Reduced Surfactant Contributes to Increased Lung Stiffness Induced by Rapid Inspiratory Flow

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Abstract

Introduction The mechanism of fast inspiratory flow rate (V_I') induced lung injury is unclear. As fast V_I' increases hysteresis, a measure of surface tension at the air–liquid interface, surfactant release or function may be important. This experimental study examines the contribution of impaired surfactant release or function to dynamic-VILI.

Methods Isolated perfused lungs from male Sprague Dawley rats were randomly allocated to four groups: a long or short inspiratory time (Ti = 0.5 s; slow V_I' or Ti = 0.1 s; fast V_I') at PEEP of 2 or 10 cmH$_2$O. Tidal volume was constant (7 ml/kg), with f = 60 breath/min. Forced impedance mechanics (tissue elastance (Htis), tissue resistance (Gtis) and airway resistance (Raw) were measured at 30, 60 and 90 min following which the lung was lavaged for surfactant phospholipids (PL) and disaturated PL (DSP).

Results Fast V_I' resulted in a stiffer lung. Concurrently, PL and DSP were decreased in both tubular myelin rich and poor fractions. Phospholipid decreases were similar with PEEP. In a subsequent cohort, laser confocal microscopy-based assessment demonstrated increased cellular injury with increased V_I' at both 30 and 90 min ventilation.

Conclusion Rapid V_I' may contribute to ventilator induced lung injury (VILI) through reduced surfactant release and/or more rapid reuptake despite unchanged tidal stretch.

Keywords Lung mechanics · Ventilator induced lung injury · Cytokines · Inspiratory flow rate · Surfactant · Confocal microscopy

Introduction

Ventilator-induced lung injury (VILI) has become recognized as a major adverse effect of ventilator assistance. Lung injury predisposes to VILI as associated lung collapse, inhomogeneity of aeration and reduced recruitability result in greater stress and strain on the lung [1]. Following pivotal basic and clinical research, current approaches to protective ventilation focus on static factors—tidal volume, plateau pressure and positive end-expiratory pressure (PEEP).

Inspiratory flow rate (V_I') and respiratory rate are dynamic factors that also contribute to VILI, and to the power that is dissipated during ventilation [1, 2]. While the respiratory rate and V_I' are related through the inspiratory time and inspiratory to expiratory (I:E) ratio, it appears that V_I' plays a greater role in VILI [3]. Indeed, analyzing the components of mechanical power, Gattinoni et al. suggest that tidal volume, driving pressure and V_I' have similar contributions [1, 2]. While the mechanisms of lung injury due to excessive volume and pressure have been extensively investigated [4], relatively little is known regarding lung injury due to high V_I'.

Protti et al. [5] suggest that high V_I' lung injury may be due to amplified viscoelastic behavior. They report greater dynamic hysteresis and greater stress relaxation at high V_I' consistent with Eissa et al. [6] who had earlier reported an increase in stress relaxation both in the acute respiratory distress syndrome (ARDS) and with increasing V_I'. While Protti et al. [5] suggested that this led to mechanical failure of the lung cytoskeleton, other factors including surface tension and inhomogeneity of ventilation also determine non-linear behavior and stress relaxation.

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The amplitude, magnitude and rate of cell deformation led to stress failure and cell wounding of cultured alveolar type II epithelial cells, as measured by increased uptake of the membrane impermeable marker propidium iodide, and are associated with increased lipid trafficking [7–9]. As the wounded plasma membrane upregulates inflammatory gene expression and growth factor release, high strain or strain rate may lead to important changes prior to obvious cell injury. However, while these results have been confirmed in the whole lung where high strain amplitude (high V_T) lung results in cell wounding [10], the whole lung effects of high strain rate (high V_I') have not been previously examined. Therefore, we used an isolated perfused lung model to examine the effects of high vs low V_I' on cell wounding and pulmonary surfactant release.

Materials and Methods

Animals

Specific pathogen-free male Sprague Dawley rats (body weight (BW) = 200 − 290 g) were used. All experiments were approved by the Flinders University Animal Welfare Committee and performed according to the National Health and Medical Research Council of Australia Guidelines on Animal Experimentation.

Isolated Perfused Lung Preparation

Rats were anesthetized with thiopentone sodium (120 mg/ kg IP; Abbott Australasia, Kurnell, Australia) and a femoral vein and artery were catheterized. The trachea was cannulated, and the lungs ventilated via a computer controlled small animal ventilator (Flexivent, SCIREQ Scientific Respiratory Equipment, Montreal, Canada) with a mixture of 5% CO_2, 20% O_2, balanced with N_2, at a tidal volume of 7 ml/kg BW, breathing frequency (f) of 60 min^{-1}. The pulmonary artery and left atrium were cannulated and without interrupting the circulation, the lungs were perfused (flow rate of 20 ml/min, left atrial pressure of 2 cmH$_2$O referenced to the top of the lungs) with Krebs bicarbonate solution (4.5% bovine serum albumin). The lungs and heart were removed from the chest, placed in a humidified chamber at 37 °C and allowed to stabilize for 2 min before the start of each protocol [11].

Isolated perfused rat lungs were randomly allocated to four groups (n = 10); Long or short inspiratory time (Ti = 0.5 s or Ti = 0.1 s) and PEEP of 2 or 10 cmH$_2$0. Airway, pulmonary arterial and left atrial pressures were monitored continuously using a MacLab System (AD Instruments, Australia).

Lung Mechanics

Forced impedance mechanics (airway (Newtonian) resistance, Raw; tissue resistance, Gtis; and tissue elastance, Htis) were measured at 30, 60 and 90 min by measuring the lung's impedance (Z) using the computer-controlled ventilator, as previously [12]. Briefly, 2 min after a recruitment maneuver ($2.5 \times V_T$), impedance of the respiratory system was measured following a forced oscillation. The data were fitted to a constant phase model [13] where Z = Raw + jI + (Gtis–jHtis)/ $(2\pi f)^\alpha$, where I is inertance, j is the imaginary unit, f is frequency, and $\alpha = (2/\pi)$arc tan (Htis/Gtis). Inertance was negligible and is therefore not reported.

Preparation and Infusion of Radiolabels

Radiolabeled red blood cells (RBC), albumin and diethylenetriamine pentaacetic acid (DTPA) were prepared, as previously and added to the perfusate 10 min before the end of the experiment [11]. Activities were: ^{51}Cr-RBC 5 µCi/100 g BW; ^{125}I-albumin 0.1 µCi/100 g BW; ^{99m}Tc-DTPA 0.4 µCi/100 g BW.

Assessment of Lung Injury

The perfusate was sampled every 30 min for determination of gas tensions (ABL-5 Blood Gas Analyzer, Radiometer, Denmark) and samples stored at − 80 °C for analysis of cytokines. After 90 min ventilation the right upper lobe was resected for determination of wet-to-dry weight ratio and radiolabel analysis, as previously [11]. The remaining lung was degassed at 0.5 atm for 60 s and lavaged at 2 °C with 3 separate 32 ml/kg BW volumes of 0.9% sodium chloride, each instilled and withdrawn × 3. Percent recovery of lavage fluid was not different between treatment groups. The lung lavage fluid was centrifuged at 150×g for 5 min at 2 °C. A sample was taken from the supernatant, aliquoted and stored at − 80 °C until analysis for cytokines.

A further aliquot of lung lavage was taken for radiolabel analysis. Compartmentalization of radiolabels in lavage and upper right lobe tissue are expressed as a percentage volume of perfusate counts, as previously [11].

Surfactant Analysis

The remaining lavage supernatant was centrifuged at 1000×g for 25 min at 4 °C to separate the surface active tubular myelin rich (alv-1) fraction and the recycled tubular myelin poor (alv-2) fraction, which appear to show product-precursor relations with lamellar bodies [14]. Lipids were extracted [15], and total phospholipid (PL) content was

determined by measuring the amount of inorganic phosphorous [16]. Disaturated phospholipids (DSP) were separated [17] and the phospholipid content determined [18].

Cytokine Determination

Cytokine concentrations in lung lavage and perfusate were analyzed using commercially available enzyme-linked immunosorbent assays (ELISA) for interleukin (IL)-6 and tumor necrosis factor (TNF)-α (PharmMingen Opt EIA, San Diego, CA), and ELISA developed in our laboratory using matched antibodies for IL-8 (GRO/KC) and monocyte chemotactic protein (MCP)-1 (Peprotech, Israel), as previously [19].

Computation of Mechanical Power

Mechanical power was computed as described by Gattinoni et al. [2]

$$\text{Power}_{\text{rs}} = 0.098 \cdot \text{RR} \cdot \left\{ \Delta V^2 \cdot \left[\frac{1}{2} \cdot \text{EL}_{\text{rs}} + \text{RR} \cdot ((1 + \text{I:E})/60 \cdot \text{I:E}) \cdot R_{\text{aw}} \right] + \Delta V \cdot \text{PEEP} \right\}$$

where RR is the breathing rate, ΔV is the tidal volume, EL_{rs} is the elastance of the respiratory system, I:E is the inspiratory-to-expiratory time ratio, and R_{aw} is the airway resistance, and 0.098 is the conversion factor from $L \times \text{cmH}_2\text{O}$ to joules (J).

Confocal Microscopy

In a second cohort of rats, laser confocal microscopy-based assessment of cell injury was undertaken [10]. Briefly, isolated perfused lungs were prepared, as above, and mechanically ventilated at PEEP 2 cmH$_2$O and f 60 min^{-1} while perfused (flow rate 10 ml/min/kg, pulmonary artery pressure 10 cmH$_2$O; left atrial pressure 0–4 cmH$_2$O) with a red blood cell enriched Krebs bicarbonate solution containing 4 µg/ml propidium iodide (PI). The lungs were randomized: Group 1—Ti 0.5 V_T 7 ml/kg 30 min; Group 2—Ti 0.1, V_T 7 ml/kg 30 min; Group 3—Ti 0.5, V_T 7 ml/kg 90 min; Group 4—Ti 0.1, V_T 7 ml/kg 90 min; Group 5—Ti 0.5, V_T 15 ml/kg, 30 min; Group 6—Ti 0.1, V_T 15 ml/kg, 30 min. Once the ventilation protocol was complete, PI free Krebs was perfused through the lung for 3 min and the lungs and heart excised. The lungs were inflated with 10 ml air and placed in saline for confocal imaging.

Left upper lobe lung tissue was imaged using a Leica TCS SP5 confocal microscope (Leica Microsystems, Mannheim) by exciting the tissue with laser light (488 nm) and collecting emission wavelengths at 500–550 nm (auto florescence) and 600–720 nm (PI). Stacks of 8 images (1 µm apart) were taken up to a depth of 40 µm. The images were digitized (8-bit resolution;

512 × 512 pixels) and Image J was used to count the number of alveoli and PI$^+$ cells per field.

Statistical Analysis

Due to their asymmetric nature all data were log-transformed before analysis. Results are expressed as mean $\pm$ SD and $P \leq 0.05$ considered significant. Differences between groups were assessed by two-way or repeated-measures analysis of variance (ANOVA), as appropriate, and relationships between continuous variables by Pearson correlation (IBM SPSS 23.0, SPSS Inc., Chicago, IL).

Results

Respiratory Mechanics

Mechanical ventilation with fast V_I' (Ti = 0.1) increased peak airway pressure, tissue elastance and tissue resistance (Fig. 1a–d). High PEEP (10 cmH$_2$O) independently increased peak airway pressure, and decreased airway and tissue resistance over the same period. However, there was no interactive effect of V_I' and PEEP on these parameters. Tissue elastance was unchanged by increasing PEEP (Fig. 1b) and airway resistance was unchanged by increased V_I' (Fig. 1c). Pulmonary arterial and venous return pressures remained unchanged by either V_I' or increasing PEEP (data not shown).

Surfactant Content

Alv-1 and Alv-2, total PL and DSP content decreased with increased V_I' (Table 1). Higher PEEP (10 cmH$_2$O) had no effect.

Lung Injury

Although perfusate PO$_2$ was unchanged by either V_I' or PEEP (Fig. 2a), PCO$_2$ was lower and pH higher with higher PEEP (Fig. 2b, c). Wet/dry weight ratio was unchanged with either V_I' or PEEP (Fig. 2d). Consistent with this, flux of radio-labeled DTPA, albumin and RBC were unchanged by
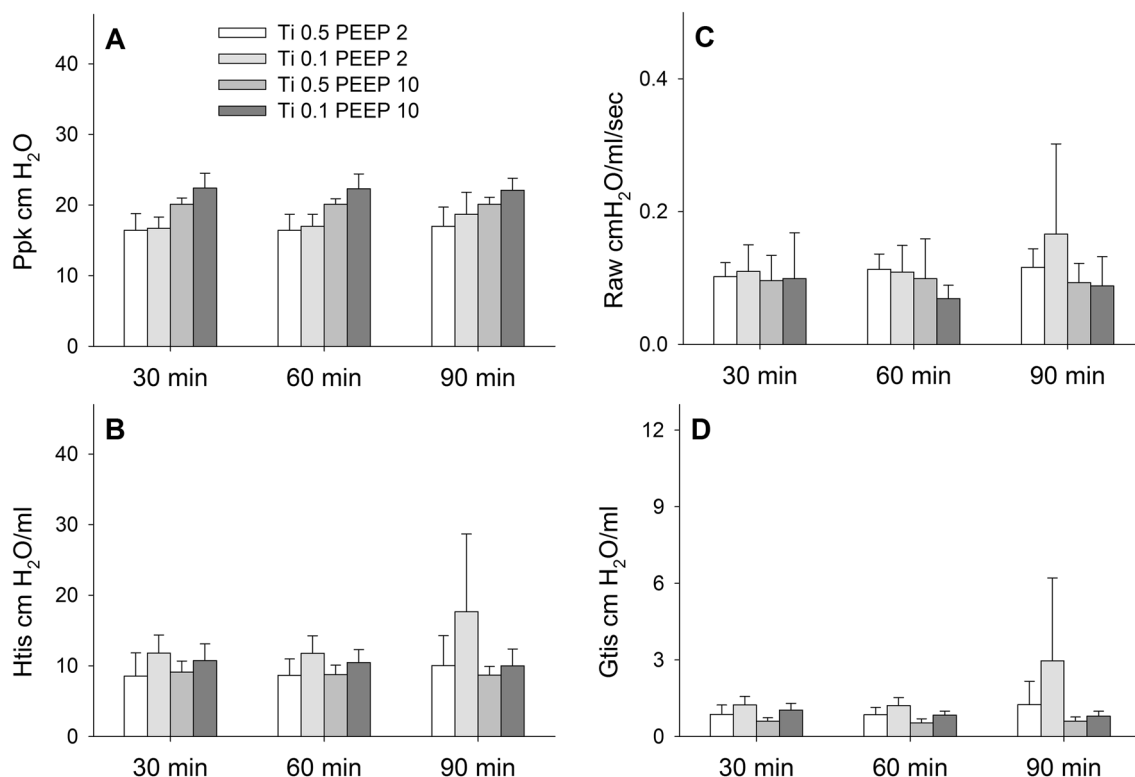
Fig. 1 Respiratory mechanics of isolated perfused rat lungs during 90 min of slow (Ti = 0.5 s) and fast (Ti = 0.1 s) V_I' with PEEP of 2 or 10 cmH$_2$O ($n=10$). Data are presented as mean ± SD and differences between groups analyzed by repeated measures ANOVA. **a** Peak airway pressure, Ppk (V_I' $P=0.02$; PEEP $P \leq 0.001$; interaction $P=0.39$; time $P=0.06$). **b** Airway resistance, Raw (V_I' $P=0.57$; PEEP $P=0.005$; interaction $P=0.32$; time $P=0.25$). **c** Tissue elastance, Htis (V_I' $P=0.001$; PEEP $P=0.24$; interaction $P=0.11$; time $P=0.09$). **d** Tissue resistance, Gtis (V_I' $P \leq 0.001$; PEEP $P \leq 0.001$; interaction $P=0.84$; time $P=0.01$)

Table 1 Surfactant content of isolated perfused rat lungs following 90 min ventilation with increasing V_I' and PEEP

	Alv 1	Alv2
Phospholipid		
Ti 0.5, PEEP 2	4035 ± 1175	3963 ± 1019
Ti 0.1, PEEP2	3404 ± 605*	3351 ± 654*
Ti 0.5, PEEP 10	4267 ± 1173	4745 ± 819
Ti 0.1, PEEP 10	3427 ± 1002*	3375 ± 978*
DSP		
Ti 0.5, PEEP 2	1851 ± 434	1662 ± 406
Ti 0.1, PEEP2	1508 ± 257*	1407 ± 404*
Ti 0.5, PEEP 10	2135 ± 586	1998 ± 563
Ti 0.1, PEEP 10	1731 ± 397*	1521 ± 464*

Values are mean ± SD and differences between groups analyzed by two-way ANOVA

Phospholipid total phospholipid μg/gm dry lung weight, *DSP* disaturated phospholipid μg/gm dry lung weight

*V_I' effect $P \leq 0.05$

V_I' (Table 2). However, higher PEEP increased the flux of DTPA into the lung and lavage, as well as RBC into the lavage.

Cytokines

All cytokines measured in the perfusate increased temporally (Fig. 3a–d). Perfusate IL-6 was increased over time with higher PEEP (Fig. 3a). However, was unchanged with increased V_I'. There was no effect of V_I' or PEEP on lavage IL-6, or lavage or perfusate TNF-α, IL-8 or MCP-1 (Fig. 3b–h).

Mechanical Power

Computed mechanical power was correlated with measured indices of lung injury. Low mechanical power at PEEP 2 was positively associated with lung edema measured as lung wet to dry weight ratio, and negatively associated with Alv 2 DSP content (Fig. 4a, b). These relationships were not apparent at higher mechanical power resulting from PEEP 10 (Fig. 4f, g). Conversely, inflammatory cytokines, IL-6, TNF-α and IL-8 in bronchoalveolar lavage were positively correlated with mechanical power at PEEP 10 (Fig. 4h–j) but not at PEEP 2 (Fig. 4c–e).
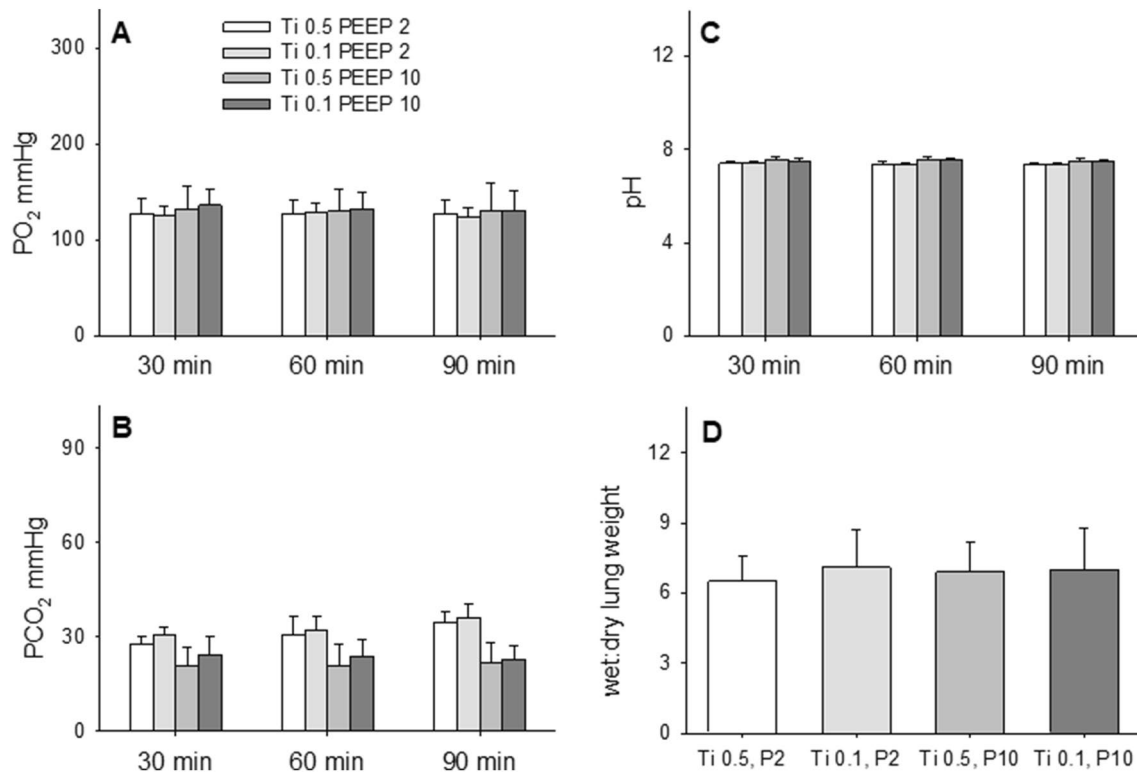
Fig. 2 Lung injury parameters of isolated perfused rat lungs during 90 min of slow (Ti = 0.5 s) and fast (Ti = 0.1 s) V_I' with PEEP of 2 or 10 cmH$_2$O ($n = 10$). Data are presented as mean ± SD and differences between groups analyzed by repeated measures ANOVA (**a–c**) or two-way ANOVA (**d**). **a** Perfusate oxygen pressure, PO$_2$ (V_I' $P = 0.76$; PEEP $P = 0.68$; interaction $P = 0.71$; time $P = 0.13$). **b** Per-fusate carbon dioxide pressure, PCO$_2$ (V_I' $P = 0.08$; PEEP $P \leq 0.001$; interaction $P = 0.59$; time $P \leq 0.001$). **c** Perfusate pH (V_I' $P = 0.58$; PEEP $P \leq 0.001$; interaction $P = 0.58$; time $P \leq 0.001$). **d** Wet to dry lung weight ratio of isolated perfused rat lungs following 90 min of slow (Ti = 0.5 s) and fast (Ti = 0.1 s) V_I' with PEEP of 2 or 10 cmH$_2$O ($n = 10$); (V_I' $P = 0.48$; PEEP $P = 0.69$; interaction $P = 0.55$)

PI$^+$ Cells

PI$^+$ cells per alveolus were increased with increased V_I' at both 30- and 90-min ventilation at V_T 7 ml/kg (Fig. 5a, c). However, increasing V_T to 15 ml/kg for 30 min equalized the degree of cellular injury discernable by this method (Fig. 5d). As reported previously [10], there was considerable heterogeneity of injury with distinct field-to-field variability in the number of PI$^+$ cells.

Discussion

In the isolated perfused rat lung, during lower V_T ventilation fast V_I' led to impaired lung mechanics, without an increase in lung edema, deterioration in gas exchange or change in either perfusate or BAL cytokines. However, there was an associated increase in epithelial cell wounding measured as increased propidium iodide uptake, and both impaired lung stiffness and reduced pulmonary surfactant content of both total and disaturated phospholipids. In addition, computed mechanical power was correlated with various indices of

lung injury, at low power (PEEP 2), and inflammation, at high power (PEEP 10), as seen previously [20].

The lack of lung edema with maintained oxygenation and inflammatory markers suggest that cell wounding and reduced pulmonary surfactant content are early changes that likely precede conventional parameters of lung injury. While others have reported lung injury and inflammation following fast V_I' [3, 5, 21, 22] it is possible that the absence of granulocytes in the lung perfusate modified the progression of lung injury in the current experiments. Pulmonary surfactant depletion using saline lavage is a common animal model of lung injury demonstrating marked changes in oxygenation, histology and lung inflammation [23]. However, consistent with the current fast V_I' data, in the absence of granulocytes only a modest increase in albumin permeability is reported [24].

While the 90-min experiment may not have been long enough for a change in cytokine release from alveolar cells, perfusate cytokines and chemokines increased over this time. Despite an increase in IL-6 with PEEP, there was no effect of increasing V_I' on any of the cytokines examined. This suggests that either exposure to the perfusate circuit

Table 2 Radiolabel compartmentalization following 90 min ventilation with increasing V_I' and PEEP

	^{99m}Tc-DTPA	^{125}I-albumin	^{51}Cr-RBC
Lung			
Ti 0.5, PEEP 2	47.2 ± 13.3	56.3 ± 9.7	43.8 ± 10.8
Ti 0.1, PEEP2	43.8 ± 15.6	50.5 ± 9.8	47.2 ± 12.5
Ti 0.5, PEEP 10	58.6 ± 12.8*	55.9 ± 11.8	53.5 ± 38.1
Ti 0.1, PEEP 10	58.0 ± 20.2*	55.4 ± 24.2	48.8 ± 24.5
Lavage			
Ti 0.5, PEEP 2	1.09 ± 0.70	1.04 ± 0.47	0.08 ± 0.05
Ti 0.1, PEEP2	1.35 ± 0.68	1.34 ± 0.39	0.08 ± 0.05
Ti 0.5, PEEP 10	2.21 ± 1.46*	1.68 ± 1.24	0.12 ± 0.07*
Ti 0.1, PEEP 10	2.35 ± 2.22*	1.76 ± 1.58	0.17 ± 0.11*
Lavage:Lung			
Ti 0.5, PEEP 2	2.30 ± 1.23	1.89 ± 0.93	0.20 ± 0.16
Ti 0.1, PEEP2	3.57 ± 2.78	2.80 ± 0.98	0.20 ± 0.15
Ti 0.5, PEEP 10	4.11 ± 3.69	3.18 ± 2.79	0.34 ± 0.28*
Ti 0.1, PEEP 10	4.08 ± 2.92	3.42 ± 2.38	0.43 ± 0.36*

Values are mean ± SD and differences between groups analyzed by two-way ANOVA

Lung cpm Lung (gm)/cpm perf (ml) × 100, *Lavage* cpm Lavage (ml)/cpm Perfusate (ml) × 100

*PEEP effect $P \leq 0.04$

or deterioration in the lung was occurring over the course of the experiment, as expected with the isolated perfused lung model. While the cell source of these proteins could be pulmonary endothelial cells or residual leukocytes including macrophages, it seems unlikely there was a contribution from epithelial cells given the lack of effect of V_I' on bronchoalveolar lavage proteins.

Examining high V_T ventilation, Gajic et al. [10] found few changes in cell wounding, lung edema, lung histology and lung stiffness until V_T was increased to 40 ml/kg (compared to control V_T 6 ml/kg, 3 cmH$_2$O PEEP). As surface tension accounts for around 70% of elastic recoil, increased lung stiffness might be due to either reduced aerated lung volume or reduced specific lung stiffness due to reduced surfactant function or content. In acute lung injury increased permeability results in inhibition of surface tension reduction by albumin resulting in, or potentiating, reduced aerated lung volume. In the isolated perfused lung used by both Gajic et al. [10] and in the current study, other factors may also inactivate surfactant function, or cell wounding and increased lipid trafficking due to excessive strain or strain rate may result in altered surfactant cycling within the alveolus.

In the current study we found increased tissue elastance and tissue resistance with fast V_I', associated with reduced pulmonary surfactant phospholipids. While reduced alveolar surfactant would produce increased surface tension and these mechanical changes, both the mechanism of reduced

surfactant phospholipid content and alternative causes of these altered mechanics need to be considered. With regard to a direct effect of V_I' on surfactant, the authors are not aware of any specific prior data. Broader studies which have touched on these effects have seen either no effect [25] or increased elastance suggesting reduced surfactant [26], consistent with our findings. It has been postulated alveolar epithelial cell injury leads to altered cellular metabolism and diminished surfactant production, thereby resulting in increased lung stiffness [26, 27]. However, as we did not examine surfactant turnover in the alveolus it is not possible to speculate whether this is due to altered release, or changes in surfactant cycling, both of which might be more likely to occur given the previous suggestion of altered lipid cycling with stretch-induced cell wounding. High V_I' might have led to lung injury independently and increased lung stiffness, however, as the perfusate was free of granulocytes, and lung water, oxygenation and inflammatory proteins were unchanged, this seems less likely.

The isolated perfused lung preparation used allows cell wounding to be measured with PI in the intact organ, and direct comparison with prior work [10]. It also minimizes potential confounders such as non-pulmonary inflammation and hemodynamic effects attributable to differences in ventilation. While these advantages allow a more focused examination of normal lung effects and potential mechanism, they also reduce the direct translation to clinical effects that might be gained by work with intact circulations, lung injury models, and clinical research.

To separate slow from fast V_I' we used Ti of 0.5 s and 0.1 s with a constant V_T of 7 ml/kg and a respiratory rate of 60 breaths/min. Again, these values were chosen to examine the effect and mechanism of fast V_I' in the rat lung, and do not suggest direct clinical translation. However, as Nakano et al. [28] reported a baseline Ti of 0.17 s with a V_T of 5.8 ml/kg in control rats the range of V_I' examined appear appropriate, allowing some general conclusions to be drawn.

The association of cell wounding, reduced alveolar surfactant content and impaired lung mechanics with fast V_I' in the current study suggest a mechanism of strain rate related VILI. The associated decrease in surface tension would lead to amplified viscoelastic behavior consistent with Protti et al. [5], and this mechanism would likely be enhanced in an already injured lung with abnormal surface tension and heterogeneous aeration. Indeed, Gattinoni et al. [2] calculated that V_I' was of similar importance to V_T in the manifestation of VILI. As VILI is modified by the underlying state of the lung there may be only minor impact in healthy lungs but in injured or inflamed lungs, particularly given the heterogeneous nature of lung injury, it is likely that there will be an impact of clinical inspiratory flows. In addition, the absence of perfusate granulocytes in the isolated lung model likely reduced evidence of
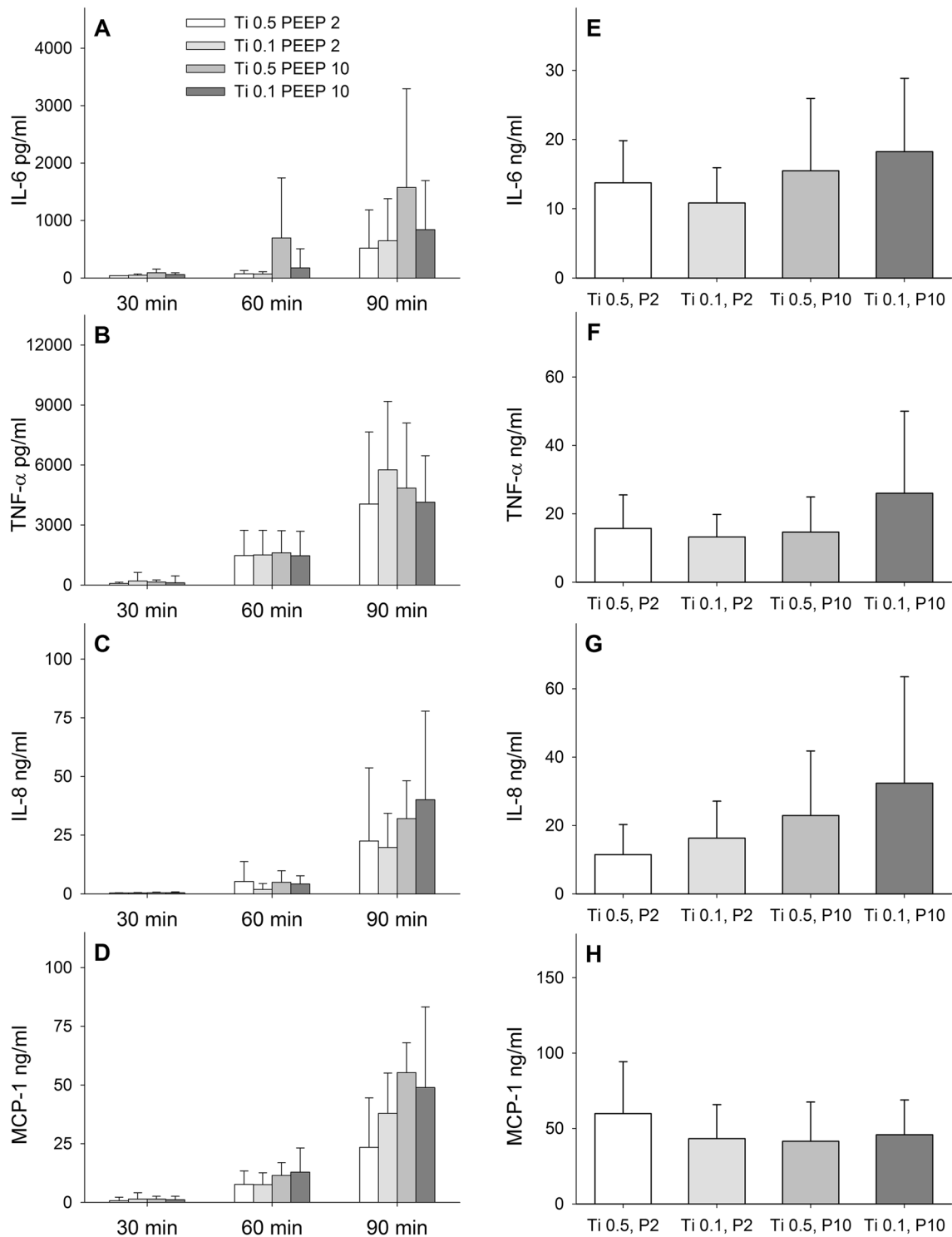
Fig. 3 **a–d** Cytokine concentrations of perfusate from isolated perfused rat lungs during 90 min of slow (Ti = 0.5) and fast (Ti = 0.1) V_I' with PEEP of 2 or 10 cmH$_2$O (n = 10). Data are presented as mean ± SD and differences between groups analyzed by repeated measures ANOVA. **a** IL-6 (V_I' P = 0.78; PEEP P = 0.03; interaction P = 0.16; time $P \leq 0.001$). **b** TNF-α (V_I' P = 0.80; PEEP P = 0.36; interaction P = 0.54; time $P \leq 0.001$). **c** IL-8 (V_I' P = 0.73; PEEP P = 0.11; interaction P = 0.54; time $P \leq 0.001$). **d** MCP-1 (V_I' P = 0.98; PEEP P = 0.12; interaction P = 0.31; time $P \leq 0.001$). **e,**

f Cytokine concentrations of lung lavage from isolated perfused rat lungs following 90 min of slow (Ti = 0.5) and fast (Ti = 0.1) V_I' with PEEP of 2 or 10 cmH$_2$O (n = 10). Data are presented as mean ± SD and differences between groups analyzed by two-way ANOVA. **e** IL-6 (V_I' P = 0.83; PEEP P = 0.65; interaction P = 0.48). **f** TNF-α (V_I' P = 0.53; PEEP P = 0.77; interaction P = 0.23). **g** IL-8 (V_I' P = 0.61; PEEP P = 0.35; interaction P = 0.61). **h** MCP-1 (V_I' P = 0.70; PEEP P = 0.57; interaction P = 0.57)

Fig. 4 Pearson correlations of mechanical power (J/min) calculated as per Gattinoni et al. [2] against indices of lung injury from isolated perfused rat lungs during 90 min of slow (Ti = 0.5) and fast (Ti = 0.1) V_1' with PEEP of 2 or 10 cmH$_2$O (n = 10). **a**, **f** lung wet to dry weight ratio. **b**, **g** Alveolar surfactant (Alv 2) desaturated phospholipid (DSP). **c**, **h** interleukin (IL)-6. **d**, **i** tumor necrosis factor (TNF)-α. **e**, **j** IL-8. The r value represents the correlation coefficient, and P, the respective P value
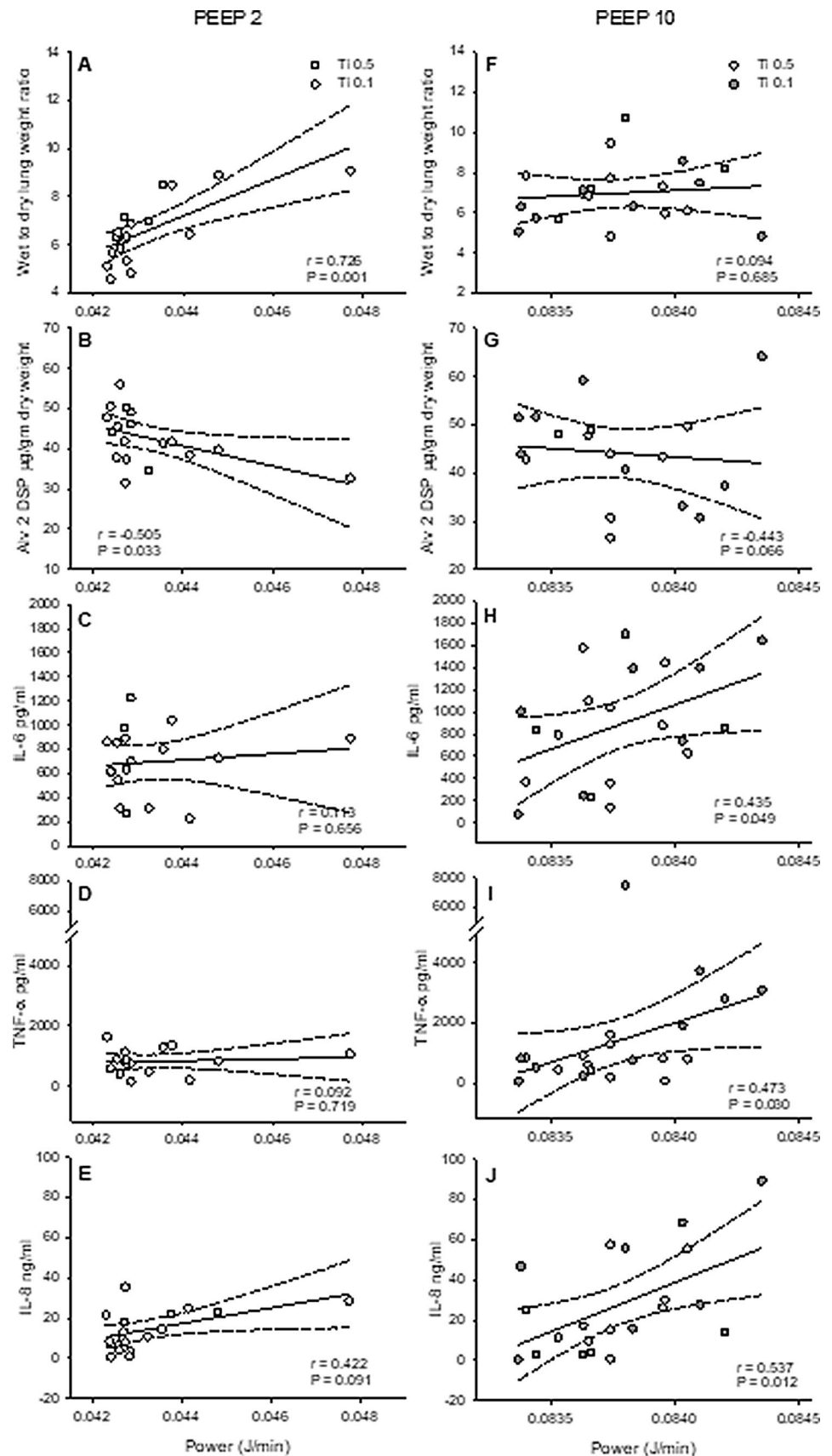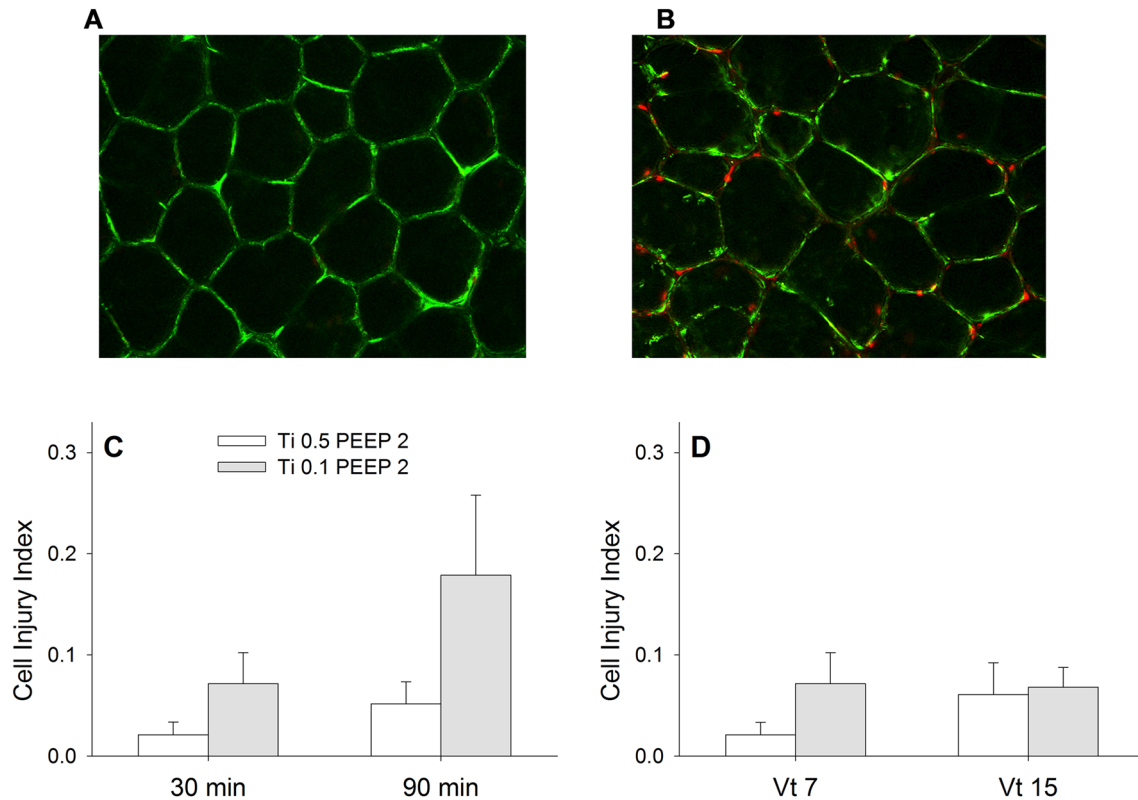
Fig. 5 **a**, **b** Confocal images of subpleural alveoli (left, control— V_I' (Ti = 0.5); right, injury— V_I' (Ti = 0.1)). The red nuclei mark the injured cells (propidium iodide (PI)—positive nuclei). **c**, **d** Average number of PI$^+$ cells per alveolus as assessed from ten random subpleural fields per animal. **c** V_T—7 ml/kg: VI' $P \leq 0.001$; time $P \leq 0.001$; interaction $P = 0.007$. **d** At 30 min: V_I' $P = 0.001$; V_T $P = 0.03$; interaction $P = 0.01$. Data are presented as mean ± SD and differences between groups analyzed by two-way ANOVA

associated lung injury [24, 29], however, this does suggest that high strain rate related cell wounding is a very early mechanical consequence.

These data support the suggestion of using low constant V_I', as opposed to decelerating flow waveforms which enforce an initial high V_I', as suggested by Gattinoni et al. [1]. However, further studies are needed to examine surfactant turnover in the alveolus, lipid cycling, the impact of granulocytes in the pulmonary circulation and the direct relevance to clinical practice.

Author Contributions ADB, DLD conceived and designed the analysis. MK, KG collected the data. DLD performed the analysis. AB, DLD wrote the paper. AB, MK, KG, DLD revised and approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval All experiments were approved by the Flinders University Animal Welfare Committee and performed according to the National Health and Medical Research Council of Australia Guidelines on Animal Experimentation.

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