Superimposing positive end-expiratory pressure during partial liquid ventilation in experimental lung injury

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ABSTRACT This study was undertaken to determine the effects of superimposing incremental levels of positive end-expiratory pressure (PEEP) during partial liquid ventilation (PLV) on gas exchange, respiratory mechanics and morphological changes in experimental acute lung injury (ALI).

In a prospective trial, six pigs weighing 30 ± 5 kg (mean $\pm$ SD) were tracheotomized, submitted to pressure-controlled mechanical ventilation (pc-CMV) and depleted of surfactant by lung lavage. Animals were then mechanically ventilated with three levels of PEEP: 0.5, 1.0 and 1.5 kPa. PLV was then initiated by intratracheal instillation of $30 \text{ mL} \cdot \text{kg}^{-1}$ perfluorocarbon, followed by pc-CMV with PEEP 0.5, 1.0 and 1.5 kPa. Computed tomography (CT)-based analyses of lung volumes and density were obtained after lung lavage, in PLV and during the combined application of PLV and PEEP. Simultaneously, haemodynamics, gas exchange, dynamic compliance (C_{dyn}) and dynamic resistance (R_{dyn}) were determined. Statistical analysis was performed using multivariate analyses of variance for repeated measures ($p < 0.05$).

In ALI and before PLV, the application of PEEP significantly reduced cardiac output and intrapulmonary shunt. Arterial oxygen tension (P_{aO_2}) was increased from 6.9 kPa (52 (42, 54) mmHg) (median, (25th and 75th percentile)) to 8.6 kPa (65 (52, 133) mmHg) (PEEP 1.0 kPa) and 15.6 kPa (117 (90, 195) mmHg) (PEEP 1.5 kPa) ($p < 0.05$). The lung volume obtained by CT increased, CT density was reduced ($p < 0.05$), C_{dyn} tended to increase and R_{dyn} to decrease (nonsignificant). PLV increased arterial carbon dioxide tension and reduced pH ($p < 0.05$). CT lung volume and lung density were increased ($p < 0.05$). Superimposing PEEP on PLV increased P_{aO_2} from 9.3 kPa (70 (52, 124) mmHg) (PEEP 0.5 kPa) to 12.9 kPa (97 (55, 233) mmHg) (PEEP 1.0 kPa) and 40.3 kPa (303 (64, 426) mmHg) (PEEP 1.5 kPa) ($p < 0.05$), but had no significant effect on CT lung volume and density.

It was concluded that in experimental lung injury, positive end-expiratory pressure provided alveolar recruitment. The combined application of positive end-expiratory pressure and partial liquid ventilation significantly augmented oxygenation and might eventually allow either a reduction in the volumes of perfluorocarbons required, or a reduction in positive end-expiratory pressure necessary to maintain pulmonary gas exchange in acute lung injury.

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In acute lung injury (ALI), mechanical ventilation with positive end-expiratory pressure (PEEP) is a mainstay in therapy, preventing alveolar end-expiratory collapse, and thus increasing end-expiratory lung volumes and functional residual capacity (FRC) [1]. Recent studies have demonstrated that in acute respiratory distress syndrome (ARDS) airway collapse concentrates in dependent lung regions owing to the increased weight of the infiltrated lung. The recruiting effects of PEEP are therefore mainly due to a counterforce mechanism preventing compression atelectasis [2].

Partial liquid ventilation (PLV), first described by Fuhrman *et al.* [3], is a novel technique to assist mechanical ventilation, in which the lungs are filled with perfluorocarbons (PFC) to a volume approximating the

FRC or smaller and then gas ventilated using a standard mechanical ventilator.

PFC are radiopaque, dense liquids with high spreading coefficients and an excellent capacity for carrying oxygen and carbon dioxide. Intrapulmonary distribution of PFC is inhomogeneous and likely to follow the gravitational gradient, preferentially pooling in dependent lung areas. The beneficial effects of PLV on gas exchange are partly attributed to the filling and distending of dependent alveoli with the noncompressible PFC, thereby reopening air spaces and preventing end-expiratory collapse. In ALI, PLV might thus provide a vertically graded PEEP-like effect [4]. Consequently, it has been suggested that during PLV, additional recruitment of alveoli by PEEP might be of less importance [5].

Despite an extensive number of studies on PLV, the issue of characterizing appropriate PEEP levels during PLV has not been systematically evaluated. Moreover, in ALI, the *in vivo* intrapulmonary distribution of PFC during PLV combined with PEEP has to be established.

This study was undertaken to clarify the physiological and morphological response to the combined application of PLV and incremental levels of PEEP in an animal model of ARDS.

Material and methods

Animal preparation

Six female pigs (Deutsches Hausschwein) with a body weight of 30 ± 5 kg were included in the study. The experimental protocol was approved by the local government and the experiments were performed in accordance with the Helsinki convention for the use and care of animals.

Animals were included in the study only when no signs of infection were perceptible prior to the experiment. Pre-medication was achieved with ketamine ($10 \text{ mg} \cdot \text{kg}^{-1} \text{ i.m.}$), midazolam ($0.5 \text{ mg} \cdot \text{kg}^{-1} \text{ i.m.}$) and atropine ($0.01 \text{ mg} \cdot \text{kg}^{-1} \text{ i.m.}$). After peripheral venous access, anaesthesia was induced with *i.v.* methohexital ($8 \text{ mg} \cdot \text{kg}^{-1}$) and fentanyl ($0.01 \text{ mg} \cdot \text{kg}^{-1}$) and maintained with a continuous infusion of methohexital ($50 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) and fentanyl ($3 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$). All animals received muscle relaxation by an initial bolus of pancuronium bromide ($0.15 \text{ mg} \cdot \text{kg}^{-1}$) followed by a continuous infusion ($2.5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) throughout the experiment. Immediately after induction, the pigs were tracheotomized and intubated with a 7.5–8.0 mm endotracheal tube, fitted with a heat moisture exchanger with bacterial filter.

Animals were then submitted to pressure-controlled mechanical ventilation (pc-CMV) (Servo 900C; Siemens Elema, Lund, Sweden) limiting peak inspiratory pressure to ≈ 40 kPa, at a frequency of $18 \cdot \text{min}^{-1}$, with an inspiratory oxygen fraction (F_{I,O_2}) of 1.0, an inspiratory/expiratory ratio of 1:1 and a PEEP of 0.5 kPa. The inspiratory pressure support and the respiratory rate were adjusted after obtaining two arterial blood gas analyses separated by 20 min in order to keep the animals normocapnic (arterial carbon dioxide tension (P_{a,CO_2}) = 5.3 ± 0.8 kPa (40 ± 6 mmHg)). Temperature was maintained within $\pm 0.5^\circ\text{C}$ of the presudy range using a heating pad, and a continuous infusion of balanced electrolyte solution ($5 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$) was given during the study. During the study, no cardiotoxic or vasoactive drugs were administered.

Arterial and central venous access were performed percutaneously. The pulmonary artery catheter (model 93A-431-7.5 F; Baxter Healthcare Corporation, Irvine, CA, USA) was advanced from the femoral vein using a 8.5 Fr sheath (Baxter). An 18 G arterial line (Vygon, Ecouen, France) was introduced into the femoral artery. The arterial line and the pulmonary artery catheter were used for blood sampling and the collection of haemodynamic data. Cardiac frequency was determined from the blood pressure curve. Central venous pressure (CVP), mean arterial pressure (MAP), mean pulmonary artery pressure (PAP) and pulmonary artery wedge pressure (PCWP) were assessed using a disposable transducer (Baxter) and a moni-

toring system (Model 66 S; Hewlett-Packard; Böblingen, Germany). Measurements were taken in the supine position with zero reference level at the midaxilla, and vascular pressures were the average taken at end-expiration from three successive respiratory cycles. Cardiac output (CO) was determined with standard thermodilution techniques (Baxter) and expressed as the mean of four measurements using injections of saline (10 mL at $1\text{--}5^\circ\text{C}$) arbitrarily performed during different phases of the respiratory cycle.

All blood samples (arterial and mixed venous) were collected anaerobically, placed in ice and analysed within 5 min using standard blood-gas electrodes (ABL 520; Radiometer, Copenhagen, Denmark), and spectrophotometry (OSM 3 Hemoximeter; Radiometer) was performed to obtain the total haemoglobin concentration, arterial oxygen saturation (S_{a,O_2}) and mixed venous oxygen saturation (S_{v,O_2}).

The following calculations were made. Arterial and mixed venous oxygen content ($\text{mL} \cdot \text{dL}^{-1}$) were calculated from the following:

$$(\text{Hb} \times 1.34 \times (\% \text{O}_2\text{-Saturation}/100)) + (P_{\text{a},\text{O}_2} \times 0.0031)$$

where Hb denotes haemoglobin and arterial oxygen tension (P_{a,O_2}). The arteriovenous oxygen content difference (ΔvDO_2) was calculated from: arterial oxygen content (Ca,O_2) - mixed venous oxygen content (Cv,O_2). The pulmonary right-to-left shunt ($Q_{\text{s}}/Q_{\text{t}}$) was calculated from:

$$(\text{Cc},\text{O}_2 - \text{Ca},\text{O}_2) / (\text{Cc},\text{O}_2 - \text{Cv},\text{O}_2)$$

where Cc,O_2 denotes pulmonary end-capillary oxygen content, with the assumption that end-capillary P_{O_2} is equivalent to alveolar P_{O_2} (P_{A,O_2}) ("alveolar" during PLV refers to the gas adjacent to the fluid layer according to the suggestions of MATES *et al.* [6]). During PLV, P_{A,O_2} (at an F_{I,O_2} of 1.0) was estimated as: Barometric pressure - P_{A,CO_2} - water vapour pressure ($P_{\text{H}_2\text{O}}$) - PFC vapour pressure (P_{PFC}) (8.1 kPa (61 mmHg) for FC 3280).

$$\text{Arterial oxygen delivery } (\text{DO}_2) (\text{mL} \cdot \text{min}^{-1}) = \text{cardiac output} \times \text{arterial oxygen content} \times 10$$

$$\text{Oxygen uptake } (\text{VO}_2) (\text{mL} \cdot \text{min}^{-1}) = \text{cardiac output} \times (\text{arterial oxygen content} - \text{mixed venous oxygen content}) \times 10$$

Flow and volume data

Measurements of airflow (V) were performed using a heated Fleisch No. 2 pneumotachograph (Metabo, Epalinges, Switzerland) placed between the y-piece of the respiratory tubing and the endotracheal tube (ETT), connected to a differential pressure transducer (Fumes Controls, Bexhill, UK). The pneumotachograph was calibrated before each experiment. Airway pressure (P_{aw}) was measured with a probe placed at the outer end of the ETT and connected to a pressure transducer (Sensym, Milpitas, USA) via a 20 cm sampling line. Both P_{aw} and V were sampled with a frequency of 100 Hz converted by an analogue-digital converter and stored on a personal computer. The pressure and flow waves and the pressure-volume (PV) loops obtained from the integrated flow signal were displayed on a computer screen.

Respiratory mechanics were determined using a least-square fitting method for selected parts of the PV loop according to the method of GUTTMANN, described in detail elsewhere [7]. Since the ETT imposes a significant additional resistance on the respiratory system, the pressure drop across the ETT (P_{ETT}) was also calculated according to the method described by GUTTMANN [8]. Using this method, tracheal pressure (P_{trach}) was calculated as $P_{trach} = P_{aw} - P_{ETT}$. For all calculations of respiratory mechanics, the calculated P_{trach} was used, thereby eliminating the pressure drop due to the imposed resistance of the ETT. At the end of each step of the protocol, respiratory mechanics data of at least 10 consecutive breaths were averaged and stored for analyses.

Induction of acute lung injury

The lavage procedure was performed according to the method of surfactant depletion described by LACHMANN and co-workers [9]. After disconnection from the ventilator, the animal was positioned in the supine, reverse Trendelenburg position at an angle of 60–70° and the lungs were completely filled with warmed saline (0.15 M, 37°C, 40 mL·kg⁻¹) via the ETT. The animal was then tilted 45° with the head down and the lavage fluid was drained by gravitational force until mean arterial pressure markedly decreased and mean pulmonary artery pressure increased. After each lavage, the animals were reconnected to the ventilator for at least 20 min before the next lavage was performed. A minimum of four lavages was performed in the supine position, followed by three to four lavages in the prone position. Beginning with the third lavage onwards, arterial blood gas samples were taken at each lavage and the ventilator settings were adapted in order to achieve a pH >7.20. Subsequent arterial blood gas samples and haemodynamic measurements were taken every 15 min. An increase in P_{aO_2} within 60 min resulted in repetitive lavages until arterial P_{aO_2} was persistently <13.3 kPa (100 mmHg), considered to indicate severe ALI.

Experimental protocol

The PFC used in this study (FC 3280; 3M Chemical Products, Neuss, Germany) is a highly purified radiopaque C₈F₁₈ molecule. At 25°C, FC 3280 has a density of 1.75 g·cm⁻³, a viscosity of 0.7 centistokes, a vapour pressure of 8.1 kPa (61 mmHg) and a surface tension of 12 mN·m⁻¹. It dissolves 40 mL O₂ and 192 mL CO₂ per 100 mL of PFC (3M product data).

After induction of ALI, animals were transferred to the computed tomography (CT) suite using a transportable ventilator (Servo 900C) and monitoring system (Hewlett Packard, 66S). The following experimental protocol was performed at the CT suite. After recording initial CT scans representing ALI and simultaneously measuring haemodynamics, gas exchange and lung mechanics during pc-CMV with different levels of PEEP (0.5, 1.0 and 1.5 kPa), 30 mL·kg⁻¹ of FC 3280 was instilled into the trachea with the animal in the supine position during inspiration using a swivel-connector (Portex, Kent, UK). Pc-CMV was resumed thereafter without changes in ven-

tilator settings except for PEEP. After instillation, CT scans of the lungs, gas exchange, haemodynamics and lung mechanics were obtained during pc-CMV at three different levels of PEEP: 0.5, 1.0 and 1.5 kPa. Each step of data acquisition at each level of PEEP lasted for 10 min, so that the entire measurements lasted for approximately 60 min.

Computed tomography protocol

CT of the lungs was performed with a Somatom plus CT scanner (Siemens, Erlangen, Germany). The scans were performed in a spiral CT technique at 165 mA and 137 kV with a slice thickness of 10 mm. The system enables 24 rotations at 1 rotation·s⁻¹. Table feed was 10 mm. Thus, whenever the craniocaudal diameter of the lungs exceeded 23 cm, a second spiral CT was performed to cover the entire lung. Spiral CT scans of the whole lungs were performed at the following respirator settings: fixed expiration ("expiratory hold" on Servo 900C) at PEEP settings of 0.5, 1.0, and 1.5 kPa. An initial CT scan series was performed immediately before the administration of PFC (following ALI) and scans were repeated immediately after administration of 30 mL·kg⁻¹ PFC. Owing to lung lavage-induced pulmonary oedema and the significant amounts of PFC administered, automatic contour-finding programmes could not be implemented to calculate lung volume. In the CT scans acquired, lungs had to be isolated from the surrounding body by manually drawn regions of interest (ROI) and in each CT slice, intraparenchymal densities and lung volume were measured. Total lung volume calculations were based on both gas-filled lung regions and intraparenchymal opacities and were calculated as sum of the lung volume of all CT slices. Whole intraparenchymal densities (including pulmonary blood volume and volume of the interstitium) were calculated as the volume-weighted mean of the ROI measurements of all CT slices.

Statistical analyses

Results are expressed as the median and 25th and 75th percentiles in order to give an indication of the sample distribution. Data are presented as measured during baseline (prelavage, not used for statistical comparison), after induction of ALI (postlavage) and during pc-CMV with incremental levels of PEEP (0.5, 1.0 and 1.5 kPa), followed by PLV at the same levels of PEEP (0.5, 1.0 and 1.5 kPa). Statistical analyses were performed using the SPSS for Windows software package (release 6.0, SPSS Inc., Chicago, IL, USA). Multivariate analyses of variance (MANOVA) for repeated measures was used. The statistical design for the MANOVA procedure was based on two within-factors, *i.e.* PEEP and PFC (indicating instillation of FC 3280), with PEEP having three and PFC having two levels. The levels of PEEP were 0.5, 1.0, and 1.5 kPa during both ALI and PLV. The levels of PFC were ALI (prior to instillation of FC 3280) and PLV. To analyse further the effect of CO on P_{aO_2} and Q_s/Q_t measurements, MANOVA was performed using CO as a time-dependent covariant. A p-value ≤0.05 was considered significant.

Table 1. – Haemodynamic data during the protocol

		PEEP kPa	Baseline		ALI		PLV		MANOVA
<i>f</i> C	min ⁻¹	0.5	84	(70, 117)	89	(81, 137)	138	(125, 166)	
		1.0			109	(71, 145)	145	(134, 179)	
		1.5			99	(72, 143)	142	(122, 181)	
MAP	mmHg	0.5	84	(72, 113)	93	(84, 106)	103	(80, 121)	
		1.0			90	(80, 137)	96	(81, 110)	
		1.5			92	(74, 127)	101	(72, 117)	
PAP	mmHg	0.5	20	(14, 22)	27	(23, 33)	38	(31, 42)	
		1.0			30	(23, 38)	34	(32, 41)	
		1.5			31	(28, 37)	37	(31, 38)	
CVP	mmHg	0.5	8	(5, 13)	8	(4, 10)	7	(5, 12)	
		1.0			6	(4, 11)	7	(6, 12)	
		1.5			8	(7, 11)	9	(5, 12)	
PCWP	mmHg	0.5	11	(5, 12)	11	(7, 13)	5	(5, 12)	
		1.0			10	(6, 13)	6	(6, 12)	
		1.5			8	(6, 12)	8	(6, 13)	
CO	L·min ⁻¹	0.5	3.7	(3.5, 5.3)	4.4	(2.9, 5.7)	5.5	(4.6, 6.8)	PEEP*, p=0.04
		1.0			3.7	(3.0, 6.2)	5.5	(4.3, 6.4)	
		1.5			3.7	(2.5, 5.4)	5.2	(3.7, 6.3)	
SVR	dyn s·cm ⁻⁵	0.5	1398	(1132, 2150)	1775	(1181, 2491)	995	(772, 2134)	
		1.0			2197	(1203, 2770)	1123	(850, 1981)	
		1.5			2338	(1517, 2590)	947	(822, 2526)	
PVR	dyn s·cm ⁻⁵	0.5	174	(149, 192)	276	(205, 500)	431	(213, 579)	
		1.0			499	(236, 550)	386	(247, 628)	
		1.5			584	(374, 628)	409	(236, 550)	PEEP/PFC*, p=0.03
DO ₂	mL·min ⁻¹	0.5	525	(426, 627)	280	(236, 459)	571	(381, 914)	
		1.0			378	(282, 591)	464	(446, 859)	
		1.5			478	(252, 544)	604	(447, 864)	
VO ₂	mL·min ⁻¹	0.5	174	(97, 187)	168	(116, 215)	201	(72, 263)	
		1.0			173	(115, 208)	165	(129, 205)	
		1.5			181	(118, 206)	220	(150, 247)	

Data are medians (and 25th, 75th percentile). Baseline indicates parameters prior to lung lavage (not used for statistical comparison); ALI: acute lung injury; PLV: partial liquid ventilation; MANOVA: multivariate analysis of variance. *: H0 hypothesis of equality of means of the measured data for variations of the respective interventions is rejected on a level of significance of 0.05 (PEEP*: significant effect of PEEP; PFC*: significant effect of FC 3280; PEEP/PFC*: significant effect of combined application and p-values). *f*C: cardiac frequency; MAP: mean arterial pressure; PAP: mean pulmonary artery pressure; CVP: central venous pressure; PCWP: pulmonary capillary wedge pressure; CO: cardiac output; SVR: systemic vascular resistance; PVR: pulmonary vascular resistance; DO₂: oxygen delivery; VO₂: oxygen uptake. 1 mmHg=0.133 kPa.

Table 2. – Summary of gas exchange, respiratory mechanics and radiographical data

		PEEP kPa	Baseline		ALI		PLV		MANOVA
pH		0.5	7.46	(7.41, 7.49)	7.40	(7.32, 7.49)	7.21	(7.18, 7.36)	
		1.0			7.37	(7.32, 7.44)	7.25	(7.21, 7.33)	
		1.5			7.36	(7.27, 7.58)	7.25	(7.21, 7.36)	PFC*, p=0.03
PaO ₂	mmHg	0.5	571	(510, 632)	52	(42, 54)	70	(52, 124)	PEEP*, p=0.03
		1.0			65	(52, 133)	97	(55, 233)	
		1.5			117	(90, 195)	303	(64, 426)	PEEP/PFC*, p=0.04
PaCO ₂	mmHg	0.5	38	(33, 45)	47	(38, 58)	72	(52, 92)	
		1.0			50	(42, 59)	69	(55, 83)	PFC*, p=0.01
		1.5			57	(40, 69)	63	(51, 87)	PEEP/PFC*, p=0.02
Q _s /Q _t	%	0.5	10	(6, 15)	61	(46, 62)	43	(39, 77)	PEEP*, p=0.02
		1.0			49	(40, 51)	46	(40, 62)	
		1.5			30	(27, 46)	29	(12, 44)	
VT	mL	0.5	287	(268, 453)	434	(352, 496)	326	(292, 501)	PEEP*, p=0.05
		1.0			346	(272, 408)	370	(314, 440)	
		1.5			330	(283, 464)	311	(268, 407)	
PIP	mbar	0.5	15	(13, 17)	30	(27, 32)	30	(22, 36)	PEEP*, p=0.01
		1.0			30	(24, 39)	34	(27, 35)	
		1.5			35	(32, 38)	38	(30, 39)	
C _{dyn}	mL·mbar ⁻¹	0.5	32	(31, 36)	13	(12, 20)	20	(16, 23)	
		1.0			18	(13, 21)	16	(14, 22)	
		1.5			20	(17, 22)	19	(16, 26)	
R _{dyn}	mbar* s·L ⁻¹	0.5	1.2	(1.1, 1.3)	6.0	(3.8, 9.0)	8.5	(6.9, 13.8)	
		1.0			4.0	(2.5, 9.8)	7.8	(5.8, 10.2)	
		1.5			2.9	(1.7, 5.3)	7.7	(4.4, 9.1)	
CT lung volume	mL	0.5	-		1328	(1092, 1532)	1528	(1187, 1798)	PEEP*, p=0.009
		1.0			1459	(1251, 1813)	1774	(1364, 1881)	PFC*, p=0.003
		1.5			1713	(1440, 1945)	1750	(1493, 1951)	
CT lung density	HU	0.5	-		675	(551, 735)	1037	(991, 1089)	PEEP*, p=0.009
		1.0			610	(504, 675)	995	(924, 1131)	PFC*, p=0.003
		1.5			509	(442, 609)	949	(849, 1089)	

Data are medians (and 25th, 75th percentile). Baseline indicates parameters prior to lung lavage (not used for statistical comparison). ALI: acute lung injury; PLV: partial liquid ventilation; MANOVA: multivariate analysis of variance. *: H0 hypothesis of equality of means of the measured data for variations of the respective interventions is rejected on a level of significance of 0.05 (PEEP*: significant effect of PEEP; PFC*: significant effect of FC 3280; PEEP/PFC*: significant effect of combined application and p-values). PaO₂: arterial oxygen tension; PaCO₂: arterial carbon dioxide tension; Q_s/Q_t: pulmonary right-to-left shunt; VT: tidal volume; PIP: peak inspiratory pressure; C_{dyn}: dynamic compliance; R_{dyn}: dynamic resistance; CT: computed tomography; 10HU: Hounsfield units. 1 mmHg=0.133 kPa; 1 mbar=0.1 kPa.

Results

Prior to lung lavage, animals were comparable with regard to body weight and prestudy conditions. Following induction of ALI, PAP, Q_s/Q_t and P_{a,CO_2} were increased (tables 1 and 2). A marked decrease in P_{a,O_2} and dynamic compliance (C_{dyn}) was observed in all animals, whereas dynamic resistance (R_{dyn}) was increased (table 2).

In ALI and before PLV, application of PEEP induced a significant decrease in CO and Q_s/Q_t . P_{a,O_2} was increased (tables 1 and 2). PEEP increased peak inspiratory pressure (PIP) and decreased tidal volumes (V_T) ($p < 0.05$) (table 2). C_{dyn} was increased and R_{dyn} decreased by administration of PEEP (NS).

Initiation of PLV significantly increased P_{a,CO_2} and decreased pH. There was a tendency for R_{dyn} to increase following PLV (NS). Superimposing PEEP on PLV induced a significant increase in P_{a,O_2} , and a reduction in P_{a,CO_2} and pulmonary vascular resistance (PVR) ($p < 0.05$). C_{dyn} and R_{dyn} were unchanged (tables 1 and 2).

To analyse further the effect of CO on Q_s/Q_t and P_{a,O_2} measurements during the study, MANOVA was performed using CO as a time-dependent covariant, demonstrating a significant effect of CO on Q_s/Q_t during pc-CMV with PEEP ($p < 0.05$). No statistical significant dependence of P_{a,O_2} values on CO could be verified.

After lavage, CT scans of the lungs revealed a patchwork of normal and dense areas with generally well-defined boundaries. In all animals, dense areas of variable size were visible in the dependent lung regions (fig. 1a). In ALI and before PLV, application of PEEP resulted in a significant decrease in intraparenchymal lung density. Lung volume was significantly increased (table 2, fig. 1b, c).

The radiodensity of FC 3280 was determined at 553 Hounsfield units (HU). Initiation of PLV induced a significant increase in both CT lung volume and intraparenchymal densities (table 2), with PFC predominantly pooling in dependent lung regions creating a vertical gradient in density (fig. 2a). Administration of PEEP during PLV induced a spreading of PFC from the tracheobronchial system into the lung periphery, e.g. the bronchial system and the alveoli (fig. 2b, c).

Fig. 1. — a) Computed tomography (CT) of the lung following induction of acute lung injury (ALI) (pressure-controlled mechanical ventilation (pc-CMV), positive end-expiratory pressure (PEEP) 0.5 kPa). Table position is at the level of the lower lobes, 4 cm below the carina. Scans showing opacities representing collapsed areas distributed throughout the lungs in a nonhomogeneous manner, but predominantly concentrating in dependent (posterior) lung regions. Note the air bronchograms in the dependent and in the middle regions of the lung. b) CT of the lung following induction of ALI (pc-CMV, PEEP 1.0 kPa) showing an increase in the air content of the lung. Note a reduction in consolidations in the dependent (posterior) lung with increasing levels of PEEP. c) CT of the lung following induction of ALI (pc-CMV, PEEP 1.5 kPa) showing a further increase in the air content of the lung. Opacities representing collapsed areas in the dependent (posterior) lung have almost disappeared.

Fig. 2. — a) Computed tomography (CT) of the lung following partial liquid ventilation (PLV) with $30 \text{ mL} \cdot \text{kg}^{-1}$ FC 3280 (pressure-controlled mechanical ventilation (pc-CMV), positive end-expiratory pressure (PEEP) 0.5 kPa). Table position as in fig. 1. The CT scans show the nonhomogeneous distribution of the highly radiopaque perfluorocarbons (PFC) in both lungs. Opacities, predominantly in the posterior lung, represent a combination of PFC and oedema/lavage fluid remaining in the airways, eventually floating on top of the dense PFC and filling alveoli above the PFC-filled regions of the lung. The larger airways of the posterior lung are completely filled with PFC, whereas air bronchograms are shown in the middle regions of the lung. b) CT scan of the lung following PLV with $30 \text{ mL} \cdot \text{kg}^{-1}$ FC (pc-CMV, PEEP 1.0 kPa) demonstrating a reduction in opacities with a more solidly opaque portion in the right posterior lung. In larger airways, small levels of PFC can be seen. c) CT scan of the lung following PLV with $30 \text{ mL} \cdot \text{kg}^{-1}$ FC 3280 (pc-CMV, PEEP 1.5 kPa) showing a further reduction in opacities and a more solidly opaque portion of PFC in the right posterior, and less in the left posterior lung with air bronchograms. The amount of intrapulmonary PFC liquid appears to be decreased as a result of distribution from the tracheobronchial system into the lung periphery, e.g. the bronchial system and the alveoli.

Discussion

In the present study, CT of the acutely injured lung revealed a significant increase in lung volume and a reduction in lung density by PEEP, presumably as an equivalent of alveolar expansion. PLV alone significantly increased CT lung volume and lung density. Coapplication of incremental levels of PEEP during PLV was highly effective in terms of oxygenation, although neither a further increase in CT lung volume nor a significant reduction in lung density was demonstrated. Radiographically, a distribution of PFC mainly into dependent lung regions was confirmed and the administration of PEEP during PLV induced a spreading of PFC from the tracheobronchial system into the lung periphery, e.g. into the bronchial system and the alveoli. It should be pointed out that lung volume calculations by CT included lung interstitium and pulmonary blood volume. This reduced the accuracy of CT lung volume calculations since PLV and the different levels of PEEP applied may have altered the intrapulmonary blood volume. Furthermore, alveolar expansion could not be dis-

criminated from compression of atelectatic lung regions by overexpansion of adjacent ventilated lung areas. In a preliminary report, GOLDNER *et al.* [10] recently demonstrated that in healthy lungs, stepwise instillation of doses of PFC, each equalling one-third of FRC, induced a dose-dependent decrease in aerated lung volume. Total FRC (gas-filled and PFC-filled, determined by helium dilution), however, was increased at higher doses of PFC (approximating two-thirds of FRC). The authors speculated that PLV associated changes in transpulmonary pressure might induce volume loss after the first dosing of PFC.

In ARDS, it has been shown that PEEP-induced changes in CT intraparenchymal opacities were well correlated with changes in oxygenation [11]. During PLV (with an FiO_2 of 1.0) the concentration of O_2 in PFC-filled alveoli is reduced compared with that in gas-ventilated alveoli (FC 3280 dissolves a maximum of 40 vol.% of O_2 , compared with 100 vol.% present in pure oxygen gas). Thus, it has been suggested that the beneficial effects of PLV on gas exchange are attributed to the filling, distending and stabilization of alveoli with the noncompressible PFC,

thereby reopening air spaces and preventing them from end-expiratory collapse, an effect similar to that of PEEP [4]. In ALI, this might be particularly true in dependent zones of the lung, where the expanded weight of the infiltrated lung causes airway closure and alveolar collapse along the vertical axis of the thorax, concentrating compression atelectasis in these dependent regions [2]. In PLV, the same gravitational force providing recruitment of dependent alveoli may, furthermore, redirect pulmonary blood flow towards nondependent, ventilated lung regions [12]. Improved homogeneity of $V'A/Q'$ resulting from the recruitment of alveoli and redirection of blood flow should therefore mainly account for improvements in oxygenation during PLV.

In the present study, however, application of PEEP alone was more effective than PLV alone, significantly increasing P_{a,O_2} along with an increase in CT lung volume, indicating recruitment due to a reduction of collapsed alveoli with zero ventilation/perfusion ratio ($V'A/Q'$). Superimposing PEEP on PLV significantly increased pulmonary gas exchange, probably through an improved matching of $V'A/Q'$. The high spreading coefficients and low surface tensions of PFC produce surfactant-like activity by reducing surface tension at air liquid interfaces. In ALI, PFC maintain low, but constant air PFC surface tensions for any given alveolar radius. If the alveolus is not completely filled with PFC, end-expiratory alveolar stability at low end-expiratory volumes (low FRC) could not be presumed and end-expiratory collapse may occur. It may be speculated that during PLV in ALI three different compartments might be separated within the lung: 1) a PFC-filled compartment in the dependent parts of the lung; 2) an intermediate compartment where alveoli are partially PFC-filled; and 3) a non-PFC-filled compartment in nondependent lung regions. In the latter ones, alveolar stability at low end-expiratory volumes is dependent on a functioning endogenous surfactant system [13] and application of high enough levels of PEEP will prevent end-expiratory collapse. The data indicate that in ALI, despite high-dose PLV (30 mL·kg⁻¹), significant $V'A/Q'$ -mismatching occurs and higher levels of PEEP are essential to provide appropriate end-expiratory lung volume. This hypothesis is partly supported by a previous study by SALMAN *et al.* [14], demonstrating alveolar collapse during weaning back from PLV (*i.e.* at low volumes of PFC) if insufficient levels of PEEP were applied.

HERNAN *et al.* [5] assessed the cumulative effects of PEEP levels between 0.6–1.4 kPa at a constant tidal volume during PLV as insignificant for pulmonary gas exchange in healthy sheep. In their experiments the authors defined end-inspiratory pressure and tidal volume as determinants of oxygenation. In normal sheep undergoing PLV, FRC might already be recruited during mechanical ventilation with a PEEP of 0.6 kPa and application of higher levels of PEEP will cause overexpansion of alveoli. Nevertheless, after initiation of PLV, HERNAN *et al.* [5] described PEEP as a means to clear proximal airways of PFC, thus preventing pressure-spikes during gas inflation. In their study, a second group of adult sheep (58±8 kg) with hydrochloric acid-induced ALI was enrolled, receiving volume-controlled CMV (PEEP 1.0 kPa, PIP 6.2±0.8 kPa) resulting in a P_{a,O_2} of 13.3 kPa (<100 mmHg) before randomization. PLV significantly improved oxygenation, but

the effects of incremental levels of PEEP during PLV were not investigated in this group of sheep.

In the present protocol, application of incremental levels of PEEP in ALI significantly impaired CO, probably mainly owing to an increase in intrathoracic pressure and a reduction in venous return. PEEP-induced circulatory effects are, however, considered to depend on many factors, including lung and chest-wall compliance, ventricular loading conditions, contractility and the sympatho-adrenergic compensatory response [15]. During PLV and PLV/PEEP, CO tended to be higher than during ALI, however, the difference was not significant. To analyse further the effects of CO on Q_s/Q_t , MANOVA was performed using CO as a time-dependent covariant. During pc-CMV with PEEP a significant effect of CO on Q_s/Q_t was demonstrated. Previously, PEEP-induced depression of CO has been identified as a mechanism of shunt reduction in ALI. This was attributed to a decrease in perfusion of unventilated lung [16]. In the present study, the increase in CO during PLV was not associated with an increase in Q_s/Q_t , suggesting no increase in the fractional perfusion of units with low $V'A/Q'$.

Initiation of PLV had no adverse effects on haemodynamics in this series. On the contrary, CO and consequently DO_2 tended to be higher during PLV (NS), an observation previously reported in larger animals [5]. Coapplication of PEEP in PLV significantly decreased PVR, probably through a concomitant improvement in oxygenation and a reduction in P_{a,CO_2} which may have resulted in a decrease in hypoxic pulmonary vasoconstriction.

Initiation of PLV during pc-CMV (with an essentially unchanged V_T) induced a significant increase in P_{a,CO_2} , suggesting an increase in alveolar dead space ventilation. Superimposing PEEP during PLV significantly reduced P_{a,CO_2} , indicating an improvement in alveolar ventilation. Total liquid ventilation has been demonstrated potentially to reduce effective alveolar ventilation, thus impairing CO₂ removal. This has been primarily attributed to diffusion limitation, caused by the "diffusion dead space" [17]. During PLV, however, CO₂ removal might be effectively improved by increasing minute ventilation.

In the piglets in the present study, PIP was limited to 4.0 kPa, although the pressures required to normoventilate the animals prior to ALI were much lower (table 2). This mode of ventilation resulted in tidal volumes of approximately 8–10 mL·kg⁻¹ at baseline (healthy pigs). After induction of lung injury, PIP was increased to values >4.0 kPa, resulting in V_T of up to 14 mL·kg⁻¹; however, normoventilation was not accomplished and mild hypercapnia was accepted. High V_T has been shown potentially to induce volutrauma in ALI [18]. Alveolar overinflation, increased shear forces and finally alveolar disruption are consequences of volutrauma. We did not evaluate ventilator-induced lung injury in this study; however, current concepts of mechanical ventilation in ALI propagate CMV at low airway pressures and low V_T [19].

In ALI, an increase in R_{dyn} has been attributed to an increase in the viscoelastic resistance of tissue and a reduction in accessible lung volume [20]. Initiation of PLV induced an additional increase in R_{dyn} , probably due to the fluid viscosity of PFC in small airways, using the first portion of airway pressure applied to move the dense liquid into the alveoli. Thus, the lung becomes functionally a slower compartment, clinically apparent as pressure-spike

readings following the implementation of gas ventilation [5]. In our series the application of incremental levels of PEEP did not counterbalance the increase in R_{dyn} . The flow and volume data reported are considered to apply purely to the animals' respiratory mechanics, excluding the effects of additional resistance due to the ETT [7]. The accuracy of P_{trach} corrections for the ETT during PLV have, however, not been systematically evaluated and PFC mucus formation might have significantly affected the accuracy of P_{trach} corrections for the ETT.

In oleic acid-induced ALI, BARBAS *et al.* [21] demonstrated that PLV (with doses of PFC approximating FRC) induced a significant increase in regional (dependent) pleural pressure and an increase in the vertical pleural pressure gradient. Moreover, the authors showed that levels of PEEP up to 2.7 kPa could decrease the vertical pleural pressure gradient, possibly owing to a more uniform distribution of alveolar gas. These preliminary data suggest that during PLV and during its combined use with PEEP, regional changes in pleural pressures could generate regional alterations in lung volumes and respiratory mechanics. Thus, calculations of compliance using standard pressure-volume measurements will not necessarily reflect the regional behaviour of lung mechanics. Further research is needed to specify regional alterations in transpulmonary pressure and surface tension and their effects on respiratory mechanics during PLV.

In conclusion, it was demonstrated that in experimental ALI, PEEP effectively provided alveolar recruitment and improved oxygenation. During PLV, incremental levels of PEEP provided a significant improvement in gas exchange and were haemodynamically well tolerated. It is tempting to speculate that the combined application of PLV and PEEP might eventually allow either a reduction in the volumes of PFC required or in the PEEP necessary to maintain pulmonary gas exchange in ALI.

Prior to its clinical usage in patients with acute respiratory distress syndrome, further research should help to clarify both the optimal dosing of perfluorocarbons and the appropriate ventilatory settings during partial liquid ventilation.

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