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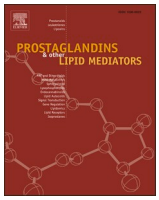

Plasma and bronchoalveolar lavage fluid oxylipin levels in experimental porcine lung injury

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ABSTRACT

Inflammatory signaling pathways involving eicosanoids and other regulatory lipid mediators are a subject of intensive study, and a role for these in acute lung injury is not yet well understood. We hypothesized that oxylipin release from lung injury could be detected in bronchoalveolar lavage fluid and in plasma. In a porcine model of surfactant depletion, ventilation with hyperinflation was assessed. Bronchoalveolar lavage and plasma samples were analyzed for 37 different fatty acid metabolites (oxylipins). Over time, hyperinflation altered concentrations of 4 oxylipins in plasma (TXB_2, PGE_2, 15-HETE and 11-HETE), and 9 oxylipins in bronchoalveolar lavage fluid ($PGF_{2\alpha}$, PGE_2, PGD_2, 12,13-DiHOME, 11,12-DiHETrE, 13-HODE, 9-HODE, 15-HETE, 11-HETE). Acute lung injury caused by high tidal volume ventilation in this porcine model was associated with rapid changes in some elements of the oxylipin profile, detectable in lavage fluid, and plasma. These oxylipins may be relevant in the pathogenesis of acute lung injury by hyperinflation.

1. Introduction

Mechanical ventilation (MV) is an indispensable tool in the treatment of acute respiratory distress syndrome (ARDS) and other forms of respiratory failure. MV may, however, cause injury to the lungs through overstretching of relatively intact alveoli, especially when large tidal volumes are applied [1–3]. Diseased lungs, and in particular ARDS lungs, appear to be more prone to develop ventilator-induced lung injury (VILI) [4]. ARDS lungs are typically consolidated in major portions, rendering them functionally small or "baby" lungs [5,6]. The remaining aerated "baby lungs" receive all of the tidal ventilation, predisposing for overstretching the healthy areas [5]. Despite the overwhelming evidence for the benefits of low tidal volume ventilation in ARDS, volutrauma or VILI still occurs [7,8].

Cyclic overdistension of aerated alveoli, and repetitive closure and reopening of small airways, is strongly associated with VILI [9,10]. This process results in inflammation with influx of neutrophils, local and systemic release of inflammatory mediators, alveolar flooding, fibrin deposition, and activation of coagulation [11,12]. The clinical manifestation can be severely impaired respiratory mechanics and gas exchange, as well as systemic inflammation that may be complicated by multiple organ dysfunction syndrome, MODS [12,13]. The inflammatory processes in the development of VILI, with subsequent tissue damage, seem to be dependent on several different signaling pathways, which are not completely understood [14].

Inflammatory signaling pathways involving eicosanoids and other regulatory lipid mediators are well recognized [15]. These numerous lipids are metabolites of the ω-6 polyunsaturated fatty acids (PUFAs) arachidonic acid (AA), linoleic acid (LA) and dihomo-γ-linolenic acid (DGLA), as well as the ω-3 PUFA α-linoleic acid (ALA) that can be metabolized to the precursor lipids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [16]. Many different bioactive signaling lipids may be formed from these precursor PUFAs and these metabolites can have both pro- and anti-inflammatory effects. A role for these as predictive clinical biomarkers is not yet well understood. Previous studies of lipid mediators, collectively known as oxylipins, in

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inflammatory diseases including ARDS, VILI, and even Covid-19, have included focus on metabolites derived from the cyclooxygenase, COX, and lipoxygenase, LOX, pathways [17–22]. AA, as well as other fatty acids, may also be metabolized through the cytochrome P450 (CYP) pathway to yield both pro-inflammatory mediators as well as anti-inflammatory epoxy-fatty acids (EpFAs). These may, in turn, be deactivated by soluble epoxide hydrolase, sEH [23]. This regulatory system is the target site for numerous drug-based therapeutic interventions, including anti-inflammatory drugs with actions on COX and LOX [17]. In the last decades, many publications have focused on sEH, and several inhibitors with promising anti-inflammatory properties have been introduced [17,23]. The CYP metabolite contribution to the development of VILI or ARDS has not been extensively studied.

These different inflammatory mediators are presumed to be increased at different times in the process, and potentially change in concentration quickly. In the context of acute lung injury and VILI, there has been little reporting on the biological expression of oxylipins in all three metabolic pathways, COX, LOX and CYP. We hypothesized that bronchoalveolar lavage fluid (BALF) oxylipin levels would increase as early inflammatory signals in response to hyperinflation volutrauma in an experimental acute lung injury model. We hypothesized that oxylipin release from lung injury could be detected in plasma.

2. Materials and methods

2.1. Animals

With ethical permission from the Umeå Animal Experimental Ethics Committee (A43–12), juvenile female Yorkshire/Swedish landrace pigs were used. All procedures were carried out in accordance with the EU Directive 2010/63/EU for animal experiments.

2.2. Preparation

Premedication was administered with intramuscular ketamine 10 mg/kg (Ketalar® 10 mg/mL, Pfizer AB, Sweden), xylazine 2.2 mg/kg (Rompun® vet 20 mg/mL, Bayer Animal Health, Denmark), and atropine sulphate 0.05 mg/kg (Atropin® Mylan 0.5 mg/mL, Mylan AB, Sweden). An induction dose of intravenous (IV) pentobarbital 10 mg/kg (Pentobarbitalnatrium, Apoteksbolaget, Stockholm, Sweden) was given, and anesthesia was maintained by an infusion of fentanyl 20 μg/kg/h (Fentanyl, Braun, Melsungen, Germany), midazolam 0.3 mg/kg/h (Dormicum, Roche, Basel, Switzerland), and pentobarbital 5 mg/kg/h. After orotracheal intubation, the animals were mechanically ventilated (Evita 4, Dräger, Kiel, Germany) with 8 mg/kg tidal volume, ratio of inspiration to expiration (I:E) 1:2, positive end expiratory pressure (PEEP) 5 cmH_2O, FiO_2 0.4 and respiratory rate set to maintain normocapnia. A warming gel pad was used to maintain rectal temperature at 38 °C. Ringer's acetate (Ringer-Acetat® Baxter Viaflo, Baxter Medical AB, Sweden) was given at a rate of 4–5 mL/kg/h. If hypovolemia was suspected, a bolus of 250 mL of hydroxyethyl starch solution (Voluven®, Fresenius-Kabi, Uppsala, Sweden) was administered. Under sterile conditions, a central venous catheter was percutaneously inserted through the external jugular vein, and an arterial catheter was placed in the carotid artery after a cut-down procedure.

To render the animals' lungs susceptible for VILI in a 2-hit model of lung injury, surfactant depletion was performed by saline lavage in all animals. After preoxygenation with 100% inspired oxygen, 30 mL/kg of 38 °C 0.9% sterile normal saline was instilled into the trachea and then allowed to drain passively through the tracheal tube over several seconds. The procedure was repeated 4 times, each interspaced by a short recovery period to allow the arterial oxygen saturation to return to at least 96%.

2.3. Protocol

Immediately after surfactant depletion, the animals were randomized by blinded choice of an allocation lot to either protective or injurious ventilation. In the group with low tidal volume (LTV) ventilation, tidal volumes were kept at 8 mL/kg with I:E 1:2 and PEEP was elevated to 8 cmH20. Respiratory rate was adjusted to normocapnia, and FiO2 was set to achieve normoxemia. The group with high tidal volume (HTV) ventilation received tidal volumes of 20 mL/kg with I:E 1:2, respiratory rate 20 bpm, ZEEP (zero end expiratory pressure), and FiO2 1,0. Extra tubing was added as needed between the endotracheal tube and the Y-piece to increase dead space to achieve normocapnia. The experimental period and sampling lasted 6 h, and then the animals were euthanized by injection of potassium chloride under continuous deep anesthesia. Samples of blood and bronchoalveolar lavage fluid (BALF) were collected at the following protocol points: before surfactant depletion, and after 1, 2, 4 and 6 h of ventilation. Biopsies from upper and lower lobes of both lungs were collected post-mortem. Lung biopsies were evaluated by a pathologist, blinded in regard to group allocation, according to previously published guidelines [24]. The presence of widened alveolar septae, intracapillary neutrophils, alveolar neutrophils, edema and intraalveolar fibrin or hyaline membranes was evaluated and lung injury was graded as no lung injury = 0, mild injury = 1, moderate injury = 2 and severe injury = 3. For each animal, an average of the 4 samples was calculated Fig. 1.

2.4. Sampling procedures

2.4.1. Bronchoalveolar lavage fluid, BALF

The BALF samples were collected using a flexible fiberoptic bronchoscope. The bronchoscope was inserted through the tracheal tube and carefully wedged into a bronchus. As sampling was repeated, care was taken to avoid multiple samples from the same location. Three aliquots of 50 mL sterile 0.9% saline was injected and then gently recovered into an iced glass vial. After immediate filtration through a 100 μm nylon filter, the recovered volume was noted and the BALF was centrifuged for 15 min at 400 g and 4 °C. The supernatants were separated from the cell pellets, divided into aliquots, and immediately frozen to − 70 °C for later analysis of bioactive lipids. The cell pellets were resuspended in phosphate buffer saline to achieve a cell concentration of 10^6 cells/mL for later differential cell counts.

2.4.2. Blood samples

Blood samples were collected from the arterial catheter into standard EDTA collection tubes. The blood samples were chilled and centrifuged for ten minutes at 2000 g and 4 °C. The plasma was then recovered and immediately frozen at − 70°C.

2.5. Analysis of oxylipins

2.5.1. Chemicals and reagents

The following native, internal, and recovery standards were used for oxylipin analysis: Prostaglandin $F_{2\alpha}$ – $PGF_{2\alpha}$, Prostaglandin E_2 – PGE_2, Thromboxane B_2 – TXB_2, Prostaglandin D_2 – PGD_2, 5(6)-epoxy-

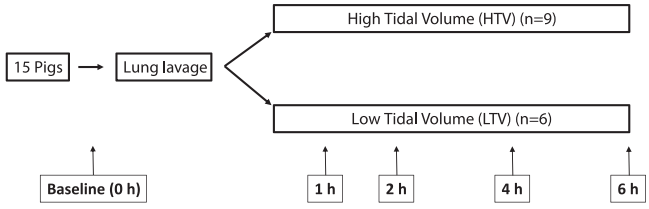

Fig. 1. Study flow chart. After lavage, the animals were immediately randomly allocated to one of the two ventilation regimens.

eicosatrienoic acid – 5(6)-EpETrE, 8(9)-EpETrE, 11(12)-EpETrE, 14(15)-EpETrE, 5,6-dihydroxy-eicosatrienoic acid – 5,6-DiHETrE, 8,9-DiHETrE, 11,12-DiHETrE, 14,15-DiHETrE, 9(10)-epoxy-octadecenoic acid – 9(10)-EpOME, 12(13)-EpOME, 9,10-dihydroxy-octadecenoic acid – 9(10)-DiHOME, 12(13)-DiHOME, 5-hydroxy-eicosatetraenoic acid – 5-HETE, 8-HETE, 9-HETE, 11-HETE, 12-HETE, 15-HETE, 20-HETE, 9-hydroxy-octadecadienoic acid – 9-HODE, 13-HODE, 15-hydroxy-eicosatrienoic acid – 15-HETrE, 12,13-dihydroxy-octadecenoic acid – 12-HEPE, 17-hydroxy-docosahexaenoic acid – 17-HDoHE, 5-oxo-eicosatetraenoic acid – 5-oxo-ETE, 12-oxo-ETE, 15-oxo-ETE, 13-oxo-octadecadienoic acid – 13-oxo-ODE, Leukotriene B_4 – LTB_4, 7,16,17-trihydroxy-docosahexaenoic acid – Resolvin D2, 7,8,17-trihydroxy-docosahexaenoic acid – Resolvin D1, 12-[[(cyclohexylamino)carbonyl]amino]-dodecanoic acid (CUDA), 12(13)-DiHOME-d_4, 12(13)-EpOME-d_4, 9-HODE-d_4, PGE_2-d_4, and TXB_2-d_4 (Cayman Chemicals, Ann Arbor, MI, USA) as well as 9,10,13-TriHOME and 9,12,13-TriHOME (Larodan Malmö, Sweden). LC-MS grade acetonitrile (ACN) and methanol (MeOH) were used (Merck, Darmstadt, Germany). LC-MS grade isopropanol (PROLABO, Fontenay-sous-Bois, France) was used. Acetic acid, (Aldrich Chemical Company, Inc. Milwaukee, WI, USA), butylhydroxytoluene (BHT) (Cayman Chemical, Ann Arbor, MI, USA), ethylenediaminetetraacetic acid (EDTA) (Fluka Analytical, Sigma-Aldrich, Buchs, Switzerland) and glycerol (Fischer Scientific, Loughborough, UK) were used. Water was purified by a Milli-Q Gradient system (Millipore, Milford, MA, USA).

2.5.2. Oxylipin quantification

A previously published solid phase extraction (SPE) protocol was used for extraction of oxylipins from plasma and modified for BALF samples [25,26]. Limit of detection for individual analytes is shown in supplemental tables S3 (BALF) and S4 (plasma) in online supplements. On the day of extraction, the samples were thawed at room temperature. SPE Waters Oasis HLB cartridges (60 mg sorbent, 30 μm particle size) were first washed with 2 mL of ethyl acetate, 4 mL of methanol, and 4 mL of 95:5 v/v water/MeOH with 0.1% acetic acid (WS). The BALF (2–2.7 mL) and plasma (0.7 mL) samples were loaded onto the SPE cartridges and spiked with 10 μL of internal standard solution (50 ng/mL for 12(13)-DiHOME-d_4 and 12(13)-EPOME-d_4, and 25 ng/mL for 9-HODE-d_4, PGE_2-d_4 and TXB_2-d_4) and 10 μL antioxidant solution (0.2 mg/mL BHT/EDTA in methanol/water (1:1)). The SPE cartridges were then washed with 4 mL of WS, dried under high vacuum for about 1 min, and eluted with 2 mL methanol and 2 mL ethyl acetate into polypropylene tubes containing 6 μL of a glycerol solution (30% in methanol). Glycerol operates as a trap solution for the analytes. Eluates were evaporated under vacuum (MiniVac system, Farmingdale, NY, USA) and residues were then reconstituted in 100 μL methanol and tubes were vortexed. The solutions were transferred to LC vials and 10 μL of the recovery standard solution was added (5 ng/mL CUDA) and UPLC-AJST-ESI-MS/MS analysis was performed immediately in randomized order. The Agilent UPLC system (Infinity 1290) was coupled to an Agilent 6490 Triple Quadrupole system equipped with the iFunnel Technology source (Agilent Technologies, Santa Clara, CA, USA). The UPLC column used was a Waters BEH C_{18} column (2.1 mm × 150 mm, 2.5 μm particle size), and 10 μL injection volume was employed. Different mobile phase composition and different gradients were compared to achieve optimal separation for all compounds, especially between critical isomer pairs. The optimal conditions were found with 0.1% acetic acid in MilliQ water (A) and acetonitrile:isopropanol (90:10) (B) using the following gradient: 0.0–3.5 min 10–35% B, 3.5–5.5 min 40% B, 5.5–7.0 min 42% B, 7.0–9.0 min 50% B, 9.0–15.0 min 65% B, 15.0–17.0 min 75% B, 17.0–18.5 min 85% B, 18.5–19.5 min 95% B, 19.5–21 min 10% B, 21.0–25.0 min 10% B with constant flow rate of 0.3 mL/min. The autosampler temperature was kept at 10 °C and the column at 40 °C. The mass analysis was done in negative mode (AJST-ESI). Integration of all peaks was manually performed using the MassHunter Workstation software.

2.6. Statistics

To be included in group analysis, we required that metabolites could be quantified in at least 80% of the samples in plasma and BALF, respectively.

For univariate statistics, all variables were subjected to the Shapiro-Wilk test for normal distribution (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Study group characteristics were compared using Mann Whitney U test for continuous variables and Fischer's exact test for nominal data. For non-normally distributed metabolite data (which included the measurements of all oxylipins), Box-Cox transformation was used. Box-Cox transformation factors were calculated using the Boxcox routine in the MASS package for R. The command line *output variable*< -boxcox(lm(*metabolite*~*treatment group***time point*,data=*data set*)) was used. Transformed values of metabolites that were deemed to meet the normality assumption were subjected to mixed linear models in R using the lmerTest package with the command line *output variable* <- lmer(*metabolite* ~ *treatment group***time point* + (1|*Case*), data=*data set*, REML=FALSE). Residuals were plotted and examined for heteroscedasticity and fit to a Q-Q-plot. P values for interaction between time and treatment group were noted for metabolites with residuals that were adequately distributed to meet the assumption of normality. Baseline means (transformed data) were compared using 2-tailed independent group t-test (IBM SPSS). Finally, correction for multiple testing was done by submitting all p-values to false discovery rate (FDR) set at 10% [27]. Statistical significance for the parametric test results was set at $P < 0.05$.

Principal component analysis (PCA) in SIMCA v14 (Umetrics, Umeå, Sweden) was used to exclude the presence of batch effects.

3. Results

3.1. Characteristics of study groups

In total, 15 animals were included in the study, and 9 were randomized to the HTV group. In the HTV group, 3 animals expired before completing the last assessment, and one animal did not complete the last two assessment points. All 6 animals in the LTV group completed the protocol. Oxygenation was more impaired in the HTV group, but low lung compliance was noted in both groups after surfactant depletion. Arterial carbon dioxide levels were not different between the groups, though pH was lower in the HTV group. Average total fluid administration per hour was higher in the HTV group. Table 1.

Recovery rates of BAL fluid were not different between groups or time points, although there was a possible trend towards lower recovery rates in later time points in the HTV group (Supplements Fig. S3). The concentration of cells in BALF was clearly reduced after the lung lavage at baseline but did not significantly differ over time between groups (Fig. S4), although there was a trend towards higher total cell concentration in the HTV group. In the HTV group, there was a significant change of differential cell counts at 2, 4 and 6 h with an increase of the fraction of neutrophils and a corresponding significant decrease in the fraction of macrophages, which was the otherwise dominant cell type (Fig. S5 and S6). There were no significant time-dependent group differences in the fraction of lymphocytes or eosinophils (Fig. S7 and S8). At histological evaluation, all animals in the LTV group exhibited no or mild lung injury, whereas all but one animal in the HTV group had moderate or severe lung injury (Fig. 2).

3.2. Bronchoalveolar lavage fluid metabolites

In BALF, 37 metabolites were detected and quantified. Of those, 17 metabolites were excluded from further analysis due to detection in less than 80% of the samples (supplemental table S3). Grouped values for all metabolite concentrations were non-normally distributed. The PCA analysis did not identify differences between analytical batches for

Table 1
Median (interquartile range). A normal distribution could not be assumed for any of the variables. Abbreviations: FiO2 - fraction inspired oxygen, P/F- ratio partial pressure of oxygen in arterial blood (kPa) and simultaneous FiO2, PCO2- partial pressure arterial carbon dioxide, BALF- bronchoalveolar lavage fluid.

Variables	LTV n = 6	HTV n = 9	p value
Weight (kg)	35.5 (28–50)	32.5 (30–37)	0.78
Measured Tidal volume (mL)	280 (228–400)	680 (600–747)	**< 0.001**
Respiratory rate (breaths/min)	30 (26–38)	20 (20–20)	**0.003**
Mean airway pressure at last assessment	11 (10 – 11)	16 (14–18)	**< 0.001**
Peak inspiratory pressure (cmH2O) at last assessment	22 (20–23)	45(41–47)	**< 0.001**
Compliance (mL/cmH2O)	14.3 (7.0–21.7)	14.9 (9.2–20.6)	0.60
FiO2	0.35 (0.29–0.40)	1.0 (1.0–1.0)	**< 0.001**
Worst P/F ratio (kPa)	46 (34–52)	9.7 (7 – 19)	**0.008**
Last P/F ratio	53 (43–64)	21 (8–28)	**0.012**
Last PCO2 (kPa)	4.6 (3.8–5.0)	7.0 (5.1–7.3)	**0.018**
Last pH	7.52 (7.47–7.55)	7.37 (7.31–7,.45)	**0.005**
Total intravenous crystalloid (mL)	2000 (975–3000)	2500 (2000–3000)	0.53
Total intravenous colloid (mL)	0 (0–500)	500 (250–500)	0.145
Average fluid administration (mL/kg/h)	7.7 (4.4–8.2)	10.0 (9–12.0)	**0.026**
Average temperature (°C)	39.4 (38.3 –	38.4 (38.2–39.1)	0.220.088
Recovered BALF volume (%)	39.7)78 (63 – 84)	70 (64 – 78)	
Lung injury score (histopathology)	**0.125 (0 – 0.44)**	**2.75 (2.5 – 2.75)**	**0.044**

either BALF or plasma samples. After transformation according to Box-Cox analysis, 11 metabolites (9,10,13-TriHOME, $PGF_{2\alpha}$, PGE_2, PGD_2, 12,13-DiHOME, 9,10-DiHOME, 11,12-DiHETE, 13-HODE, 9-HODE, 15-HETE and 11-HETE) met the assumption of normality with heteroscedasticity in residuals and were included in baseline comparisons and mixed model analysis. One metabolite, $PGF_{2\alpha}$, was lower in the VILI group at baseline. FDR was calculated at $p < 0.0514$ for BALF metabolites. The 9 different metabolites which showed positive interactions between time and group were the following: $PGF_{2\alpha}$, PGE_2, PGD_2, 12,13-DiHOME, 11,12-DiHETrE, 13-HODE, 9-HODE, 15-HETE, 11-HETE (Fig. 3 A-I and Supplemental Table S1).

3.3. Plasma

From the serial plasma samples in each animal, 37 metabolites were detected and quantified in at least one of the samples. Among these, 13 were excluded from further analysis due to detection in less than 80% of the samples (supplemental table S4). All metabolites were non-normally distributed. After transformation according to Box-Cox analysis, 21 metabolites (TXB_2, 9,12,13-TriHOME, 9,10,13-TriHOME, $PGF_{2\alpha}$, PGE_2, 12,13-DiHOME, 9,10-DiHOME, 14,15-DiHETrE, 11,12-DiHETrE, 8,9-DiHETrE, 20-HETE, 13-HODE, 9-HODE, 15-HETE, 13-oxoODE, 11-HETE, 15-oxoETE, 5-HETE, 12,13-EpOME, 9,10-EpOME and 5,6-EpETrE) met the assumption of normality, and were included in baseline comparisons and mixed model analysis. There were no differences between groups at base line. The correction for multiple testing gave a statistical significance level at $p < 0.012$. Interactions between time and group were identified for 4 metabolites: TXB_2, PGE_2, 15-HETE and 11-HETE (Fig. 4A-D and Supplemental Table S2).

4. Discussion

The findings in this exploratory study pinpointed responsive oxylipins due to volutrauma insult in an acute lung injury model. Nine oxylipins were increased in BALF (Fig. 3) and four in plasma (Fig. 4), three of which (PGE_2, 15-HETE and 11-HETE) were detected in both sample types. The analysis included assessments of 37 metabolites (Fig. 5) at four time points, and the different metabolites had own patterns for concentration changes. The findings confirm the hypothesis that some oxylipins involved in acute lung injury and volutrauma can be detected early after diffuse lung insult in plasma and potentially serve as easily accessible biomarkers of progression of lung injury.

The study design allowed detection of oxylipin concentration changes as an exploration, and there was no attempt to correlate severity of injury with the biomarker levels. In this way, the clinical relevance of these findings is unclear. These findings demonstrate that studying oxylipins simultaneously in different body compartments can be a promising future line of study [28]. Additionally, epoxide-diol ratio assessment where possible has showed promise [29].

The experimental setup included a two hit VILI model, to study the detrimental effects of injurious ventilation after surfactant depletion. Before surfactant depletion, the two experimental groups were similar as far as oxylipin levels for all the 37 assessed substances, with the exception of BALF levels of $PGF_{2\alpha}$.

The hyperinflation lung injury model performed well in causing severe lung injury, built on a surfactant-depletion situation, with severely impaired gas exchange and severe histological lung injury in the HTV group, and minimal lung injury in the LTV group. The extreme illness generated by the HTV intervention did limit sampling at later time points, which is a limitation of this model concerning response over time. Still, this experimental design allowed sampling of signals in oxylipin concentration changes (Figs. 3 and 4).

Elevated TXB_2 levels in plasma were observed in the HTV group, even though BALF TXB_2 results were too unevenly distributed for formal significance testing even with transformed data. TXA_2 is a potent bronchoconstrictor that also mediates platelet aggregation and vasoconstriction [30]. TXA_2 is non-enzymatically degraded to its more stable metabolite TXB_2, of which elevated levels have been demonstrated in numerous studies on inflammatory airway diseases [30]. Elevated levels of TXB_2 have also previously been demonstrated in ARDS [31,32]. TXB_2 levels have also been shown to correlate with early worsening of oxygenation in experimental guinea pig ARDS [33].

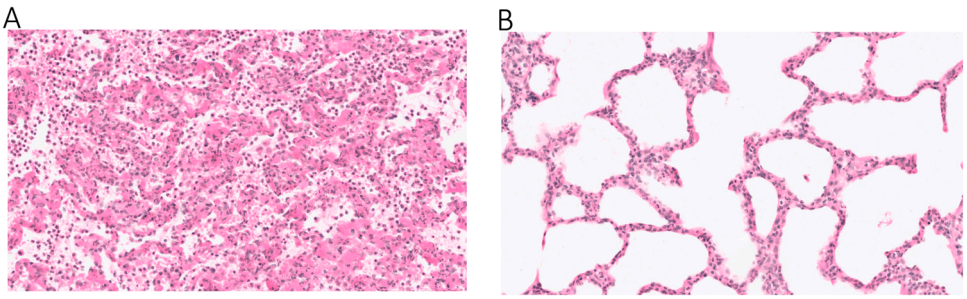

Fig. 2. Panel A shows representative image of lung tissue from HTV group. Panel B shows representative image of lung tissue in LTV group.

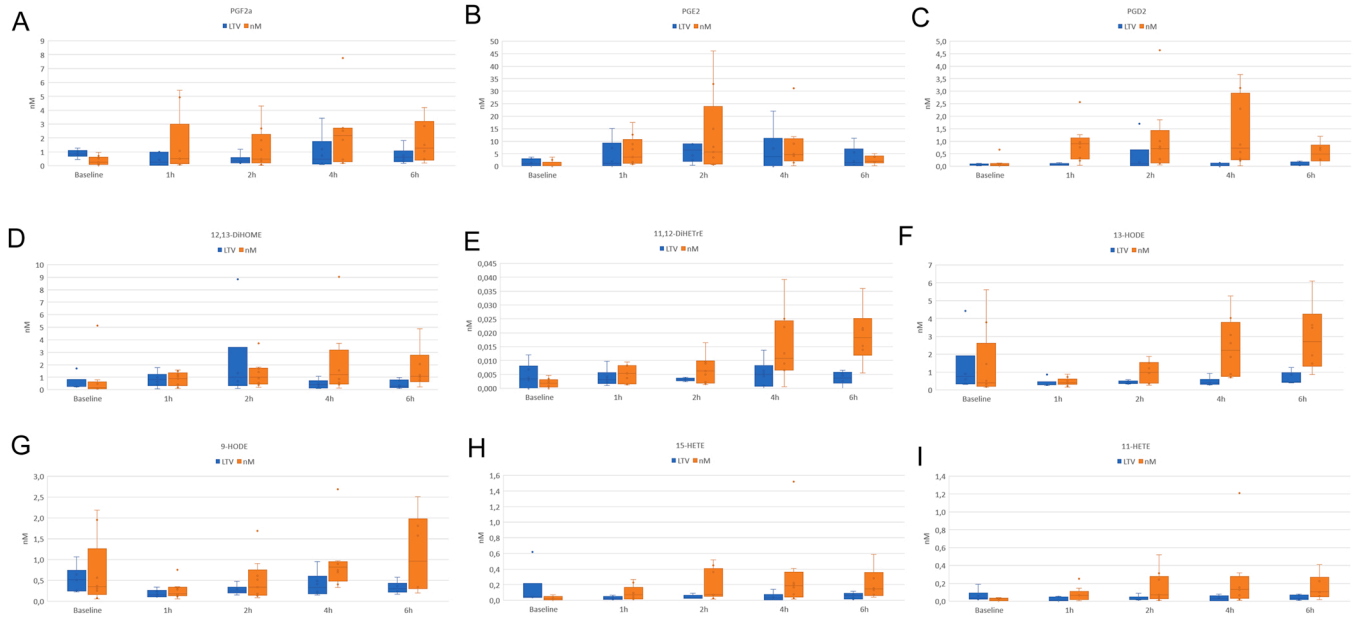

Fig. 3. Panels A-I. Substances in bronchoalveolar lavage fluid where there were differences between lung volume treatment groups. Median metabolite concentrations are shown for different time points. Boxes and whiskers represent interquartile range and range, respectively. P values for grouped comparisons at specific time points are presented in supplemental Table S1. Abbreviations: LTV – Low tidal volume group, HTV – high tidal volume group.

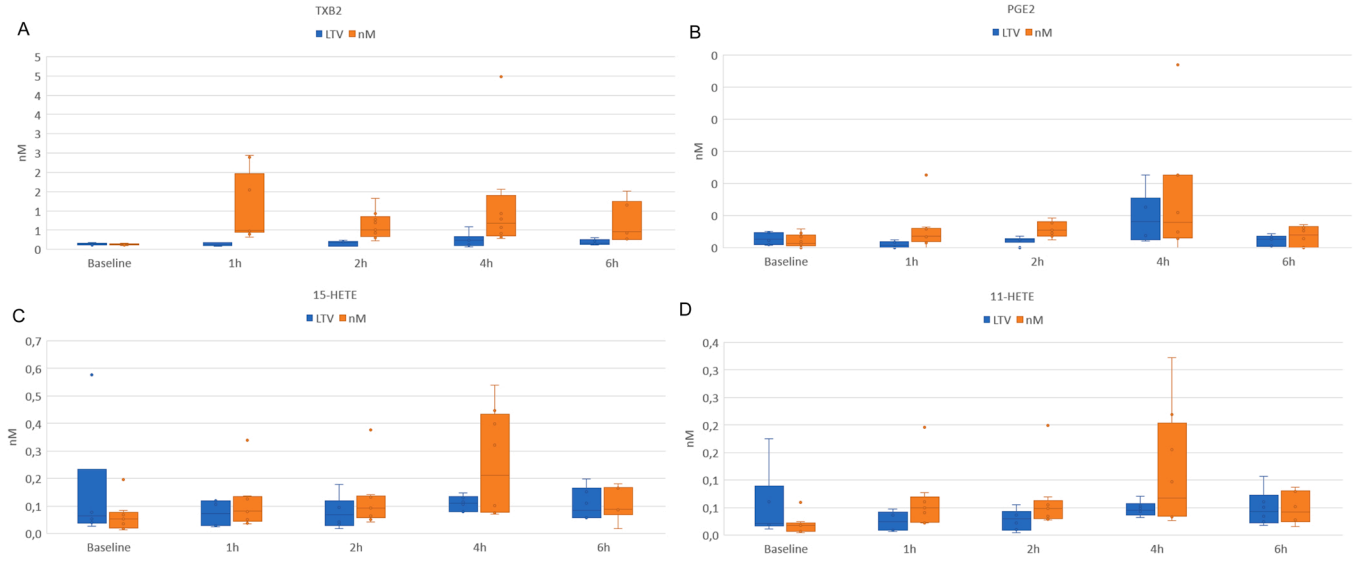

Fig. 4. Panels A-D. Substances in plasma where there were differences between lung volume treatment groups. Median metabolite concentrations are shown for different time points. Boxes and whiskers represent interquartile range and range, respectively. P values for grouped comparisons at specific time points are presented in supplemental Table S2. Abbreviations: LTV – Low tidal volume group, HTV – high tidal volume group.

PGD_2 in BALF increased early in the response to lung injury in our study. PGD_2 increases have previously been reported to have possible anti-inflammatory and protective effects in this context [34], as well as in the resolution phase of inflammation [35]. $PGF_{2\alpha}$ also increased in BALF in response to HTV ventilation. $PGF_{2\alpha}$ is also associated with the resolution phase of inflammation [36], but with unclear effects in human ARDS [37]. $PGF_{2\alpha}$ has, however, been shown to exert effects on neutrophil chemotaxis [38]. It is also a potent vaso- and broncho-constrictor [39] and has been shown to increase in cultured pig pulmonary artery segments in response to endotoxin, as well as to proinflammatory cytokines [37].

The LOX metabolites 13-HODE and 15-HETE increased in BALF in HTV ventilation, which is consistent with previous reports, where 13-HODE is thought to damage the respiratory epithelium in response to interleukin 13 (IL-13) mediated upregulation of 15-LOX [40]. 15-HETE is generated by airway epithelial cells [41] and has been implicated as a mediator of pulmonary artery thrombus formation [42]. It is known to prevent thrombin induced-platelet aggregation inhibition [43] and may be relevant to lung injury pathogenesis in the context of vascular dysfunction [44]. The LOX metabolites have also been proposed as key mediators of neutrophil cell recruitment [45], which is an important feature of acute lung injury pathogenesis. Furthermore, DiHOMEs are synthesized by neutrophils and induce neutrophil chemotactic activity at relatively low doses [46–48], while at higher doses they inhibit the neutrophil respiratory burst [49]. This is interesting since it implies that DiHOMEs may provide a negative feedback-loop for limiting the inflammation, given the difference in dose required for the inhibitory and chemotactic effects [48]. Activation of inflammatory leukocytes

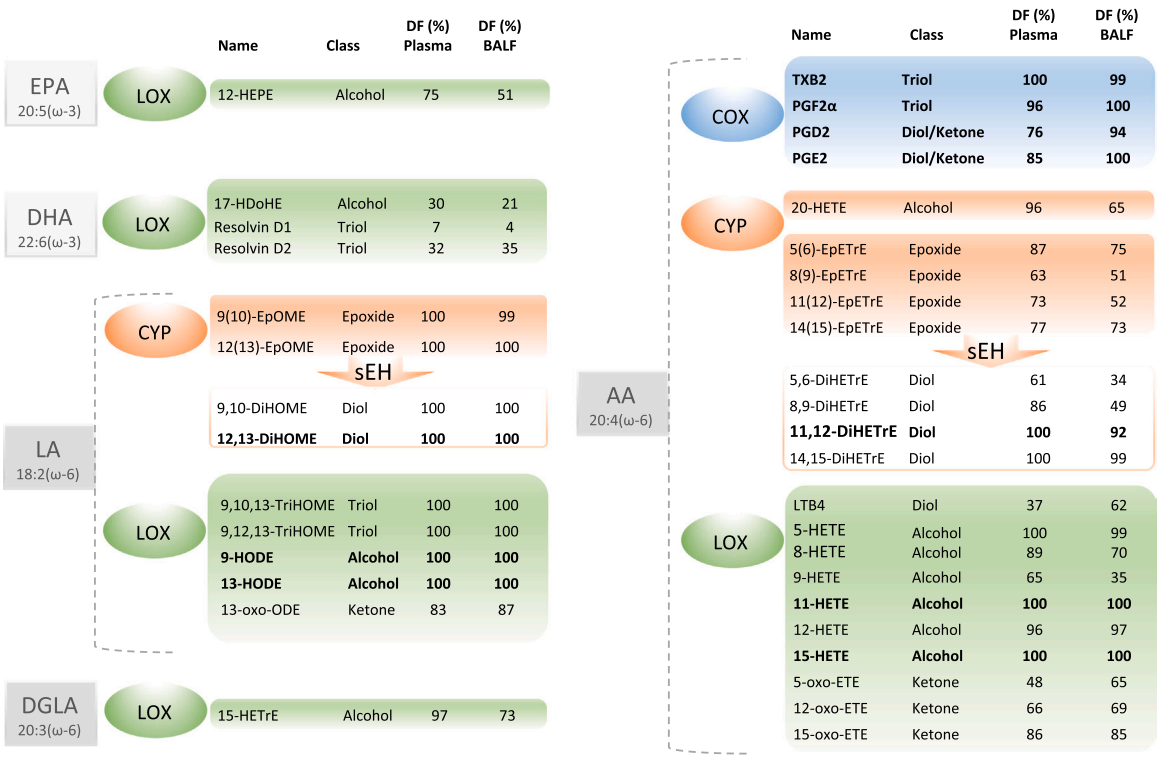

Fig. 5. Overview of investigated oxylipins and their respective origin and enzymatic pathway. Fatty acid precursors are shown in grey boxes: ω-3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), as well as ω-6 linoleic acid (LA), dihomo-γ-linolenic acid (DGLA) and arachidonic acid (AA). Detection frequency (DF) is shown for plasma and BALF separately. Of these 37 substances, 20 had DF of at least 80% in BALF and 24 had DF of at least 80% in plasma. LOX – lipoxygenase, CYP – cytochrome P450, sEH – soluble epoxide hydrolase, COX – cyclooxygenase. Oxylipins in bold indicate significant elevation in HTV group over time.

such as neutrophils can also result in production of PGE_2 [50].

PGE_2 may be the most extensively studied prostaglandin [30], and this oxylipin was elevated in plasma in the HTV group. PGE_2 is thought to exert different types of effects under different conditions [30,36,51]. In ARDS, PGE_2 has been shown to mediate/participate in formation of pulmonary edema in response to PAF [52]. Given the requirement of proinflammatory signals to support early and persistent neutrophil influx to the site of inflammation followed by a resolution phase, a temporal shift of oxylipins with both pro- and anti-inflammatory, as well as pro-resolving properties, was expected. Accordingly, individual patterns were observed in a subset of the 37 investigated oxylipins. Responsive oxylipins were derived from both LA and AA fatty acid precursors via LOX-, COX-, and CYP pathways (Fig. 5), which exert their effects in concert with key inflammatory cells, and thereby contribute to modulation of the inflammatory response in lung injury. Since oxylipins possess context-dependent properties and may even change function from pro-inflammatory to pro-resolution in a dose-dependent manner as illustrated by DiHOMEs above, further studies are needed for conclusions on the contributions of each oxylipin in lung injury. The current study may assist in selection of candidate biomarkers for such attempts and showed usefulness of BALF and plasma measurements. Increases in levels of both 15-HETE and 11-HETE were evident in BALF earlier than the increases in plasma (Figs. 3 and 4), suggesting that sampling directly from the lung may facilitate earlier detection of some biomarkers produced in an injured lung. Contrary to this, the immediate (after 1 h) increase in plasma TXB_2 shows that oxylipin biomarkers may also, at least in some cases, be detectable in plasma early after injury.

Study design limitations included a relatively small number of observations. As an exploratory study, a conservative statistical analysis approach was chosen to reduce risk for data false positive findings. Levels for oxylipins measured but not discussed are available in the online supplements (Supplements, Table S3 and 4, Figs. S1 and 2).

In conclusion, experimental induction of acute lung injury by high tidal volume ventilation in this porcine model is associated with rapid changes in some elements of the oxylipin profile, detectable in lavage fluid, and plasma for a subset of the measured oxylipins. Further studies are needed to test possible clinical predictive value of oxylipin testing in blood for acute lung injury.

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Declarations of Interest

Niklas Larsson has received speaker fees for lecturing in clinical symposia sponsored by Dräger Medical, and not related to this specific topic.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.prostaglandins.2022.106636.

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