory flow and volume were periodically assessed with a pneumotachometer and signal integrator, respectively. Positive end-expiratory pressure (PEEP) was applied by immersion of the distal expiratory port to 5 cmH2O. Carotid and pulmonary arterial (Pa) pressures were recorded, and CO was determined in triplicate via thermodilution. All protocols were approved by the University of Washington (Seattle) Animal Care and Use Committee.

PLV. Warmed, non-proxenogenated perfluorbron (CF3Br; LiquiVent, Alliance Pharmaceutical, San Diego, CA) was instilled intratracheally via the side port of the endotracheal tube. Each dose of PFC was trickled into the airway over 4-5 min during 100% O2 breathing, delivering one-third of the dose in each of the right and left lateral and supine positions in an attempt to distribute evenly the fluids in the lung. Hourly doses of 2 ml/kg were given to replace evaporative losses of PFC (see Appendix A).

O2 and CO2 gas-exchange analysis. Blood-gas measurements were made with a pH/blood-gas analyzer (Corning model 150), which showed a linear PaO2 response to tonometered blood up to 700 Torr with and without perfluorbron vapor present. In several experiments, duplicate blood samples were obtained to determine the effect of intersample differences. These were found to be negligible for O2 and CO2. The subroutines of Olszowska and Farhi (22), which are based on the homogams of Dill et al. (3), were used to calculate O2 and CO2 blood contents (C02 and CCO2, respectively; in ml/100 ml blood) corrected for temperature, hemoglobin concentration, pH, PCO2, and base excess. The subroutines are based on the concept that if temperature, base excess, and hemoglobin concentration are known, then CO2 and CCO2 can be calculated from PO2 and PCO2.

O2 shunt was calculated with the Berggren shunt equation

\[ \frac{Q_S}{Q_T} = \frac{C_{O2} - C_{O2g}}{C_{CO2} - C_{vO2}} \]

where \( Q_S \) is shunt flow (in l/min), \( Q_T \) is total pulmonary blood flow (in l/min), \( C_{O2} \) is peripheral arterial O2 content, \( C_{O2g} \) is mixed venous O2 content, and \( C_{CO2} \) is end-capillary O2 content, with the assumption that end-capillary PO2 is equivalent to alveolar PO2 (PAO2). PAO2 during 100% O2 breathing was estimated as PO2 - PH2O - PCCO2 - PFFC, where PO2 is barometric pressure, \( PH2O \) is water vapor pressure, and PFFC is PFC vapor pressure. "Alveolar" during PLV refers to the gas adjacent to the fluid layer and does not necessarily represent gas tensions in resident PFC. The PO2, values of the infused MIGET gases were considered negligible (see Appendix B). (a-a)DCCO2 was calculated as PAO2 minus measured PAO2.

(a-a)DCO2 was determined from PAO2 and end-tidal PCO2 values. Exhaled CO2 was continuously monitored with a Novametrix model 7000 infrared analyzer situated in-line between the piglet and the solenoid valve (Fig. 1). The analyzer showed excellent linear correlation (R = 0.998) with a medical mass spectrometer (Perkin-Elmer model 1100) with respect to PCO2 in the presence of both water vapor and perfluorbron vapor. Alveolar PCO2 (PAPO2) was taken as the peak of the expired capnogram over 5-10 breaths before blood-gas sampling. Typically, this was the value at end expiration due to the usual positive slope of the expirogram. However, in some cases during PLV, peak PAPO2 occurred in the first 40% of an exhaled breath, and the expirogram had a negative slope, in which case the highest value of expired PCO2 was chosen. End-tidal expired PCO2 is often used as an estimate of "true" PAPO2 (alveolar gas in dose proximity to PFC in these experiments) because it is presumed to represent gas exhaled from distal regions of lung, minimally diluted by dead space.

During PLV, there are regional alterations in lung mechanics due to the presence of a low-surface-energy fluid, and it is reasonable to assume increased heterogeneity of alveolar emptying times and of PAPO2 in the context of variable distributions of PFC. We have previously discussed the implications of this with regard to "measuring" PAPO2 at the mouth (18). We have chosen to use peak expired PCO2 to represent PAPO2 for the purposes of estimating (a-a)DCO2, although one cannot truly represent it as a single number. (a-a)DCO2 calculated this way underestimates the difference in alveoli with lower PAPO2 values and actually represents the minimum (a-a)DCO2 that exists in PFC-filled lungs.

MIGET. This technique, subject to a number of assumptions, allows one to distinguish shunt, dead space, and the general pattern of Va/Q distribution in a lung (8, 33). It is based on the elimination of six inert gases of varying blood solubilities (\( \beta \); in ml solute:100 ml solvent\(^{-1}\)·mmHg\(^{-1}\)) and blood-to-gas partition coefficients (\( \lambda = \beta_I/\beta_g \) where \( \beta_I \) is the blood solubility and \( \beta_g \) is the gas solubility) as they pass through the lung. Measured retention (R = Pa/PP, where PP is mixed venous pressure) and excretion (E = Pe/PP, where Pe is expired pressure) of each of the six gases is compared with a multicompartement mathematical model that provides estimates of shunt, dead space, and Va/Q heterogeneity. The assumptions underlying this model are that steady-state conditions exist with respect to gas exchange, that ventilation and perfusion to gas-exchange compartments are constant, and that there is complete diffusive equilibrium between end-capillary blood and alveolar gas.

In applying MIGET to PLV, reevaluation of these assumptions is necessary in light of the presence of PFC in the alveolar space. In a previous communication, Mates et al. (17) discussed the fact that SF6 is not in a steady state with respect to gas flux under shunt conditions. Using a two-compartment model describing inert gas exchange in a PFC-filled alveolus with perfusion but no ventilation, Mates et al. calculated a time constant of \( \sim 3 \) h for SF6 equilibration between blood and PFC. The next longest equilibration time was 18 min for ethane (17). In some additional unpublished calculations with a three-compartment model of a PFC-filled alveolus with ventilation as well as perfusion, we found much shorter equilibration times for ventilated units, ranging from 7 s for acetone to 133 s for SF6 (Mates, Hildebrandt, and Hlastala, unpublished observations). Our conclusion from these calculations was that SF6 is unsuitable for MIGET during PLV due to lack of steady-state conditions, but the remaining five gases satisfy this condition. MIGET results presented in this paper are based on five gases, with the lowest solubility gas, SF6, eliminated. This will affect the results in two ways: MIGET shunt will be invalid because SF6 retention is the most sensitive measure of shunt. Second, overall sensitivity of the modeling technique will be reduced by elimination of one of the six gases. Va/Q predictions will not be systematically biased by the absence of SF6 but will be more affected by noise in the other five data points. The overall effect will be to make it more difficult to find statistically significant trends in Va/Q heterogeneity.

Biological fluctuations in blood flow, ventilation, etc. are additional sources of error in this technique because MIGET model solutions are based on the assumption of continuous
blood flow and ventilation. Wagner (31) analyzed 400 duplicate pairs of inert gas samples from multiple experimental conditions and reported excellent reproducibility of MIGET dispersion indexes despite physiological variation within and between experiments. During PLV, an additional source of variation would be redistribution of the PFC within alveoli. We do not feel that this is a large source of error within the time frame of our measurements. Continuous PEEP was applied, which has the effect of pushing the PFC-air interface into distal airways throughout the respiratory cycle, reducing back-and-forth mixing of the fluid in larger airways. Dependent drainage of PFC does occur over the course of a 3- to 4-h experiment, but it occurs slowly and is not likely to affect hourly measurements.

Diffusion equilibrium between blood and alveolar gas may be hampered by the presence of PFC as we predict it might for O2 and CO2. This would have the effect of increasing the inert gas (a-A)D area that, as we discuss below, is the variable we have chosen to predict V˙A/Q˙ heterogeneity. In an as yet unpublished study, we examined the potential role of diffusion limitation in creating (a-a)D values for O2, CO2, and the inert gases (Mates, Hildebrandt, and Hlastala, unpublished observations). Briefly, this model consists of a spherical gas-exchange unit the size of a terminal sac with capillary perfusion in the outer shell, an evenly distributed shell of PFC, and an inner sphere of alveolar gas. Inert and respiratory gases were modeled as diffusing from the capillary bed to alveolar gas along radial lines. Capillary-to-alveolar gas partial pressure gradients were calculated for a variety of PFC thicknesses and alveolar gas volumes. Inert gas partial pressure gradients were found to be significantly lower than those of O2 and CO2 and <5% of the driving pressure over the range of PFC thickness estimated for the evenly distributed 30 ml/kg dose of PFC in ventilated gas-exchange units. Inert gas (a-a)D area should not be affected by diffusion disequilibrium with PFC present as long as gas penetrates the unit (i.e., it is ventilated). If little or no gas is present in a gas-exchange unit, R and E of the gases will approximate shunt, as would be appropriate.

Graphic presentation of V˙A/Q˙ heterogeneity as determined by MIGET has been expressed in a variety of ways. The most recognized format as well as the most intuitively satisfying is the presentation of continuous distributions of alveolar ventilation (V˙A) and blood flow (Q˙) vs. V˙A/Q˙ as described by Wagner et al. (33) (Fig. 2A). This approach uses a 50-compartment model with mathematical smoothing functions to generate a solution describing shunt, dead space, and a distribution of V˙A/Q˙ that may have up to three modes. One of the main criticisms of this analysis is that the contours are not unique solutions and are often overinterpreted (10, 11). Hlastala and Robertson (10) described a simplified approach to analysis that computes the inert gas (a-A)D area from a four-compartment model fit to measured R and E data (Fig. 2B). The four compartments consist of shunt, dead space, and two intermediate V˙A/Q˙ compartments that are chosen by the algorithm as a best fit to measured data points. It is a simpler solution and avoids the pitfalls of the transformation mathematics of Wagner et al. (33). Inert gas (a-A)D area is the area between the measured R and E curves and the predicted R and E curves from the four-compartment homogeneous lung model (Fig. 2C). (a-A)D area increases with increased heterogeneity as the measured R and E values deviate from the ideal R and E values. The R and E components of the (a-A)D area reflect perfusion heterogeneity and ventilation heterogeneity, respectively. Hlastala and Robertson (10) also found that the peak of the (a-A)D area curve shifts left on the solubility curve with predominantly low V˙A/Q˙, shifts right along that axis with predominantly high V˙A/Q. and is centered around a solubility of 1 in healthy lungs. We have chosen to use the method of Hlastala and Robertson to analyze inert gas data gathered during PLV. The four-compartment fit was felt to be a more robust technique for measuring changes in heterogeneity in the face of elimination of SF6. The measurement of one fewer inert gases would only exacerbate the problem of nonunique solutions in the technique of Wagner et al. (33). We emphasize that the inert gas (a-A)D area is not a measure of diffusion limitation in the lung; rather it measures the goodness of fit of a four-
compartment homogeneous lung model to the experimental R and E values.

The theory and methodology of MIGET have been discussed at length elsewhere (8, 10, 31–33). The average solubilities of MIGET gases in blood and perfluorobron are listed in Table 1. Solubilities of the gases in pig blood and perfluorobron were measured with the double-extraction technique of Wagner et al. (32), and the average values of six trials are presented. There are no chemical reactions between the MIGET gases and PFC, and we did not measure a net loss of inert gases during PLV. Mass balance as determined by the ratio of expired inert gases to arteriovenous inert gas difference (Ve·E/(1 – R)·Q·l, where Ve is expired ventilation) was within 2% of 1 (perfect mass balance) for 90% of all gases and all runs. Heated lines and gas-collection chambers prevented loss of inert gases due to condensation of PFC or water vapor.

A dilute solution of five inert gases (ethane, cyclopropane, halothane, ether, and acetone) in 5% dextrose was continuously infused into the jugular vein (33). Expiratory gas was collected in a heated 1-liter flow-through glass chamber with a three-way solenoid valve triggered by the ventilator to separate the inspiratory and expiratory pathways. All tubing leading to the glass chamber was heated as well to prevent water and PFC condensation. Mixed venous blood, arterial blood, and mixed expired gas samples were collected simultaneously after the time period allowed for the establishment of a steady state. Before our discovery of the longer than expected equilibration time constants, an average of 40 min was allowed between MIGET sampling and any change in PFC volume. In the later studies (four of nine animals in the graded-dose protocol), 50–60 min were allowed to establish a steady-state equilibrium. In animals given a single dose of PFC, the time between samples was >60 min in all cases. Inert gas concentrations in the blood and expired air samples were determined by gas chromatography with a flame ionization detector according to the method of Wagner et al. (32). Expired gas samples were maintained at >40°C before analysis to avoid water and PFC condensation and the loss of highly soluble gases.

Experimental protocol. After instrumentation, baseline arterial blood gases were assessed, and an inert gas infusion was initiated. Inert gas equilibration during gas ventilation (GV) was continued for 30–40 min, and the first set of MIGET samples was drawn. Arterial and mixed venous blood gases; airway, arterial, and pulmonary arterial pressures; and CO were recorded immediately after inert gas sampling during both GV and PLV. In 9 of 14 animals, PFC was administered in 10 ml/kg doses up to a maximum dose of 30 ml/kg, followed by drainage of 10 ml/kg to repeat the 20 ml/kg condition (the graded-dose protocol). After each change in PFC volume, 40–60 min were allowed for inert gas equilibration before MIGET samples were drawn. Inert gases were subsequently analyzed by gas chromatography. A 30 ml/kg dose of PFC is approximately equal to the functional residual capacity (FRC) of these animals, which was verified by visualization of a fluid level in the endotracheal tube at midchest level at an airway pressure of zero. Fuhrman et al. (4) described verification of an FRC fluid volume by visualization of a PFC meniscus in the endotracheal tube at the anterior chest wall. We feel that this method is in error because the trachea is located at approximately the midchest level and FRC is defined as the volume of gas in the lung at zero intratracheal pressure. A PFC meniscus at the anterior chest wall implies positive intratracheal pressure to support a fluid column in the endotracheal tube. A fluid level at the midchest level more accurately represents zero endotracheal pressure and an FRC volume. It is important to realize, however, that this volume is not going to exactly equal the gas FRC due to changes in lung recoil forces secondary to an alteration of surface forces with the presence of PFC. It should be viewed as an approximation used to standardize dosing regimens to make comparisons between experimental protocols possible.

To control for the effect of time on gas exchange during PLV, 5 of 14 animals were given a single 20 ml/kg dose of PFC after baseline GV measurements. Blood and gas samples, inert gas samples, and cardiovascular parameters were measured at hourly intervals during 3 h of PLV. In this group of animals, periodic rotation into right and left lateral and supine positions was done throughout the experiments to facilitate redistribution of fluid. MIGET data were not analyzed due to a potential disturbance of steady-state conditions with position changes.

Statistics. Repeated-measures analysis of variance (RM-ANOVA) was used to determine statistical trends in (A-a)DO2, Berggren shunt, and (A-a)DCO2 vs. PFC dose in nine animals and vs. time in five animals. RM-ANOVA was also used to determine trends in MIGET inert gas (A-a)D area and the R and E components of the (A-a)D area with PFC dose. Student’s two-tailed t-test was used to determine significant changes in hemodynamic parameters and blood-gas data. Average data are expressed as means ± SD unless otherwise noted. The statistical software package STATVIEW II (Abacus Concepts, Berkeley, CA) was used for all calculations.

RESULTS

Of 21 animals studied, 4 died due to puncture of the right ventricle during pulmonary artery catheter placement. 1 was excluded due to improper placement of the pulmonary artery catheter, and 2 were excluded for incomplete protocols, leaving 14 animals from which data are reported for the 2 protocols. The mean weight of nine animals in the graded-dose protocol was 2.54 ± 0.6 kg and of five animals in the single-dose protocol was 3.05 ± 0.8 kg. Cardiovascular status was stable throughout all experiments, with no statistically significant changes in Pa or CO; however, there was a small decline in pulmonary arterial wedge pressure in the single-dose protocol (Table 2). Mean peak proximal airway pressure was 19.5 cmH2O during the graded-dose protocol and 21 cmH2O during the single-dose protocol and did not change from GV to PLV in either case. Average tidal volume and frequency were 17.1 ± 2.9 ml/kg and 20 ± 4 breaths/min, respectively, for the graded-dose protocol and 16.0 ± 2.3 ml/kg and 24 ± 4 breaths/min, respectively, the single-dose protocol. Av-

Table 1. Solubility of 6 inert gases in pig blood and PFC

<table>
<thead>
<tr>
<th>Gas</th>
<th>βG</th>
<th>βPFC</th>
<th>λPFC/Bl</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF6</td>
<td>0.00974 ± 0.000049</td>
<td>0.410 ± 0.065</td>
<td>420.0</td>
</tr>
<tr>
<td>Ethane</td>
<td>0.016 ± 0.0047</td>
<td>0.234 ± 0.026</td>
<td>20.2</td>
</tr>
<tr>
<td>Cyclopropane</td>
<td>0.079 ± 0.0078</td>
<td>0.791 ± 0.052</td>
<td>10.6</td>
</tr>
<tr>
<td>Halothane</td>
<td>0.396 ± 0.047</td>
<td>0.826 ± 0.066</td>
<td>2.09</td>
</tr>
<tr>
<td>Ether</td>
<td>1.34 ± 0.036</td>
<td>5.08 ± 0.19</td>
<td>3.80</td>
</tr>
<tr>
<td>Acetone</td>
<td>38.4 ± 0.42</td>
<td>3.86 ± 0.37</td>
<td>0.101</td>
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</tbody>
</table>

Values are means ± SE; n = 9 determinations for blood (b) and 6 determinations for perfluorocarbon fluid (PFC). β, Solubility (in ml gas·100 ml solvent·1·mmHg·1); λ, blood-to-gas partition coefficient; SF6, sulfur hexafluoride.
(A-a)DO2 increased throughout PLV (Fig. 3), showing a rise with dose by RM-ANOVA (P < 0.05) but was well maintained at >300 Torr in all animals (Table 3). PaO2, and mixed venous PO2 (PvO2) were unchanged from GV to PLV. *Significantly different values from baseline GV, P < 0.05.

Average PEEP values were 4.6 and 5.2 cmH2O for the graded- and single-dose protocols, respectively.

Table 2. Hemodynamic changes in both graded- and single-dose protocols

<table>
<thead>
<tr>
<th>Condition</th>
<th>PaO2, mmHg</th>
<th>CO2, ml/min</th>
<th>Pwedge, cmH2O</th>
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</thead>
<tbody>
<tr>
<td>Graded dosing (n = 9)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Baseline GV</td>
<td>70.9 ± 19.0</td>
<td>430 ± 86</td>
<td>10.4 ± 4.8</td>
</tr>
<tr>
<td>10 ml/kg</td>
<td>74.6 ± 14.4</td>
<td>463 ± 160</td>
<td>9.78 ± 4.3</td>
</tr>
<tr>
<td>20 ml/kg</td>
<td>62.3 ± 8.7</td>
<td>433 ± 150</td>
<td>10.6 ± 5.0</td>
</tr>
<tr>
<td>30 ml/kg</td>
<td>56.2 ± 11.9</td>
<td>454 ± 160</td>
<td>10.8 ± 5.9</td>
</tr>
<tr>
<td>20 ml/kg</td>
<td>53.7 ± 12.2</td>
<td>461 ± 170</td>
<td>9.00 ± 4.9</td>
</tr>
<tr>
<td>Single dose (n = 5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-h GV</td>
<td>67.8 ± 16.5</td>
<td>608 ± 170</td>
<td>9.50 ± 1.3</td>
</tr>
<tr>
<td>1-h PLV</td>
<td>68.0 ± 13.0</td>
<td>723 ± 74</td>
<td>7.50 ± 1.3*</td>
</tr>
<tr>
<td>2-h PLV</td>
<td>64.5 ± 9.6</td>
<td>773 ± 83</td>
<td>8.00 ± 1.8*</td>
</tr>
<tr>
<td>3-h PLV</td>
<td>60.3 ± 10.5</td>
<td>703 ± 55</td>
<td>8.75 ± 4.4*</td>
</tr>
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</table>

Values are means ± SD; n, no. of piglets. Pa, arterial pressure; CO, cardiac output; Pwedge, pulmonary arterial wedge pressure; GV, gas ventilation; PLV, partial liquid ventilation. *Significantly different from baseline GV, P < 0.05.

Gas exchange in piglets given graded doses of PFC. Shunt and (a-A)DCO2 data from eight of the nine animals in this group were previously reported (17) and are repeated here for ease of comparison with the additional gas-exchange analysis presented here. PaO2 decreased significantly compared with baseline GV throughout PLV (P < 0.05) but was well maintained at >300 Torr in all animals (Table 3). PaO2 and mixed venous PO2 (PvO2) were unchanged from GV to PLV. (a-A)DO2 increased throughout PLV (Fig. 3), showing a statistically significant change with PFC dose by RM-ANOVA (P < 0.05). Drainage of PFC from the lung to return to the 20 ml/kg dose produced an (a-A)DO2 value insignificantly different from values obtained at the 20 ml/kg dose earlier in experiments. Berggren shunt also showed a rise with PFC dose (Fig. 3) and a statistically significant change with the progressive addition of PFC by RM-ANOVA (P < 0.05). As with (a-A)DO2, drainage of PFC from the lung resulted in O2 shunt insignificantly different from that of the first 20 ml/kg dose.

PaCO2 and PvO2 rose significantly during PLV (P < 0.05), arterial pH fell with the onset of PLV (P < 0.05), and PaCO2 was unchanged. Despite the rise with PLV, PaCO2 remained within physiological limits (<45 Torr) throughout all experiments. (a-A)DCO2 also demonstrated a statistically significant increase throughout PLV (Fig. 4) and showed a significant dose response by RM-ANOVA (P < 0.05). Drainage of 10 ml/kg of PFC after the 30 ml/kg dose resulted in an a-ADCO2 not statistically different from that of the first 20 ml/kg dose.

. MIGET inert gas (a-A)D area, a unitless index of VA/Q heterogeneity, increased roughly 50% during PLV (Fig. 5). There was a statistically significant change with dose by RM-ANOVA (P < 0.05), but the changes do not appear to be linear with dose. There was a significant rise in the E component of the inert gas (a-A)D area throughout PLV (P < 0.05) but no significant change in the R component by RM-ANOVA (Fig. 5). There were no significant differences in any MIGET parameters between the 20 ml/kg dose and drainage of 10 ml/kg of PFC from the lung after the 30 ml/kg dose. The (a-A)D area vs. solubility curve was symmetrical around a solubility of 1 for most experiments, with no systematic shifts along the abscissa. Dead space ventilation (Vd/VT) as determined by MIGET was 0.31 ± 0.06 during GV and was unchanged throughout PLV by RM-ANOVA. The mean sum of squares for all MIGET solutions reported (9 experiments with 5 runs each)

Table 3. Blood-gas data for graded- and single-dose protocols

<table>
<thead>
<tr>
<th>Condition</th>
<th>PaO2, Torr</th>
<th>PaO2, mmHg</th>
<th>PV2O2, Torr</th>
<th>PCO2, Torr</th>
<th>PV2CO2, Torr</th>
<th>pHa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graded dose (n = 9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline GV</td>
<td>520 ± 42</td>
<td>680 ± 10</td>
<td>41 ± 9</td>
<td>35 ± 5</td>
<td>32 ± 3</td>
<td>7.45 ± 0.05</td>
</tr>
<tr>
<td>10 ml/kg</td>
<td>448 ± 59*</td>
<td>661 ± 12</td>
<td>40 ± 5</td>
<td>40 ± 7*</td>
<td>33 ± 4</td>
<td>7.40 ± 0.06*</td>
</tr>
<tr>
<td>20 ml/kg</td>
<td>379 ± 92*</td>
<td>660 ± 12</td>
<td>38 ± 6</td>
<td>42 ± 6*</td>
<td>32 ± 3</td>
<td>7.37 ± 0.08*</td>
</tr>
<tr>
<td>30 ml/kg</td>
<td>327 ± 77*</td>
<td>658 ± 14</td>
<td>39 ± 5</td>
<td>44 ± 9*</td>
<td>31 ± 4</td>
<td>7.34 ± 0.07*</td>
</tr>
<tr>
<td>20 ml/kg</td>
<td>412 ± 91*</td>
<td>659 ± 16</td>
<td>44 ± 10</td>
<td>43 ± 8*</td>
<td>31 ± 4</td>
<td>7.37 ± 0.06*</td>
</tr>
<tr>
<td>Single dose (n = 5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-h GV</td>
<td>452 ± 43</td>
<td>676 ± 13</td>
<td>40 ± 9</td>
<td>37 ± 3</td>
<td>32 ± 2</td>
<td>48 ± 6</td>
</tr>
<tr>
<td>1-h PLV</td>
<td>422 ± 98*</td>
<td>660 ± 10</td>
<td>46 ± 7</td>
<td>40 ± 6</td>
<td>33 ± 3</td>
<td>51 ± 9</td>
</tr>
<tr>
<td>2-h PLV</td>
<td>394 ± 61*</td>
<td>661 ± 10</td>
<td>46 ± 9</td>
<td>40 ± 6</td>
<td>32 ± 5</td>
<td>48 ± 3</td>
</tr>
<tr>
<td>3-h PLV</td>
<td>414 ± 42*</td>
<td>662 ± 10</td>
<td>47 ± 8</td>
<td>40 ± 6</td>
<td>31 ± 4</td>
<td>49 ± 8</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of piglets. PaO2, PaCO2, and PaCO2, arterial, mixed venous, and alveolar PO2, respectively; PaCO2, PaCO2, and PaCO2, arterial, mixed venous, and alveolar PCO2, respectively; pHa, arterial pH. *Significantly different from baseline GV, P < 0.05.
was 0.5469 ± 0.5782. Of the 45 runs, 35 had a residual sum of squares, 6 were <10.0, 2 were <20.0, and 2 were eliminated due to inert gas sampling errors.

Gas exchange in piglets given a single dose of PFC. Similar to the graded-dose protocol, PaO₂ decreased significantly from baseline GV throughout PLV (Table 3). PvO₂ was slightly but insignificantly higher during PLV compared with GV. In contrast to the graded-dose scheme, PaCO₂ did not rise significantly over baseline during PLV. PaCO₂ was unchanged from pre- to post-PLV and did not change with PLV or time. (A-a)DO₂ increased significantly with the addition of PFC as did O₂ shunt (P < 0.05), but there were no systematic trends with time in either parameter (Fig. 6). (a-A)DCO₂ increased slightly but insignificantly from GV to PLV (Fig. 7) and did not vary over 3 h of PLV.

DISCUSSION

The aim of this study was to determine the effect of a PFC-filled lung on gas-exchange efficiency in the healthy animal. In applying the standard tools of gas-exchange analysis to the study of PLV, it is important to carefully examine the assumptions under which these analytic tools were derived and determine whether they were satisfied in the case of PLV. It is also important to note that the large body of information about gas exchange during FTLV is not directly applicable to PLV due to fundamental differences in ventilation (i.e., fluid vs. gas).

Shunt during PLV. As discussed in our previous publication (17), Berggren shunt increased in all animals during PLV and did so in a dose-dependent manner. In this publication, we show (A-a)DO₂ alongside O₂ shunt (Figs. 3 and 6) because the (A-a)Do₂...
represents the fundamental disturbance that occurred due to the presence of PFC. O₂ shunt is calculated from the Berggren equation, which assumes complete equilibration of O₂ tension across alveolar membranes in ventilated areas. It is a measure of blood flow past unventilated regions, attributing all (A-a)DO₂ to blood flow past collapsed air space or anatomically unventilated areas (i.e., bronchial circulation). During PLV, one must consider a second mechanism of increased (A-a)DO₂ (and the derived parameter shunt): diffusion limitation in PFC that can potentially lead to a partial pressure difference between alveolar gas and capillary blood. The presence of liquid in alveoli presents a significant diffusion barrier to O₂, with a five order of magnitude reduction in diffusion coefficients of O₂ and CO₂ in PFC (9, 29). There is probably an alveolar-to-arterial gradient of O₂ due to diffusion limitation in ventilated alveoli, a situation different from the purely gas-filled lung. It must be kept in mind that what we call shunt in gas-filled gas-ventilated lungs must be thought of as a combination shunt and diffusion limitation in lungs partially filled with liquid fluorocarbon.

To be thorough, we must also examine the underlying assumption that an FIO₂ of 1.0 eliminates Va/Q heterogeneity as a cause of increased (A-a)DO₂ during PLV. West (34) explains that high FIO₂ abolishes (A-a)DO₂ due to Va/Q heterogeneity by greatly increasing driving pressure and eliminating diluent gas N₂. During PLV, inspired O₂ penetrates the lower airways, probably at the level of the terminal alveolar sac, mixing with resident gas and PFC. PAO₂ during PLV approximates atmospheric pressure minus PH₂O, PA CO₂, and PPFC, a combination shunt and diffusion limitation in lungs.

In this study, we found that shunt increased in proportion to the volume of PFC in the lung. Our time-control trials confirmed the fact that the dose dependence of shunt seen here was not confounded by time-dependent changes during prolonged periods of PLV (Fig. 6). We hypothesize that the relationship between PFC volume and shunt is due to creation of shunt regions by the pooling of excess PFC and/or an increased diffusion limitation throughout the lung. Ongoing work in the area of mathematical modeling of gas diffusion in PFC-filled alveoli in our laboratory may help determine whether diffusion limitation is a significant contributor to (A-a)DO₂ or a minor component. These data seem to suggest that lower volumes of fluid (i.e., less than the FRC doses used in recent clinical trials) provide a gas-exchange advantage. It must be kept in mind, however, that we are comparing PLV and GV in piglets with a healthy baseline. In subjects with lung injury, it has been shown many times that shunt improves dramatically from GV to PLV with an FRC dose of fluid in the lung (2, 13, 14). Injured lungs contain collapsed or edematous regions that would presumably open and participate in gas exchange with the addition of low-surface-energy liquid. Our data may apply to the situation in which patients whose lung function is improving are weaned from PLV. We speculate that the optimum PFC dose will fall as lung disease improves.

(A-a)DCO₂ during PLV. In healthy lungs, (A-a)DCO₂ is close to zero. As shown in our previous work (17) and here, (A-a)DCO₂ increases >300% from GV to PLV in animals given graded doses of PFC. The mechanism(s) underlying these increases can be attributed to Va/Q heterogeneity and/or diffusion limitation. West (34) describes increased (A-a)DCO₂ in the gas-filled lung as due primarily to an increased alveolar dead space that reduces PA CO₂. However, in the case where ventilation is constant and respiratory compensation is not possible (as with mechanical ventilation), changes in (A-a)DCO₂ are due to worsened Va/Q heterogeneity. We did not measure Vd/VT by the CO₂ method in these studies because the underlying assumption of complete arterial-alveolar equilibration is probably invalid. Vd/VT determined by MIGET showed no change from GV to PLV. As discussed with O₂ diffusion limitation is also an important mechanism of (A-a)DCO₂; where it would not be considered otherwise.

In the graded-dose protocol, overall Va/Q heterogeneity increased 40–50% over baseline during PLV, independent of PFC dose (Fig. 5). Some of the increase in (A-a)DCO₂ is attributable to worsening Va/Q heterogeneity, but it is impossible to calibrate the percent change in (A-a)DCO₂ due to this mechanism. As discussed in Va/Q heterogeneity during PLV, increased Va/Q heterogeneity was global and not attributable to a relative increase in low or high Va/Q. As with O₂ shunt, we are unable to determine the degree to which diffusion disequilibrium affects (A-a)DCO₂ with traditional techniques.

(A-a)DCO₂ did not change significantly from GV to PLV in animals given a single 20 ml/kg dose of PFC. In addition, the absolute value of (A-a)DCO₂ was comparable to the (A-a)DCO₂ measured in the graded-dose protocol at the 20 ml/kg dose. The explanation for differences in this measure between protocols is unclear. It may be that delivering a single large dose of PFC over a short period of time allows for a more even distribution of the fluid and reduces Va/Q heterogeneity and thereby (A-a)DCO₂. It is unfortunate that technical difficulties precluded accurate MIGET determination of Va/Q heterogeneity in these studies.

Va/Q heterogeneity during PLV. Va/Q heterogeneity increased during PLV in healthy piglets and the changes did not correlate with PFC volume. Furthermore, all of the increase in inert gas (a-A)D area was attributable to increased ventilation heterogeneity as measured by the E component of the (a-A)D area. There were no trends in the data suggesting predominance of high or low Va/Q during PLV. MIGET analysis did not show any change in Vd/VT from GV to PLV at any dose supporting the conclusion that Vd/VT is not the principle cause of measured increases in (A-a)DCO₂.
The potential mechanisms of VA/Q heterogeneity during PLV are numerous. The presence of a low-surface-energy fluid in alveoli alters local surface and interfacial tensions, alveolar-capillary configuration, and possibly local capillary blood flow (15). Ventilation distribution is altered as fluid in small airways and local compliance changes affect resistance and compliance, resulting in increased heterogeneity of ventilation time constants (18, 28). Maneuvers such as increasing PEEP to push fluid menisci into distal airways throughout ventilation may help decrease ventilation heterogeneity by minimizing back-and-forth movement of the fluid. Additional studies investigating the influence of respiratory rate, PEEP, mean airway pressure, etc. on VA/Q heterogeneity during PLV would be helpful. This study does not attempt to characterize the mechanisms responsible for heterogeneity but rather points out that in the healthy lung it is an important cause of an increased (a-A)DCO2 and would cause a significant (a-a)DO2 if the FiO2 was significantly <1.

As discussed in METHODS, the validity of MIGET is limited by its underlying assumptions, as are all modeling methodologies. The assumption that there is a complete diffusive equilibrium between end-capillary blood and alveolar gas is of particular concern during PLV because there may exist a diffusion limitation in fluid-filled alveoli. The effect of diffusion limitation would be to increase R (R = Pa/PV) and decrease E (E = gas pressure/PV) of all inert gases. MIGET results from these studies indicate a negligible role of diffusion limitation in creating error. If inert gas elimination in the liquid-filled lung were affected by diffusion limitation, one would expect the MIGET to show increased shunt and to overestimate dead space as the difference between the R and E curves widens. Shunt and dead space were not consistently or disproportionately elevated in these studies, decreasing our suspicion of error due to diffusion limitation. In addition, the sum of squares values for all experiments indicated a good fit between model predictions and measured inert gas R and E.

Summary. A moderate decrease in gas-exchange efficiency was noted in healthy piglets during PLV and can be explained by increased shunt and VA/Q heterogeneity. The effect of VA/Q heterogeneity on oxygenation is minimal if 100% O2 breathing is used during PLV but may play a role if lower FiO2 values are used. Furthermore, there may be a component of shunt due to diffusion limitation in the liquid-filled alveolus that we are currently unable to quantitate. (a-A)DCO2 increased with graded doses of PFC but not significantly with a single dose of PFC. The reason for this discrepancy is unclear, but an increase in VA/Q heterogeneity correlates with the rise in (a-A)DCO2 in the graded-dose protocol. There may also be a component of (a-A)DCO2 due to diffusion limitation.

The changes in gas exchange described here were mild, with blood gases well within the range of normal. It must be emphasized that ventilator settings were not adjusted to optimize gas exchange on the transition to PLV. This was done to compare GV and PLV with the same ventilatory parameters. One would not expect gas exchange to improve on filling healthy lungs with liquid. In fact, it is remarkable that blood-gas tensions within the normal range were achieved with an FRC volume of liquid in the lung. Adjustments to ventilator settings such as increasing the mean airway pressure or increasing the ventilator rate during PLV would most likely result in blood-gas values no different from during GV. These observations in healthy animals have relevance to the clinical management of patients with RDS who will, during recovery, reach a phase when more PFC will result in worse rather than improved gas exchange.

APPENDIX A

Estimation of evaporative losses of PFC. To arrive at the rate of evaporative loss of PFC from the lung, a rough estimate of PFC excretion is made, assuming that body temperature is 37°C and the exhaled gas (VE; in ml/h) is saturated at PPF = 10.5 Torr. The volume flow rate of PFC vapor exhaled (VPFC) is

\[ V_{E} \cdot FPFC = \frac{V_{E} \cdot PPFC}{PB} \]

where FPFC is the fraction of exhaled gas that is PFC vapor, and PB is in millimeters of mercury. Corrected to body temperature and pressure, this becomes

\[ V_{PF} = \frac{V_{E} \cdot PPFC}{PB} \cdot \frac{273}{310} \cdot \frac{760 - 47}{760 - 47} \cdot \frac{PPFC}{VE} \]

The number of moles (nPFc) of PFC exhaled is then

\[ V_{PF} \cdot 22,400 \text{ mol}^{-1} \cdot \text{mol}^{-1}. \]

Assuming a ventilatory rate of 20 breaths/min, a tidal volume of 15 ml/kg, and a dead space of 3 ml/kg (volume that is not saturated with PFC)

\[ V_{E} (\text{ml/h}) = 20 \text{ breaths/min} \cdot 60 \text{ min/h} \]

\[ = (15 - 3) \text{ ml/ breaths}^{-1} \cdot \text{kg}^{-1} = 14,000 \text{ ml/ kg}^{-1} \cdot \text{h}^{-1} \]

It follows that

\[ n_{PF} (\text{kg}^{-1} \cdot \text{h}^{-1}) = \frac{PPFC}{760 - 47} \cdot \frac{273}{310} \cdot \frac{14,000 \text{ ml/ kg}^{-1} \cdot \text{h}^{-1}}{22,400} \text{ mol/ml} \]

which for PPF = 10.5 Torr is 8.50 \times 10^{-3} \text{ mol/ kg}^{-1} \cdot \text{h}^{-1}. Finally, with the molar weight of the PFC = 499 g/mol and the density of the PFC = 1.9 g/ml, this becomes

\[ V_{PF} = 8.50 \times 10^{-3} \cdot \frac{499}{1.9} = 2.23 \text{ ml/ kg}^{-1} \cdot \text{h}^{-1} \]

APPENDIX B

Alveolar partial pressure of inert gases. The assumption that the alveolar partial pressure of inert gases is negligible was verified by calculating the partial pressure of acetone in the MIGET perfusate (5% dextrose in water). Partial pressures in the perfusate will always be greater than the partial pressures in blood, where the gases are diluted, and in alveolar space, where inert gases are excreted. Acetone
concentration in the perfusate is 1 ml acetone/250 ml dextrose solution. The \( \beta \) of acetone in water is \( 40 \text{ ml} \cdot 100 \text{ ml}^{-1} \cdot \text{mmHg}^{-1} \). With Henry's law (\( C = \beta \cdot x \cdot P \)), where \( C \) is concentration and \( x \) is gas species, the partial pressure of acetone in the perfusate is

\[
\frac{1 \text{ ml acetone/250 ml dextrose solution}}{40 \text{ ml acetone-100 ml dextrose solution}^{-1} \cdot \text{mmHg}^{-1}} = 0.01 \text{ mmHg}
\]

The other four gases have partial pressures of the same small order.

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