Eur Respir J 2001; 18: 93–99
Printed in UK – all rights reserved

European Respiratory Journal
ISSN 0903-1936

Partial liquid ventilation improves lung function in ventilation-induced lung injury

G.F. Vazquez de Anda*,#, R.A. Lachmann*, S.J.C. Verbrugge*, D. Gommers*, J.J. Haitsma*, B. Lachmann*

Partial liquid ventilation improves lung function in ventilation-induced lung injury. G.F. Vazquez de Anda, R.A. Lachmann, S.J.C. Verbrugge, D. Gommers, J.J. Haitsma, B. Lachmann. ©ERS Journals Ltd 2001.

ABSTRACT: Disturbances in lung function and lung mechanics are present after ventilation with high peak inspiratory pressures (PIP) and low levels of positive end-expiratory pressure (PEEP). Therefore, the authors investigated whether partial liquid ventilation can re-establish lung function after ventilation-induced lung injury.

Adult rats were exposed to high PIP without PEEP for 20 min. Thereafter, the animals were randomly divided into five groups. The first group was killed immediately after randomization and used as an untreated control. The second group received only sham treatment and ventilation, and three groups received treatment with perfluorocarbon (10 mL·kg^{-1}, 20 mL·kg^{-1}, and 20 ml·kg^{-1} plus an additional 5 mL·kg^{-1} after 1 h). The four groups were maintained on mechanical ventilation for a further 2-h observation period. Blood gases, lung mechanics, total protein concentration, minimal surface tension, and small/large surfactant aggregates ratio were determined.

The results show that in ventilation-induced lung injury, partial liquid ventilation with different amounts of perflubron improves gas exchange and pulmonary function, when compared to a group of animals treated with standard respiratory care. These effects have been observed despite the presence of a high intra-alveolar protein concentration, especially in those groups treated with 10 and 20 mL of perflubron.

The data suggest that replacement of perfluorocarbon, lost over time, is crucial to maintain the constant effects of partial liquid ventilation.

Eur Respir J 2001; 18: 93–99.

*Dept of Anesthesiology, Erasmus University Rotterdam, Rotterdam, The Netherlands. #Unidad de Cuidados Intensivos, Hospital de Especialidades "Dr. Bernardo Sepulveda G.", del Centro Médico Nacional Siglo XXI, Instituto Mexicano del Seguro Social, México City, México.

Correspondence: B. Lachmann, Dept of Anesthesiology (Room Ee 2393), Erasmus University Rotterdam, PO Box 1738, 3000 DR Rotterdam, The Netherlands.
Fax: 31 104089450

Keywords: Mechanical ventilation
partial liquid ventilation
ventilation-induced lung injury

Received: March 4 2000
Accepted after revision January 30 2001

This work was financially supported by the International Foundation for Clinically Oriented Research (IFCOR), and V. de Anda received a study grant from Consejo Nacional de Ciencia y Tecuología, Mexico. Equipment was made available by Siemens-Elema AB, Solna, Sweden.

It is known that modes of mechanical ventilation which allow end-expiratory alveolar collapse and/or end-inspiratory alveolar overstretching result in a decrease of lung compliance and gas exchange, and lead to atelectasis, pulmonary oedema, pneumonitis and fibrosis: for review see [1]. Development of intra-alveolar protein-rich oedema in healthy rats subjected to intermittent positive pressure ventilation at high inflation pressures, without positive end-expiratory pressure (PEEP), was first demonstrated by WEBB and TIERNEY [2] and was later confirmed by DREYFUSS and coworkers [3, 4] who suggested that high inspiratory lung volumes induce endothelial and epithelial overstretching leading to microvascular injury. Additionally, it is known that large changes in both volume and surface area result in surfactant depletion from the alveoli into the airways as well as to transformation from surface active large aggregates to inactive small aggregates [5–9]. Thus, loss of surfactant function will increase the surface tension at the air-liquid interface of the alveolar walls resulting in alveolar collapse and an increased suction force on the pulmonary interstitium which causes more alveolar oedema. The epithelial/endothelial damage results mainly from the shear forces which appear in a nonhomogeneous ventilated lung [4, 8].

It is known that perfluorocarbons (PFCs) have a surfactant-like activity due to their low surface tension (18 mN·m^{-1}), which in a surfactant-deficient lung decreases the high surface tension at the air-liquid interface [10–13]. Based on this low surface tension, the resulting peak inspiratory pressure (PIP) during volume-controlled ventilation are reduced [10, 11, 13]. Another property of PFCs is their high density which, mainly in the dependent part of the lung, recruits collapsed alveolar units [14, 15]; the combination of PFCs with gas ventilation, better known as partial liquid ventilation (PLV), also improves gas exchange in surfactant-deficient lungs [10, 11]. Additionally, because PFCs might not be affected by the presence of

plasma proteins in the alveolus, PFC might prove useful as a treatment for ventilation-induced injury [16, 17]. Therefore, the aim of this study was to establish whether PLV can re-establish lung function in ventilation-induced lung injury (VILI).

Material and methods

Animal preparation

This study was approved by the local Animal Committee at the Erasmus University Rotterdam. The study was performed in 30 adult male Sprague-Dawley rats (body weight 280 – 350 g). After induction of anaesthesia with 2% enflurane and 65% nitrous oxide in oxygen, a polyethylene catheter was inserted into a carotid artery for drawing arterial blood samples and continuous monitoring of arterial blood pressure. Before tracheotomy, the animals received 30 $mL \cdot kg^{-1}$ pentobarbital sodium, intraperitoneal (*i.p.*) (Nembutal®, Algin BV, Maassluis, the Netherlands). After tracheotomy, muscle relaxation was induced by pancuronium bromide 0.6 $mL \cdot kg^{-1}$, intramuscular (*i.m.*) (Pavulon®, Organon Teknika, Boxtel, the Netherlands) immediately followed by connection to a ventilator and a pressure transducer for continuous monitoring of arterial blood pressure. The animals were mechanically ventilated with a Servo Ventilator 300 (Siemens-Elema, Solna, Sweden) in a pressure constant time-cycled mode, at an inspired oxygen concentration (F_{I,O_2}) of 1.0, frequency of 30 breaths per minute (bpm), PIP of 12 cmH_2O, PEEP of 2 cmH_2O, and inspiratory:expiratory (I:E) ratio of 1:2. Anaesthesia was maintained with pentobarbital sodium 30 $mL \cdot kg \cdot h^{-1}$, *i.p.*; muscle relaxation was maintained with pancuronium bromide 0.6 $mL \cdot kg \cdot h^{-1}$ *i.m.* Body temperature was kept within the normal range by means of a heating pad.

Experimental design

To produce ventilation VILI, PIP was increased to 45 cmH_2O and PEEP was decreased to zero for 20 min, whereas the other ventilator settings were not changed. Thereafter, a 5-min period of hypoventilation was introduced (*i.e.* PIP decreased to 26 cmH_2O and PEEP increased to 6 cmH_2O) to increase arterial carbon dioxide (CO_2). These ventilator settings were chosen based on a pilot study (unpublished data) which showed that animals ventilated at 45/0 cmH_2O (PIP/PEEP, respectively) for 20 min and then ventilated at 30/10 cmH_2O died from severe hypocapnia.

Experimental groups

The animals were randomized to one of five groups (n = 6 per group). In the first group (untreated controls), the animals were killed after the 5-min ventilation period of 26/6 (PIP/PEEP) with an overdose of pentobarbital. In each animal the thorax and diaphragm were opened, the tracheotomy catheter was connected to a pressure transducer and a pressure/volume curve (P/V curve) was immediately recorded (see later); thereafter a bronchoalveolar lavage (BAL) was performed five times with saline-$CaCl_2$ (see later). This group was used as an untreated control group. The second group (sham-treated controls) received a sham bolus of air 28 $mL \cdot kg^{-1}$ intratracheally and was mechanically ventilated at a PIP of 30 cmH_2O, PEEP of 10 cmH_2O, I:E ratio of 1:2, F_{IO_2} 1.0, and respiratory rate of 40 bpm for 2 h. These ventilator settings were chosen based on a preliminary study which showed that pressures of 26/6 cmH_2O (PIP/PEEP, respectively) and 28/8 cmH_2O were too low to keep animals alive for a 2-h observation period. Three groups received PFC at a dose of 10 $mL \cdot kg^{-1}$ (PFC10), 20 $mL \cdot kg^{-1}$ (PFC20), or 20 $mL \cdot kg^{-1}$ plus after 60 min, an extra dose of 5 $mL \cdot kg^{-1}$ (PFC20+R); this extra dose was based on a previous experience with this model (unpublished data) and aimed to maintain oxygen tension in arterial blood (P_{a,O_2}) as stable as possible during the remainder of the study period.

Treatment with perfluorocarbons

The PFC used in this study (Liquivent®, Alliance Pharmaceutical, San Diego, CA, USA) is insoluble in water, has a specific gravity of 1.918 $g \cdot cm^{-1}$ at 25°C, a surface tension of 18.1 $dynes \cdot cm^{-1}$, vapour pressure of 3.6 torr (0.5 kPa) at 20°C and 10.5 torr (1.4 kPa) at 37°C, an oxygen solubility of 53 $mL \cdot 100\ mL^{-1}$ and CO_2 solubility of 210 $mL \cdot 100\ mL^{-1}$ at 37°C, at 1 atmosphere pressure [10, 11]. The groups PFC10 and PFC20 received a single dose of PFC intratracheally. The PFC20+R group received an initial dose of 20 $mL \cdot kg^{-1}$ of PFC and, after 60 min, an extra dose of 5 $mL \cdot kg^{-1}$ of PFC was instilled intratracheally to compensate loss of PFC due to evaporation. At instillation, animals were disconnected from the ventilator and PFC was administered directly into the endotracheal tube over 3 – 5 s; the animals were then immediately reconnected to the ventilator.

Gas exchange and haemodynamics

Arterial blood gas samples were taken in all groups before, after VILI, and at 5 min after the 26/6 period, and in the four ventilated groups at 5 min after the 30/10 period, and at 30, 60, 90 and 120 min after PFC instillation. The samples were analysed for P_{a,O_2} and arterial carbon dioxide tension (P_{a,CO_2}) by conventional methods (ABL 505, Radiometer, Copenhagen, Denmark). At the same time points, arterial pressure was recorded. Haemodynamic support was provided by infusion of 1 mL of saline (to a maximum of 2 $mL \cdot h^{-1}$) when mean arterial pressure (MAP) decreased <60 mmHg.

Pressure/volume curves

At 120 min after administration of PFC all animals were killed with an overdose of pentobarbital sodium

injected through the penile vein. Then static P/V curves were recorded. After the thorax and diaphragm were opened (to eliminate the influence of chest wall compliance and intra-abdominal pressure), the tracheotomy catheter was connected to a pressure transducer (Validyne model DP 45-32, Validyne Engineering Co., Northridge, CA, USA) with a syringe attached to it, and pressures were recorded on a polygraph (Grass model 7B, Grass Instrument Co., Quincy, MA, USA). Using a syringe filled with nitrogen the lungs were first inflated (within 10 s) to an airway pressure of 35 cmH_2O, which was maintained for 5 s, followed by deflation to an airway pressure of 0 cmH_2O. Then the lungs were re-inflated in steps of 0.5 mL until an airway pressure of 35 cmH_2O was reached. Each inflation step took 1–2 s followed by a 5-s pause to allow pressure equilibration. After this, in the same way, the lungs were then deflated until an airway pressure of 0 cmH_2O was reached. The volume of nitrogen left in the syringe was recorded. The lower inflection point (LIP) was determined from the intersection of the lines representing the minimum slope of the compliance curve and the maximum slope of the compliance curve. Maximal compliance (C_{max}) was calculated from the steepest part of the deflation limb [18]. Total lung capacity (TLC_{35}) was defined as lung volume at inflation with a distending pressure of 35 cmH_2O [19].

Gruenwald index

The Gruenwald index which characterizes the surfactant system *in situ* [20], was calculated from the P/V curve, defined as $(2V_5 + V_{10})/2V_{max}$, where V_5, V_{10} and V_{max} are the lung volumes at airway pressures of 5, 10 and 35 cmH_2O from the deflation limb, respectively.

Bronchoalveolar lavage

After the P/V curve recordings a BAL (30 $mL \cdot kg^{-1}$) was performed five times with saline-$CaC1_2$ 1.5 $mmol \cdot L^{-1}$. In the resultant BAL (crude lavage) there were two visible layers, the upper layer was composed of the saline-$CaCl_2$, oedema fluid and cell debris, and the bottom layer comprised only PFC. Thereafter, cell debris and PFC were removed from BAL by centrifugation at $400 \times g$ for 10 min. The active surfactant component in the BAL fluid was separated from the nonactive surfactant component by differential centrifugation, followed by phosphorus analysis, and the ratio of nonactive to active (small to large aggregate) surfactant was calculated [21]. Finally, the protein concentration of the BAL fluid was determined using the Bradford method (Bio-Rad protein-assay, Munich, Germany) [22].

Minimal surface tension

Minimal surface tension of the crude lavage was determined by means of a modified Wilhelmy balance (E. Biegler GmbH, Mauerbach, Austria). In this method, a tight-fitting teflon barrier reduces the surface area of a teflon trough from 100–20% at a cycle speed of 0.33 min^{-1}. Saline is used as subphase and is kept at 37°C. The force on a platinum slide (1×1 cm) is measured by a force transducer and expressed as surface tension. Further, maximal surface tension is measured at 100% surface area and minimal surface tension at 80% surface compression and expressed as milli Newton·$metre^{-1}$ ($mN \cdot m^{-1}$). Surface tension characteristics of a BAL sample are measured after application on the surface of the saline-filled trough. In this study 300 μL of BAL fluid was applied to the surface of the trough; surface tension was measured after 3 cycles [23].

Statistical analysis

Statistical analysis was performed using the Instat 2.0 biostatistics package (GraphPad software, San Diego, CA, USA). Intragroup comparisons were analysed with repeated measures using analysis of variance (ANOVA); intergroup comparisons were analysed with ANOVA. If a difference was found, a *post hoc* test was performed (Tukey-Kramer). Statistical significance was accepted at p-values <0.05. All data are expressed as mean ± standard deviation.

Results

Figure 1 shows the P_{a,O_2} levels during the whole study period. After VILI and the ventilator settings were set at 26/6 cmH_2O for 5 min the P_{a,O_2} decreased <13.3 kPa in all groups. After PFC instillation and

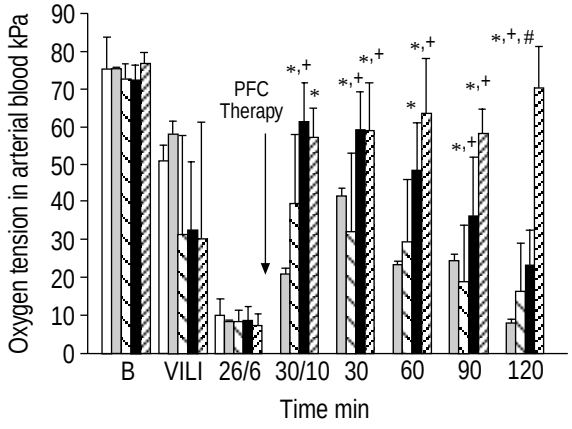

Fig. 1. – Arterial oxygen tension (mean ± SD) during the whole study period. B: baseline; VILI: ventilation with peak inspiratory pressure (PIP) of 45 cmH_2O without peak end-expiratory pressure (PEEP) after 20 min; 26/6: after 5 min at PIP 26 cmH_2O PEEP; arterial carbon dioxide tension (P_{a,O_2}) at 60 min in the group PFC_{20+R} was measured before instillation of the additional 5 mL perflubron dose; PFC: perfluorobcarbons. PFC_{10}: PFC at dose of 10 $mg \cdot kg^{-1}$; PFC_{20}: PFC at dose of 20 $mg \cdot kg^{-1}$; PFC_{20+R}: PFC at dose of 20 $mg \cdot kg^{-1}$ plus 5 $mg \cdot kg^{-1}$ after 60 min of ventilation. □: untreated; ■: sham-treated; ▨: PFC_{10}: ■: PFC_{29}; ▨: PFC_{20+R}. *: significant difference with sham-treated control group; $^{+}$: significant difference with PFC_{10}, $^{\#}$: significant difference with PFC_{20}.

Table 1. – Data on arterial carbon dioxide tension (P_{a,CO_2}) and mean arterial pressure (MAP) over time in both the treated and untreated groups

	Time min	Untreated	Sham-Treated	PFC10	PFC20	PFC20+R
P_{a,CO_2} kPa	Baseline	4.7±0.7	5.3±1.6	4.8±0.97	4.9±0.8	5.6±1.7
	VILI	2.5±0.3	2.4±0.6	2.5±0.35	2.7±0.4	2.9±0.5
	5 26/6	4.7±0.7	5.3±1.0	5.0±2.1	5.2±0.89	6.7±1.7
	5 30/10		5.3±1.3	4.3±0.4	4.8±0.7	5.7±0.8
	30		5.6±1.3	3.9±0.4	4.4±0.9	5.4±0.9
	60		6.3±1.6	4.4±1.3	4.4±0.7	4.7±1
	90		6.5±1.3	4.8±1.2	4.7±0.5	5.6±0.8
	120		6.9±1.8	4.8±2.1	4.9±0.3	4.4±0.9
MAP mmHg	Baseline	135±16	134±15	144±6	140±24	136±17
	VILI	75±25	84±32	72±25	85±22	71±21
	5 26/6	74±30	63±42	47±24	67±36	89±32
	5 30/10		111±12	88±24	106±25	97±14
	30		91±24	80±20	89±21	90±9
	60		89±19	78±17	95±15	95±37
	90		86±29	76±17	97±15	97±12
	120		89±27	85±9	73±24	80±13

Data are presented as mean±SD. Untreated: nonventilated control. Sham-treated is the Sham-treated, ventilated control; PFC10: partial liquid ventilation (PLV) with perfluorocarbons (PFC) at dose 10 mg·kg^{-1}; PFC20: PLV with PFC at dose 20 mg·kg^{-1}; PFC20+R: PLV with PFC at dose 20 mg·kg^{-1} plus 5 mg·kg^{-1} after 60 min of ventilation; VILI: ventilation induced lung injury; Baseline: measurement before VILI.

after increasing the pressures to 30/10 cmH_2O the PFC20 and PFC20+R groups showed a significant increase in P_{a,O_2} values to pre-VILI levels ($p<0.001$), but only the PFC20+R group maintained oxygen tension levels >60 kPa during the 2-h study period. In both groups with a single dose of PFC (PFC10 and PFC20) P_{a,O_2} values decreased over time. There were significant differences between the values in the ventilated and PFC10 groups compared with the values of the PFC20+R throughout the study period ($p<0.001$).

Table 1 shows that the P_{a,CO_2} values and MAP levels were comparable in all groups during the whole study period.

Table 2 shows data from BAL fluid and lung mechanics. Protein concentration was significantly higher in the PFC10 group compared with the untreated and the PFC20+R groups. The Gruenwald Index and the minimal surface tension of the crude lavage fluid from all ventilated groups were not significantly different from the untreated control group. For data on TLC35, Cmax, and LIP see table 2 and figures 2a and b. The total phosphorous concentration in the BAL fluid was not different between groups. The ratio of small to large aggregates in BAL fluid was significantly higher in the four ventilated groups compared with the untreated control group, and there was no significant difference between the sham-treated control group and all the PFC-treated groups.

Figure 2a shows the inflation limbs from the P/V curves. Both PFC groups treated with 20 mL·kg^{-1} have a significantly lower opening pressure than both the untreated and the sham-treated control groups. The PFC20+R group had a significantly higher TLC35 than both the untreated and sham-treated control groups. Figure 2b shows the deflation limbs from the P/V curves. The three PFC-treated groups had a

Table 2. – Amount of recovered bronchoalveolar ravage (BAL) fluid, protein concentration, lung volume above functional residual capacity at pressure 35 cmH_2O (TLC35), maximum compliance (Cmax), Gruenwald Index, lower inflection point of the pressure volume curve (LIP), minimal surface tension (min surf) of crude BAL fluid, total phosphorus concentration, and small aggregates (SA)/large aggregates (LA) ratio

	Untreated	Sham-Treated	PFC10	PFC20	PFC20+R
Recovery BAL fluid %	90±1	90±1	90±1	90±1	90±1
Protein concentration BAL mg·mL^{-1}	1.3±0.3*	1.4±0.4	1.9±0.2	1.8±0.3	1.4±0.1*
TLC35	35±2**	31±3*,+,**	38±4	39±4	42±5
Cmax mL·kg^{-1}	1.5±0.3*,+,**	1.4±0.2*,+,**	2.4±0.2	2.6±0.2	2.8±0.6
Gruenwald index	0.30±0.09	0.40±0.17	0.40±0.07	0.4±0.07	0.5±0.2
LIP cmH_2O	15.3±1.4*,+,**	18.2±2*,+,**	10.8±1.2**	10.6±3.3**	6.7±1.9*
Min surf mN·m^{-1}	33±3.1	31±1.6	35±2.1	35±0.5	32±3.9
Total phosphorus mmol	2.0±0.6	1.4±0.5	1.7±0.4	1.5±0.3	1.5±0.2
SA/LA ratio	1.7±1.1*,+,**	4.6±2.7	4.6±2.3	5.6±3.5	4.2±2.7

Values are presented as mean±SD. Untreated: nontreated, nonventilated control group; Sham-treated: Sham-treated, ventilated control group; PFC10: PFC 10 mg·kg^{-1}; PFC20: PFC 20 mg·kg^{-1}; PFC20+R: PFC 20 mg·kg^{-1}+5 mg·kg^{-1} after 60 min of ventilation to replace PFC loss due to evaporation; PFC: perfluorobcarbons. *: *versus* PFC10, $p<0.05$; $^{+}$: *versus* PFC20, $p<0.05$; **: *versus* PFC20+R, $p<0.05$.

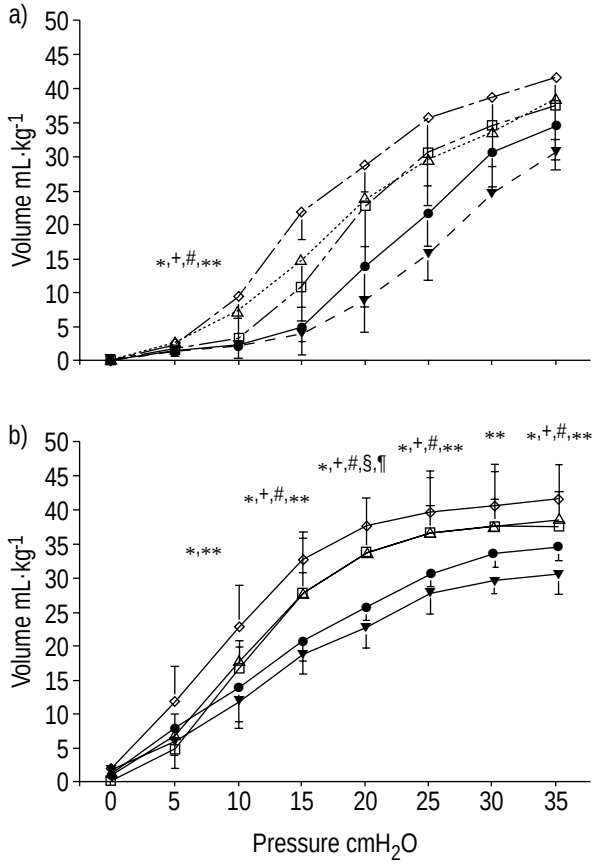

Fig. 2. – a) Inflation limbs from the pressure/volume curves (mean ± SD). Untreated: untreated, nonventilated control group. Lower inflection point. b) Deflation limbs from the pressure/volume curves (mean ± SD). PFC_{10}: PFC at dose of 10 mg·kg^{-1} PFC_{20}: PFC dose of 20 mg·kg^{-1}; PFC_{20+R}: PFC dose at 20 mg·kg^{-1} plus 5 mg·kg^{-1} ventilation after 60 min of ventilation. ●: untreated; ▼: sham-treated; □: PFC_{10}; △: PFC_{20}; ◇: PFC_{20+R}. *: significant differences between PFC_{20+R} *versus* untreated; **: significant differences between PFC_{20+R} *versus* sham-treated; $^{+}$: significant differences between PFC_{20} *versus* sham-treated; $^{\#}$: PFC_{10} *versus* untreated; ¶: significant difference between PFC_{10} *versus* sham-treated; §: significant difference between PFC_{20} *versus* untreated.

significantly higher Cmax than both untreated and the sham-treated control groups.

Discussion

This study shows that partial liquid ventilation improves P_{a,O_2} and lung mechanics in a model of ventilation-induced lung injury, despite the presence of a high intra-alveolar protein concentration. The authors have previously shown that this model of VILI is characterized by a decrease in lung mechanics and Gruenwald Index, and a high minimal surface tension in BAL fluid compared with healthy rat lungs [8]. The exact mechanism by which the lung damage is produced by artificial ventilation is not entirely clear, but the role of surfactant changes is being stressed [8, 9, 16]. The present group have shown that modes of ventilation with large tidal volume without PEEP disturb the surfactant system in a VILI model [8]. It has been demonstrated that loss of surface active molecules due to mechanical ventilation with high inspiratory lung volumes without PEEP is produced by displacement of surfactant from the alveolar air-liquid interface into the small airways [2, 5]. Moreover, the surface area changes produced by the high inspiratory lung volumes lead to an increased rate of conversion of active into nonactive surfactant subtractions [7–9]. All these mechanisms will lead to alveolar collapse and protein infiltration [2–4, 8, 9] in which the latter leads to further inactivation of surfactant [24, 25]. In the current study PLV was used to improve the disturbed lung function caused by VILI. The results showed that after VILI, PLV produced an immediate dose and time-dependent improvement in P_{a,O_2}. In the group treated with 10 mL·kg^{-1} PFC the pre-VILI values of P_{a,O_2} were never reached, whereas in both groups treated with 20 mL·kg^{-1} PFC, within 5 min there was a significant increase in P_{a,O_2} values compared with values after VILI, and these improved values were comparable with baseline values. However, P_{a,O_2} decreased over time in both groups in which PFC was not replaced. It has been shown that in surfactant-deficient animal lungs PLV provides adequate gas exchange as long as a sufficient amount of PFC is present in the lungs [10–13]. The present group has demonstrated that higher doses of PFC lead to higher levels of oxygenation in animals suffering from acute respiratory failure as a result of dose-dependent recruitment of collapsed atelectatic alveoli by PFC [10, 11]. It is also known that oxygenation deteriorates over time if no additional doses of PFC are instilled; this is attributed to evaporation of PFC which will cause affected alveoli to collapse [10, 11].

In the present study, the inflation limbs of the P/V curves showed on the one hand, a significantly lower opening pressure in the three PFC-treated groups and, on the other, a significantly higher maximal compliance compared with both control groups. It may be speculated that one of the reasons for this is that in the surfactant-deficient lungs, the decrease of surface tension at the air-liquid interface by PFC (vapour) improves the mechanical properties of the lung [10, 11, 26–29]. These findings have previously been demonstrated by KIRMSE *et al.* [14] showing that with increasing amounts of perflubron the LIP decreases to a point where additional doses of perflubron have no further influence on the detectable LIP.

A side-effect of PFC may be the constant surface tension which does not change with the changes in surface area (which is a property of natural surfactant) so that the end-expiratory stability in the PFC-treated animals (characterized by the Gruenwald Index) was not statistically different from the sham-treated control animals [13].

In the current study, the protein level from the crude BAL fluid in all ventilated groups was as high as in the untreated ventilated control group. Moreover, in both PFC-treated groups without replacement, an increase in the total protein concentration was observed. Additionally, the data show that with a larger amount of PFC (PFC_{20+R}) in the lung, the total amount of protein in the BAL fluid is less. How

PFC prevents protein infiltration and alveolar flooding is not entirely clear. DREYFUSS *et al.* [26] showed in rats that PFC partially reversed the effects of alveolar flooding (the so-called tamponade effect), but in some rats, did not reduce the permeability changes on the alveolo-capillary membrane measured by ^{125}I-labelled serum albumin. It has been suggested that as a result of the PFC-filled alveoli, the suction forces on the interstitium more or less disappear thus preventing protein influx into the PFC-filled alveoli. However, alveoli which have only a PFC film at the air-liquid interface and which may collapse during expiration will promote alveolar flooding due to their high surface tension at end-expiration; this could be one reason why the amount of proteins in the PFC group receiving only 10 mL·kg^{-1} PFC is significantly higher than in the PFC20+R group.

In conclusion, the results of the present animal study show that in ventilator-induced lung injury, partial liquid ventilation with different amounts of perflubron improves gas exchange and pulmonary function, when compared to a group of animals treated with standard respiratory care. These effects have been observed despite the presence of a high intra-alveolar protein concentration, especially in those groups treated with 10 and 20 mL of perflubron. The data suggest that replacement of perfluorocarbon lost over time is crucial to maintain constant effects of partial liquid ventilation.

Acknowledgements. The authors thank S. Majoor and E. Hendrik for expert technical assistance, and the Depts of Cardiochemistry and Paediatrics at Erasmus University Rotterdam for assisting with phospholipid measurements. They would also like to thank L. Visser-Isles for the English-language editing.

References

1. Verbrugge SJ, Lachmann B. Mechanisms of ventilation-induced lung injury and its prevention: role of surfactant. *Appl Cardiopulm Pathophysiol* 1998; 7: 173–198.
2. Webb H, Tierney D. Experimental pulmonary oedema due to high intermittent positive pressure ventilation with high inflation pressures. Protection by positive end expiratory pressure. *Am Rev Respir Dis* 1974; 110: 556–565.
3. Dreyfuss D, Basset G, Soler P, Saumon G. Intermittent positive pressure hyperventilation with high inflation pressures produces pulmonary microvascular injury in rats. *Am Rev Respir Dis* 1985; 132: 880–884.
4. Dreyfuss D, Soler P, Basset G, Saumon G. High inflation pressure pulmonary oedema: respective effects of high airway pressure, high tidal volume, and positive end *expiratory pressure. Am Rev Respir Dis* 1988; 137: 1159–1164.
5. Faridy E. Effect of ventilation on movement of surfactant in airways. *Respir Physiol* 1975; 38: 461–466.
6. Ito Y, Veldhuizen R, Yaou L, McCraig L, Bartlett A, Lewis J. Ventilation strategies affect surfactant aggregate conversion in acute lung injury. *Am J Respir Crit Care Med* 1997; 155: 493–499.
7. Ito Y, Manwell S, Kerr C, *et al.* Effects of ventilation on the efficacy of exogenous surfactant therapy in a rabbit model of acute lung injury. *Am J Respir Crit Care Med* 1998; 157: 149–155.
8. Verbrugge SJ, Böhm SH, Gommers D, Zimmerman L, Lachmann B. Surfactant impairment after mechanical ventilation with large alveolar surface area changes and effects of positive end-expiratory pressure. *Br J Anaesth* 1998; 80: 360–364.
9. Verbrugge SJ, Vazquez de Anda G, Gommers D, *et al.* Exogenous surfactant preserves lung function and reduces alveolar Evans blue dye influx in a rat model of ventilation-induced lung injury. *Anaesthesiology* 1998; 89: 467–474.
10. Tütüncü AS, Akpir K, Mulder P, Erdmann W, Lachmann B. Intratracheal perfluorocarbon administration as an aid in the ventilatory management of respiratory distress syndrome. *Anaesthesiology* 1993; 79: 1083–1093.
11. Tütüncü AS, Faithfull NS, Lachmann B. Intratracheal perfluorocarbon administration combined with artificial ventilation in experimental respiratory distress syndrome: dose dependent improvement of gas exchange. *Crit Care Med* 1993; 21: 962–969.
12. Hirschl RB, Tooley R, Parent A, Johnson K, Bartlett R. Evaluation of gas exchange, pulmonary compliance, and lung injury during total and partial liquid ventilation in the acute respiratory distress syndrome. *Crit Care Med* 1996; 24: 1001–1008.
13. Verbrugge S, Lachmann B. Partial liquid ventilation. *Eur Respir J* 1997; 10: 1937–1939.
14. Kirmse M, Fujino Y, Hess D, Kacmarek R. Positive end-expiratory pressure improves gas exchange and pulmonary mechanics during partial liquid ventilation. *Am J Respir Crit Care Med* 1998; 158: 1550–1556.
15. Quintel M, Hirschl R, Roth H, Loose R, Van Ackern K. Computer tomographic assessment of perfluorocarbon and gas distribution during partial liquid ventilation for acute respiratory failure. *Am J Respir Crit Care Med* 1998; 158: 249–255.
16. Dreyfuss D, Saumon G. Ventilator induced lung injury. Lessons from experimental studies. *Am J Respir Crit Care Med* 1998; 157: 294–323.
17. Gauger PG, Overbeck MC, Chambers SD, Cailipan CI. Partial liquid ventilation improves gas exchange and increases EELV in acute lung injury. *J Appl Physiol* 1998; 84: 1566–1572.
18. Gommers D, Vilstrup C, Bos J, *et al.* Exogenous surfactant therapy increases static lung compliance, and cannot be assessed by measurements of dynamic compliance alone. *Crit Care Med* 1993; 21: 567–574.
19. Lachmann B, Robertson B, Vogel J. *In vivo* lung lavage as an experimental model of the respiratory distress syndrome. *Acta Anaesthesiol Scand* 1980; 24: 231–236.
20. Gruenwald P. A numerical index stability of lung expansion. *J Appl Physiol* 1963; 88: 359–367.
21. Veldhuizen RAW, Inchley K, Hearn SA, Lewis J, Possmayer F. Degradation of surfactant associated protein B (SP-B) during *in vitro* conversion of large into small surfactant aggregates. *Biochem J* 1993; 295: 141–147.
22. Bradford MM. A rapid and sensitive method for the

quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Ann Biochem* 1976; 72: 248 – 254.
23. Eijking E, Gommers D, So K, de Maat M, Mouton J, Lachmann B. Prevention of respiratory failure after hydrochloric acid aspiration by intratracheal surfactant instillation in rats. *Anesth Analg* 1993; 76: 472 – 477.
24. Holm B, Enhorning G, Notter R. A biophysical mechanism by which plasma proteins inhibit surfactant activity. *Chem Phys Lipids* 1988; 49: 40 – 55.
25. Seeger W, Stohr G, Wolf H, Neuhof H. Alteration of surfactant function due to protein leakage: special interaction with fibrin monomer. *J Appl Physiol* 1985; 58: 326 – 338.
26. Dreyfuss D, Martin-Lefèvre L, Saumon G. Hyperinflation-induced lung injury during alveolar flooding in rats. Effect of perfluorocarbon instillation. *Am J Respir Crit Care Med* 1999; 159: 1752 – 1757.
27. Kaisers U, Kuhlen R, Keske U, *et al.* Superimposing positive end-expiratory pressure during partial liquid ventilation in experimental lung injury. *Eur Respir J* 1998; 11: 1035 – 1042.
28. Hartog A, Kaisers U, Gommers D, Lachmann B. Comparing the effects of four different perfluorocarbons on gas exchange and lung mechanics in an animal model of acute lung injury. *Appl Cardiopulm Pathophysiol* 1999; 8: 41 – 46.
29. Hartog A, Vazquez de And G, Gommers D, *et al.* Comparison oexogenous surfactant therapy, mechanical ventilation with high end-expiratory pressure and partial liquid ventilation in a model of acute lung injury. *Br J Anaesth* 1999; 82: 81 – 86.