

# Porcine model of sepsis-induced systemic inflammation and acute lung injury in donor lungs

Basil Saad Nasir (✉ [basilsnasir@gmail.com](mailto:basilsnasir@gmail.com))

Centre Hospitalier de L'Universite de Montreal <https://orcid.org/0000-0003-1482-832X>

Ahmed Menaouar

Centre de recherche du CHUM

Caroline Landry

Centre de recherche du CHUM

Jean-Francois Germain

Centre de recherche du CHUM

Patrick Laplante

Centre de recherche du CHUM

Shant Der Sarkissian

Centre de recherche du CHUM

Moishe Liberman

Centre de recherche du CHUM

Jean-Francois Cailhier

Centre de recherche du CHUM

Charled Leduc

Centre Hospitalier de Université de Montréal

Nicolas Noiseux

Centre de recherche du CHUM

Pasquale Ferraro

Centre de recherche du CHUM

## Research

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## Abstract

Background: The shortage of organ donors is a major challenge in lung transplantation. To expand the lung donor pool, ex-vivo lung perfusion (EVLP) has emerged as a platform for assessment and reconditioning marginal donor lungs. In this study a stable and reproducible large animal model of lipopolysaccharide (LPS) induced systemic inflammation and acute lung injury (ALI) was developed.

Methods: Pigs (n=6) were anesthetized and monitored. After infusion of LPS (20 µg/kg) for 1 hour, followed by a 90-minute response period, lungs were procured and kept on ice for 2 hours, followed by 4 hours of EVLP. Pulmonary function, inflammatory biomarkers and edema formation were measured in vivo before procurement and during EVLP. Pro and anti-inflammatory cytokines were assayed in blood and in EVLP perfusate, which were collected before and every 30 minutes after LPS administration and EVLP.

Results: LPS infusion resulted in significant hemodynamic instability, characterized by marked pulmonary hypertension, decreased systemic blood pressure and increased heart rate. This was associated with increased levels of TNFa, IL-10, IL-6, but no change in IL-1 $\beta$ . Ex vivo assessment of injured lungs showed graft dysfunction characterized by impaired gas exchange and edema formation. The inflammatory profile showed stable but elevated TNFa levels, and continuous production of interleukins during EVLP.

Conclusion: We describe a reproducible large animal model of LPS-induced systemic inflammation and ALI. EVLP alone was unable to recondition severely injured lungs. These findings suggest that the EVLP platform requires adjuncts such as targeted anti-inflammatory agents to allow reconditioning of marginal donor lungs.

## Introduction

Lung transplantation is an effective therapy for patients with end-stage lung disease<sup>1</sup>. Donor lung availability remains a limiting factor. Based on data from a Canadian multicenter study, only 28% of donor lungs offered were utilized<sup>2</sup>. The most common reason for organ refusal was organ function (79% of lungs offered). However, 27% of those lungs were deemed mildly marginal. It is estimated that two-thirds of these lungs may have been utilized, with or without alternative management approaches. Multiple strategies have been suggested, including donation from non-heart-beating donors (also known as donation after circulatory death), extended criteria donors and ex-vivo lung perfusion (EVLP)<sup>3-6</sup>.

Many donor lungs are injured during the acute process that resulted in the donor's death. Sepsis results in a significant inflammatory response within the donor. This response affects the lungs resulting in impaired gas exchange and reduced compliance, possibly increasing the incidence of primary graft dysfunction in the recipient<sup>7</sup>. Lungs that have been subjected to significant sepsis are typically considered unusable. The use of EVLP in reconditioning lungs has been previously described<sup>5,6,8,9</sup>.

Since the introduction of EVLP by Steen and associates, its use continues to grow worldwide<sup>5</sup>. Benefits in graft preservation and transport seem apparent, and its use should help increase the organ donor pool. EVLP's ability to assess graft function is well established<sup>6,8</sup>. EVLP provides clinicians with a unique opportunity to evaluate the lungs, as well as the inflammatory profile of injured grafts. Studying graft function and monitoring inflammatory biomarkers before, during, and after EVLP allows the development of predictive models to confirm the suitability of marginal donor lungs.

EVLP has the potential to recondition injured lungs. This ability, however, seems mostly theoretical. Mehaffey et al. reported on the use of EVLP in rehabilitating sepsis-induced lung injury in a porcine model<sup>9</sup>. The model described in that study utilizes an open atrial system with an acellular perfusate. There have been no studies utilizing EVLP with a cellular perfusate in this setting. We seek to study the efficacy of EVLP using a cellular prime to recondition marginal lungs using a porcine model of lipopolysaccharide (LPS)-induced systemic inflammation and acute lung injury (ALI). In this publication, we describe a large animal model of sepsis-induced lung injury that may be reconditioned using the EVLP platform. Furthermore, we aim to use this particular model to test targeted therapies aimed at limiting the sepsis-induced lung injury or possibly reverse it.

## Materials And Methods

The experimental protocol was approved by the institutional ethical committee for animal research (CIPA # 4I17025PFp) in compliance with the Canadian Council on Animal Care and the National Institute of Health's Guide for the Care and Use of Laboratory Animals.

### Donor pig management

White-hybrid pigs (55–65 kg) are used in this study. General anesthesia is induced. Under continuous electrocardiogram and blood pressure monitoring, the pigs are mechanically ventilated with an inspiration: expiration ratio of 1:2. Respiratory rate is adjusted to keep an end-tidal CO<sub>2</sub> between 35 and 45 mmHg. Saline solution is infused at a rate of 10 mL/kg/h.

In the control group (n = 5), after baseline measurement of the respiratory parameters and collection of blood samples, a sternotomy is performed, and the first lung biopsy is collected (in-situ biopsy). After clamping the ascending aorta, the right and left atrium are opened, and blood collected with *Cell Saver* (C.A.T.S., Fresenius HemoCare GmbH, Bad Homburg, Germany). The lungs are flushed with 1 liter of cold *Perfadex*–LPD solution (Vitrolife AB, Gothenburg, Sweden) through a cannula secured in the main pulmonary artery. The lungs are then procured in a standard fashion and preserved in cold *Perfadex* for 2 hours.

In the LPS group (n=6), a right carotid artery catheter is installed for systemic arterial pressure monitoring and blood sampling. A central line with a pulmonary artery catheter is installed in the right internal jugular vein for measurement of pulmonary artery pressure (PAP, mmHg), blood sampling, and drug administration. Respiratory and hemodynamic parameters are recorded continuously. An LPS infusion

induces systemic inflammation. LPS (*Escherichia coli*; serotype 0111:B4, Sigma, Saint Louis, MO, USA) is dissolved in 100 mL saline solution and infused intravenously at a concentration of 20 µg/kg over one hour, followed by a 90-minute observation period. The dose of LPS was determined based on an initial small-scale feasibility study where higher concentrations of LPS (50-100 µg/kg) over 30-60 minutes caused deleterious effects on the lungs rendering them unsuitable for EVLP perfusion. Boluses of 1 mg/ml Milrinone are administered intratracheally to treat pulmonary hypertension. Hemodynamic instability (mean arterial pressure, MAP < 65 mmHg) is treated with increasing the rate of saline infusion to 15 mL/kg/h and an infusion of phenylephrine. Blood samples are collected at baseline (before LPS administration) and at 30-minute intervals for a total of 150 minutes. The samples are centrifuged (1500g/15 min), and plasma is preserved at -80°C for later analysis of cytokines. Blood gases, chemistry, and metabolite contents are analyzed in the blood samples at baseline, at the end of LPS infusion, and before lung procurement. At the end of the observation periods, the lungs are procured and flushed with cold *Perfadex*. LPS-treated lungs are compared to the lungs from the control group.

### **Ex-vivo Lung Perfusion Procedure**

In preparation for EVLP, one cannula is secured in the main pulmonary artery and the second in the trachea. The left atria cuff is left open per the Lund EVLP protocol<sup>10</sup>. A second biopsy (Pre-EVLP biopsy) is performed. Lungs are weighed (pre-EVLP lung weight). A temperature probe and a perfusate sample catheter are secured inside the left atrium. The lungs are then connected to the perfusion system.

EVLP is performed with Vivoline LS1 (Vivoline Medical AB, Lund, Sweden) using a modified Lund protocol adapted for pig lungs. The circuit is primed with 2.6 L of a perfusion solution containing human serum albumin and 500 mL of washed red blood cells, previously collected from the donor pig during the procurement with *Cell Saver*. Before reperfusion, baseline hematocrit is adjusted to 10-12%. Heparin and antibiotics (Imipenem 100mg) are added to the perfusion solution, and pH corrected to 7.35-7.45 using tris(hydroxyl)aminomethane (THAM).

Porcine cardiac output (CO) is estimated to be 100 ml/min/kg body weight. The final target flow used in our EVLP protocol was 50% of CO, corresponding to blood flow between 2.8 and 3.2 L/min. Based on the work from Steinmeyer et al. and Roma et al., a reduced flow rate of less than 100% of CO is more suitable for the delicate lungs of young pigs<sup>11,12</sup>. This has been consistent with our experience. We noticed significant lung edema when utilizing a flow rate of 100% of CO. Since we have modified the protocol with the reduced flow rate as per Roman et al., we have seen improved outcomes using the Lund protocol. Lung reperfusion is initiated at a flow rate of 0.5 L/min and gradually increased to 1.0-1.2 L/min over the first 10 minutes or until the temperature of the lungs reaches 25°C. Bronchoscopy and bronchoalveolar lavage fluid (BALF) collection are performed. The flow rate is then increased over 30-40 minutes to 50% CO. When the perfusate temperature reaches 32°C, ventilation using 50% FiO<sub>2</sub> is started in volume-controlled mode at an initial tidal volume of 4 ml/kg of donor weight, a positive end-expiratory pressure of 5 cmH<sub>2</sub>O and a rate of 5 breaths/min. Tidal volume is increased gradually to a maximum of 6 mL/kg.

When a temperature of 37°C and a target 50% flow are reached, a recruitment maneuver is performed with an inspiratory hold on 20 cmH<sub>2</sub>O over 1 minute and repeated hourly.

### **Hemodynamic and ventilation parameters**

The following hemodynamic parameters are monitored and collected every 15 min: PAP (mmHg), perfusate flow (L/min), pulmonary vascular resistance (PVR; Dyn.s.cm<sup>-5</sup>), and temperature (°C). The ventilation parameters collected or calculated every 15 min were: peak airway pressure (cmH<sub>2</sub>O) and airway plateau pressure (cmH<sub>2</sub>O), airway resistance (cmH<sub>2</sub>O/l/sec), static and dynamic compliance (ml/cmH<sub>2</sub>O) and end-tidal carbon dioxide (mmHg).

At the end of the reconditioning phase, the evaluation phase starts with disconnecting the oxygen supply to the oxygenator, which serves to deoxygenate the perfusate using a gas mixture of 7% CO<sub>2</sub> and 93% nitrogen. Lung function is evaluated after ventilation with 100% FiO<sub>2</sub>. Blood gas analyses are performed on samples collected from the left atrium. The pO<sub>2</sub>/FiO<sub>2</sub> (P/F) ratio is calculated. Electrolytes and metabolites are also measured in these samples and the perfusates collected at the start (T0), at 2 hours and 3 hours of EVLP. The lungs are then weighed (post-EVLP lung weight) to estimate total lung water accumulated during EVLP, followed by a third biopsy (post-EVLP biopsy).

### **Acute Lung Injury Profiling**

The parameters used to evaluate the acute lung injury following LPS infusion include the weight of the graft, wet-to-dry weight ratios; the presence of edema on bronchoscopic evaluation; BALF analysis for total protein. These parameters are measured before and after EVLP.

### **Inflammation Profiling during EVLP**

Kinetics of the cytokine response is explored during two different phases: after LPS infusion in the donor pig and during EVLP. Cytokines are measured in the serially collected arterial plasma samples and perfusates. BALF samples that were collected using bronchoscopy before and after EVLP were also centrifuged and used for the analysis of cytokines. Porcine TNFa, IL-6, IL-10, and IL-1β are analyzed using kits from DuoSet® ELISA Development System (R&D Systems Inc., MN, USA).

### **Lung Histology**

Lung tissue biopsies collected in-situ, at the end of 2-hour cold ischemic time and the end of 4 hour-EVLP periods are fixed in neutral buffered 10% formalin solution for 24-48 hours. Biopsies were embedded in paraffin, sectioned at 5-μm thickness, stained with hematoxylin and eosin, and examined under light microscopy for pathologic changes.

### **Statistical Analysis**

Data in the graphs are presented as means  $\pm$  standard deviation (SD). The lung weight of the donors in control and LPS groups was compared using the unpaired t-test. Within the LPS donor group, repeated measures during the observation period were compared to baseline data using the paired t-test. For the data collected during EVLP, a 2-way ANOVA test with repeated measures was used to compare the two groups over time. Statistical analyses were performed using GraphPad Prism software (GraphPad Software Inc, La Jolla, CA, USA), and a *p*-value  $<0.05$  was considered significant.

## Results

### *First phase: LPS – infusion and in vivo evaluation*

#### Hemodynamic changes:

No significant differences were observed between the LPS group and the control group concerning baseline pig weight and ventilation parameters before the LPS infusion. LPS infusion caused hemodynamic instability with an abrupt increase in PAP during the first 10 minutes (**supplementary figure 1**). This was followed by an increase in systolic blood pressure at 10-15 minutes and heart rate at 20-30 minutes. The baseline mean PAP was  $24 \pm 1$  mmHg. The maximum mean PAP ( $52 \pm 2$  mm Hg) was reached at a mean of 25 minutes and then started to decrease slowly and stabilize at  $32 \pm 1$  mmHg by the end of 1 hour of LPS infusion until lung procurement. Systolic blood pressure and heart rate followed the same trend but lagged by 10 to 20 minutes. The hemodynamic changes were accompanied by a sustained decrease in lung oxygenation capacity by the end of LPS infusion and at the procurement of lungs ( $\Delta PO_2$  with  $FiO_2$  of 50%: 272 mmHg at baseline, 230 mmHg at the end of LPS infusion and 165 mmHg just before lung procurement).

#### Inflammation markers

Before LPS infusion, there was no significant difference between the two groups for all measured cytokines. In pigs infused with LPS, changes in circulating cytokines are summarized in **figure 1**. There were rapid increases in the plasma levels of TNF $\alpha$  at 30 minutes. The TNF $\alpha$  level peaked at 60 minutes ( $1464 \pm 252$  vs.  $89 \pm 89$  pg/ml at baseline;  $p < 0.01$ ). IL-10 levels began to increase at 30 minutes. IL-10 levels reached maximum levels at 2 hours ( $2724 \pm 687$  vs.  $18 \pm 12$  pg/ml at baseline;  $p < 0.01$ ). IL-6 showed a gradual increase starting at 90 minutes and continued to rise throughout the observation period ( $2077 \pm 364$  vs.  $0 \pm 0$  pg/ml at baseline;  $p < 0.01$ ). IL-1 $\beta$  did not demonstrate changes.

#### Markers of tissue injury

Compared to control lungs, LPS-injured lungs showed a significantly higher weight gain ( $603 \pm 34$  g vs.  $483 \pm 23$  g;  $p < 0.02$ ), higher in-situ wet to dry weight ratio ( $5.94 \pm 0.09$  g/g vs.  $5.43 \pm 0.13$  g/g;  $p < 0.01$ ), and more proteins in BALF ( $69.4 \pm 15.2$  pg/ml vs.  $26.6 \pm 5.4$  pg/ml in the control group;  $p < 0.01$ ) (**supplementary figure 2**). On bronchoscopy, we found no difference in the presence of edema between the two groups before starting EVLP reperfusion.

## **Second phase: EVLP phase**

### **Hemodynamic and ventilation assessment**

LPS-affected lungs showed significant differences in PAP, PVR, and perfusate flow, starting at 45 minutes after the initiation of reperfusion (**figure 2**). In control lungs, PVR was low and the targeted EVLP flow ( $3.0 \pm 0.2$  L/min) was reached at low PAP ( $14.0 \pm 0.8$  mmHg). In LPS-affected lungs, PVR was high and most LPS-affected lungs did not reach targeted flow ( $2.9 \pm 0.2$  L/min). Ventilation parameters are shown in **figure 3**. LPS-affected lungs showed higher airway pressures after 105 minutes reperfusion ( $p < 0.05$ ). Lung compliance in the LPS-group was lower.

### **Blood gas analysis**

LPS-affected lungs showed worse gas exchange (**figure 4**). The  $\text{pO}_2/\text{FiO}_2$  ratio decreased ( $p < 0.05$ ) during EVLP, with a final result of  $270 \pm 72$  compared to  $499 \pm 36$  mmHg in the control lungs. We found the same pattern ( $p < 0.001$ ) with the pH and bicarbonate levels at the end of EVLP.

### **Pulmonary metabolism assessment**

Glucose and lactate were measured in the perfusates. In control lungs, glucose decreased from  $12.5 \pm 0.2$  mmol/l to  $9.5 \pm 0.4$  mmol/l (**supplementary figure 3**). LPS-lungs showed higher glucose consumption, with perfusate glucose decreasing from  $13.7 \pm 0.3$  mmol/l to  $5.2 \pm 0.5$  mmol/l ( $p < 0.001$ ). Starting at the 2 hours, lactate production by LPS-affected lungs was significantly higher ( $13.3 \pm 0.5$  mmol/l versus  $0.3 \pm 0.0$  mmol/l at baseline) compared to control lungs ( $3.6 \pm 0.5$  mmol/l versus  $0.3 \pm 0.0$  mmol/l at baseline) ( $p < 0.001$ ).

### **Inflammatory markers during EVLP**

We measured cytokines in the perfusate collected at the start of EVLP and then every 30 minutes during the perfusion period (**figure 5**). Lungs from LPS group produce a large amount of TNF $\alpha$  at reperfusion ( $801 \pm 215$  pg/ml vs.  $0 \pm 0$  pg/ml;  $p < 0.01$ ) which reached a maximum after 3 hours of EVLP ( $1561 \pm 132$  pg/ml vs.  $71 \pm 45$  pg/ml;  $p < 0.01$ ). A significant amount of IL-6 was produced during EVLP of LPS lungs, going from  $314 \pm 199$  pg/ml to  $5779 \pm 1091$  pg/ml at the end of EVLP, while no IL-6 was detected in the perfusate from control lungs. In lungs from the LPS group, perfusate IL-10 increased from  $17 \pm 11$  pg/ml to reach a maximum of  $403 \pm 155$  pg/ml, while control lungs showed a small increase from 0 to  $179 \pm 31$  pg/ml. In LPS lungs, IL-1 $\beta$  was not detected in donor plasma. However, reperfusion of lungs showed an increase from 0 to a maximum of  $345 \pm 104$  pg/ml after 3 hours of EVLP, while no change was observed for the control lungs.

We detected inflammatory cytokines from LPS-affected lungs in BALF. TNF $\alpha$ , which was the only cytokine detected in the first BALF ( $561 \pm 201$  pg/ml), continued to increase during EVLP to peak at  $866 \pm 241$  pg/ml. IL-6 was not detected in the first BALF sample but abruptly increased during EVLP to reach a maximum of  $2882 \pm 560$  pg/ml. IL-10 increased from  $9 \pm 5$  p/ml to  $81 \pm 25$  pg/ml ( $p < 0.05$ ) and IL-1 $\beta$

increased from  $3 \pm 3$  pg/ml to  $149 \pm 57$  pg/ml ( $p<0.05$ ). We did not detect any cytokines in BALFs collected from control lungs (**supplementary figure 5**).

### Markers of tissue injury during EVLP

In control lungs, no weight change was observed between the start and the end of EVLP. LPS-lungs started with a higher weight ( $603 \pm 34$  g;  $p<0.001$  compared to control lungs) and continued to gain weight ( $899 \pm 76$  g;  $p<0.01$  compared to pre-EVLP lung weight) (**Figure 6**).

The control lungs had increased wet weight during cold ischemia as measured by the wet/dry weight ratio ( $5.43 \pm 0.13$  g/g *in situ* versus  $5.70 \pm 0.18$  g/g pre-EVLP), but reconditioning caused a significant decrease by the end of EVLP ( $5.17 \pm 0.28$  g/g versus  $5.70 \pm 0.18$  g/g;  $p<0.05$ ) (**Figure 6**). LPS-lungs had higher *in-situ* wet/dry weight ratio ( $5.94 \pm 0.09$  g/g versus  $5.43 \pm 0.13$  g/g in control lungs;  $p<0.05$ ). In the LPS lungs, no significant change in the wet/dry ratio was observed after EVLP.

Both control and LPS lungs showed minimal edema on bronchoscopy before the start of EVLP reperfusion. By the end of EVLP, bronchoscopy revealed massive edema and flooding in the airways in the LPS group, but not in the control group (**supplementary figure 6**).

## Discussion

This study aimed to develop a reproducible large animal model of LPS-induced systemic inflammation and ALI that could mimic the pathogenesis of human sepsis. This model is different than previously described models because it utilizes a cellular perfusate and an open atrial technique. Previous publications showed similar results using cellular and acellular perfusates in humans<sup>13,14</sup>. We sought to validate the use of cellular perfusate with an open atrial system in a large animal model of sepsis induced lung injury. The protocol is divided into two phases; an *in-vivo* phase where sepsis is induced with an LPS infusion and an *ex-vivo* phase, where the lung is procured and placed on EVLP. The present study shows that a model using LPS-induced sepsis yielded an applicable and reproducible platform that can be used to study the effect of sepsis on donor lungs.

During the first phase (*in-vivo* phase), we assessed the model's ability to reproduce sepsis by examining physiologic, histologic, and molecular parameters. We showed that the model reproduces changes associated with sepsis in all three parameters. Physiologic changes are dominated by the development of pulmonary hypertension in the LPS lungs, which was not present in the control group. Histologically, we showed that LPS lungs had increased tissue edema and increased weight during the *in-vivo* phase. Finally, we showed that overproduction of cytokines characterized systemic inflammation in plasma after LPS infusion, mostly TNF $\alpha$ , IL-10, and IL-6. These effects are similar to a more prolonged sepsis model reported by Kubiak et al<sup>15</sup>. Similarly, the same cytokine profile was published in severe human sepsis and acute respiratory distress syndrome in adults by Park<sup>16</sup>. The absence of IL-1 $\beta$  in plasma of pigs after LPS infusion could be expected as previous studies showed no change in IL-1 $\beta$  in humans with severe sepsis<sup>17</sup>.

In the second phase of the study, the ex-vivo phase, we evaluated the lungs using EVLP. We examined donor lungs using physiologic, histologic, and molecular parameters. From a physiologic standpoint, the most striking feature was the increase in PVR that was demonstrated in LPS donor lungs and limited optimal perfusion. In contrast, control lungs had much lower PVR, and optimal perfusion was uniformly reached. In many LPS lungs, target flows were not achieved. The increase in PVR during the ex-vivo phase can be explained by sustained vasoconstriction caused by prolonged action of endothelin, thromboxane and platelet activating factor<sup>18,19</sup>. The net effect of this generalized vasoconstriction in the lungs in the LPS group culminated in hypoxia of cells and deterioration of lung metabolism.

On a molecular level, we observed that cytokines might play an important role in the initiation of lung injury following reperfusion. We noted that during the introduction of perfusion, the cytokine profile is dominated by TNFa, which showed high concentrations in the perfusate and BALF samples. TNFa has been shown to destabilize tight junction proteins and affect cell membrane integrity in pulmonary epithelia, and this may result in pulmonary edema later during reperfusion<sup>20</sup>. Other measured cytokines were also increased by the end of perfusion. In line with these observations, DEVELOP-UK investigators have very recently shown during clinical EVLP that IL-β could be linked to ALI following ischemia-reperfusion<sup>21</sup>. They demonstrated that higher concentrations of IL-1β and TNF-α in perfusate after 30 minutes of perfusion differentiated declined lungs after EVLP from survival lungs.

It should be noted that assessing the effect of EVLP on donor lungs was not a primary aim of our study. Since we do not have a control group that did not undergo EVLP, we can not say whether EVLP does have a therapeutic role in the lungs subjected to sepsis. Instead, we aimed to assess the protocol's ability to reproduce sepsis in a large animal model using EVLP per the Lund protocol. The group from the University of Virginia studied the ability of EVLP to rehabilitate sepsis-induced lung injury<sup>9</sup>. In their protocol, they induced sepsis with an LPS infusion and then randomized pigs to EVLP versus standard procurement and preservation without EVLP. They showed that physiologic and molecular parameters did improve with EVLP. In contrast, we did not note improvements in physiologic or molecular lung parameters with EVLP. Many possibilities may explain the discrepancy in our findings. First, we used a fixed dose of LPS, whereas they used a physiologic determinant to determine the dose given. This may have resulted in a difference in the severity of sepsis in the two studies. Second, we used a cellular prime that contains blood from the donor, whereas they used an acellular priming fluid. Perhaps a cellular prime may further propagate the inflammatory response when compared to an acellular prime. However, previous studies did not show the superiority of acellular perfusate over cellular perfusate<sup>13,14</sup>. Furthermore, a porcine model of extended EVLP up to 24 hours showed an advantage of cellular over acellular perfusates<sup>23</sup>. Both studies were performed in a porcine model with no sepsis. The situation may be different in sepsis-induced lung injury, and perhaps an acellular perfusate may be preferred in this setting. Future applications of this protocol in our laboratory will aim at assessing whether EVLP can be used as a platform to help recondition these lungs, as well as a means to deliver other therapeutic interventions, including ventilatory strategies, molecular targets or other interventions in an attempt to rehabilitate these lungs.

# Conclusions

In summary, we believe that our described model can represent a reliable method of inducing and studying sepsis induced lung injury in donor lungs in an animal model. The model includes analysis of donor lungs on a physiologic, histologic, and molecular level. In the future, this model can be used to study the role of EVLP as a therapeutic tool or as a platform for targeted drug and cellular therapy for the amelioration of lung injury in donor lungs.

## Abbreviations

ABG	Arterial blood gas
ALI	Acute lung injury
BALF	Bronco-alveolar lavage fluid
CO	Cardiac output
EVLP	Ex-vivo lung perfusion
IL-1B	Interleukin 1 beta
IL-6	Interleukin 6
IL-10	Interleukin 10
LPS	Lipopolysaccharide
MAP	Mean arterial pressure
PAP	Pulmonary artery pressure
P/F	PaO <sub>2</sub> /FiO <sub>2</sub>
PVR	Pulmonary vascular resistance
TNF <sub>a</sub>	Tumor necrosis factor alpha
ABG	Arterial blood gas
ALI	Acute lung injury
BALF	Bronco-alveolar lavage fluid
CO	Cardiac output

EVLP	Ex-vivo lung perfusion
IL-1B	Interleukin 1 beta
IL-6	Interleukin 6
IL-10	Interleukin 10
LPS	Lipopolysaccharide
MAP	Mean arterial pressure
PAP	Pulmonary artery pressure
P/F	PaO <sub>2</sub> /FiO <sub>2</sub>
PVR	Pulmonary vascular resistance
TNF <sub>a</sub>	Tumor necrosis factor alpha

## Declarations

*Ethics approval and consent to participate:* The experimental protocol was approved by the institutional ethical committee for animal research (CIPA # 4I17025PFp) in compliance with the Canadian Council on Animal Care and the National Institute of Health's Guide for the Care and Use of Laboratory Animals.

*Consent for publication:* Not applicable

*Availability of data and materials:* Data generated or analysed during this study are included in this published article. The datasets that are not available in the manuscript are available from the corresponding author on reasonable request

*Competing interests:* The authors declare that they have no competing interests

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*Authors' contributions:* All authors (BSN, AM, CL, JFG, PL, SDS, ML, JFC, CD, NN, PF) made substantial contributions to the conception and design of the experimental protocol

BSN, AM, CL, JFG, SDS, ML, NN and PF participated in performing the experiments and data acquisition

All authors (BSN, AM, CL, JFG, PL, SDS, ML, JFC, CD, NN, PF) contributed to data analysis and interpretation of data;

All authors (BSN, AM, CL, JFG, PL, SDS, ML, JFC, CD, NN, PF) have approved the submitted version (and any substantially modified version that involves the author's contribution to the study) and agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

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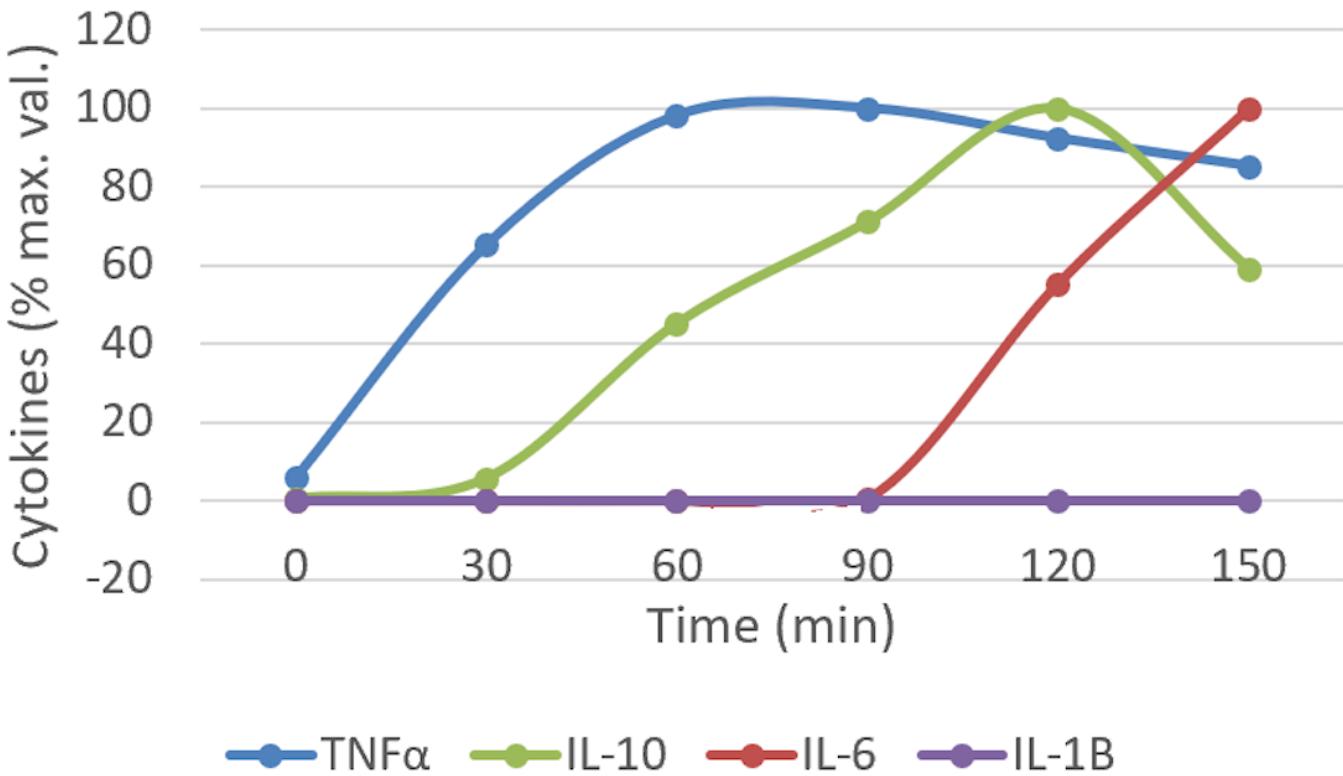
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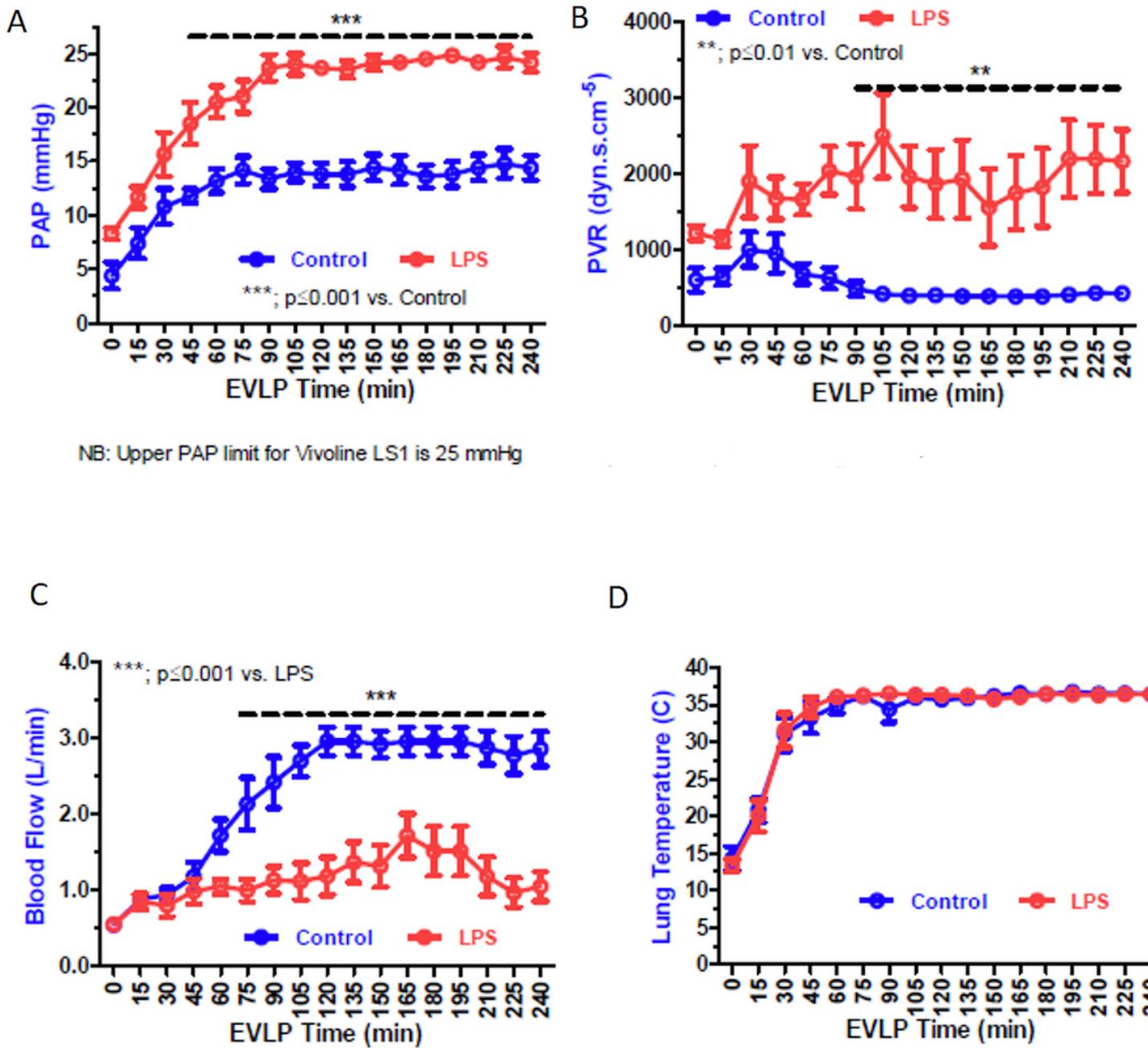
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## Figures



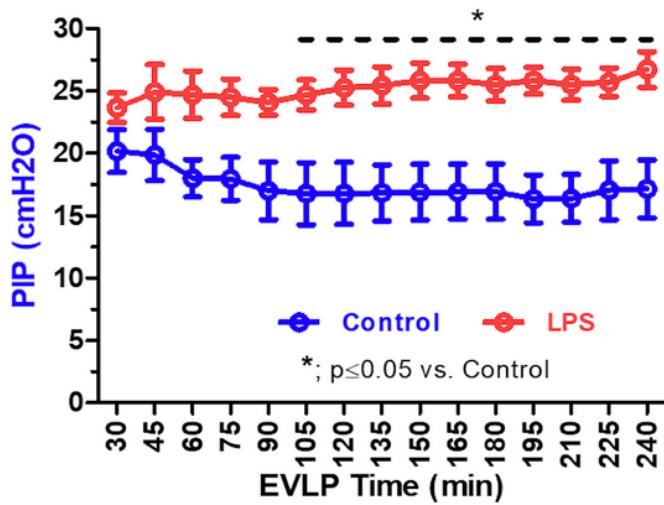
**Figure 1**

Inflammatory profile during the induction of acute lung injury in pigs. Cytokines measured in blood samples collected at baseline and every 30 minutes. Data are presented in percent of the maximum value for each cytokine. The maximum cytokine values (considered 100%) are reported in the figure

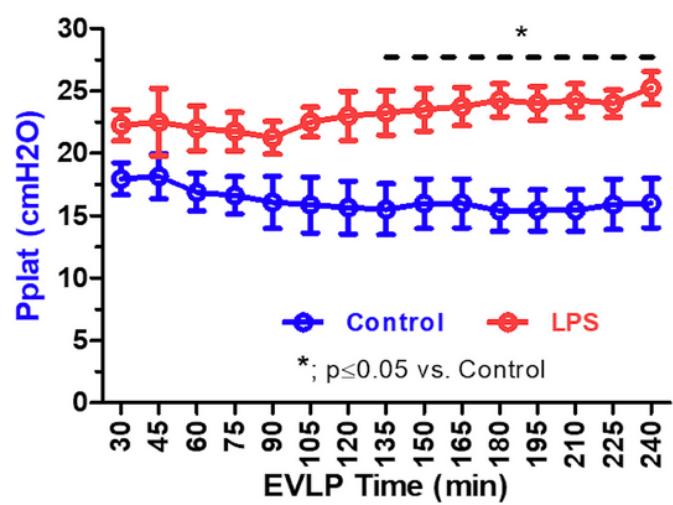


**Figure 2**

Hemodynamic parameters monitored continuously and averaged every 15 minutes over 4 hours of EVLP



NB: Upper airway pressure limit (30 cmH<sub>2</sub>O)



NB: Upper airway pressure limit (30 cmH<sub>2</sub>O)

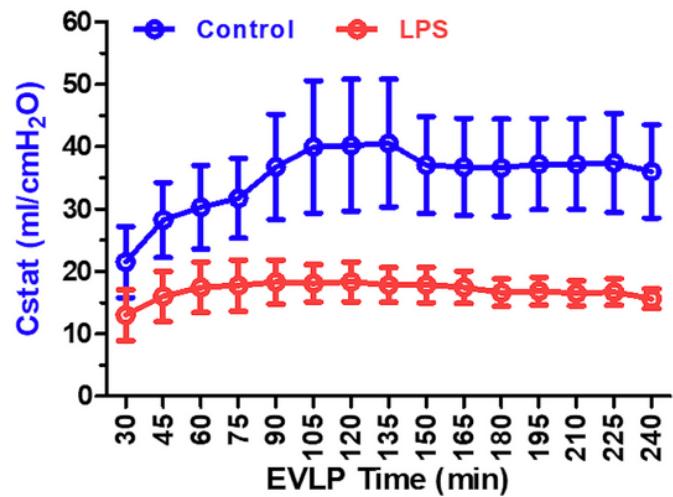
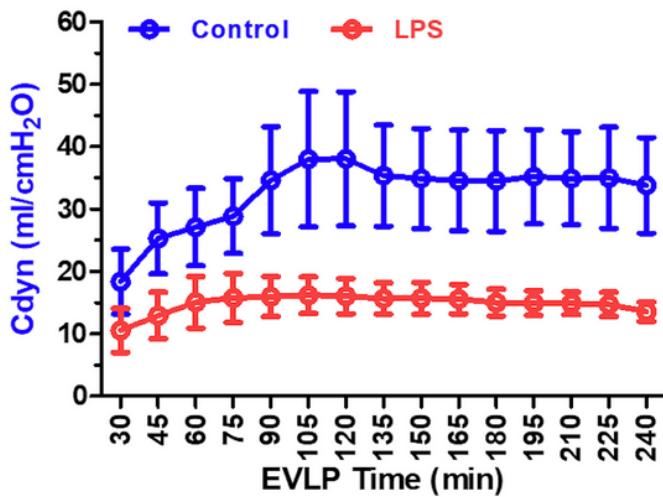
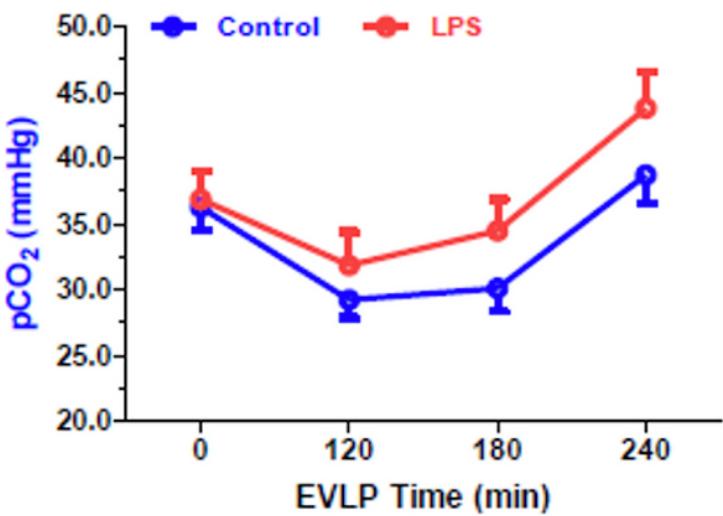
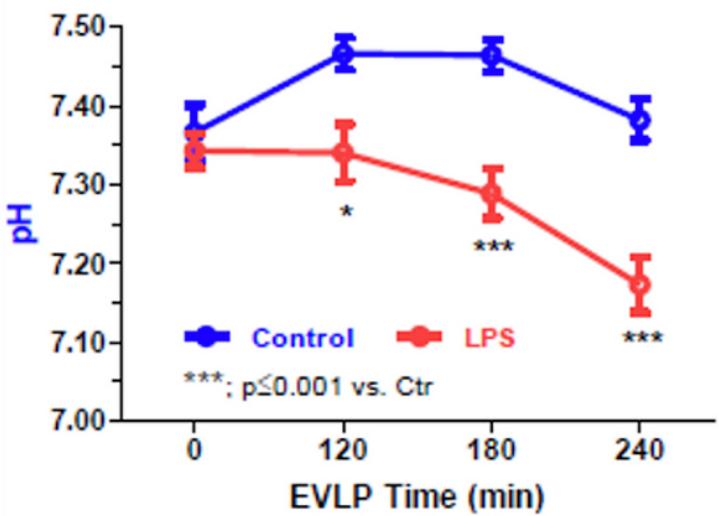


Figure 3

Ventilatory parameters during EVLP



Ctr: Control

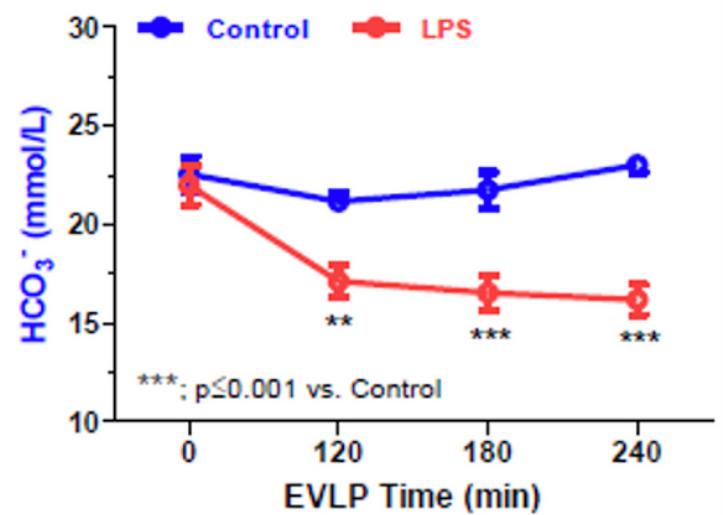
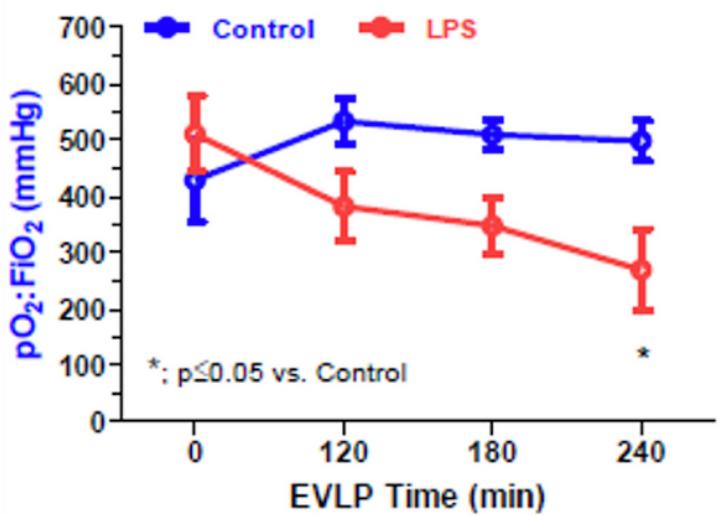
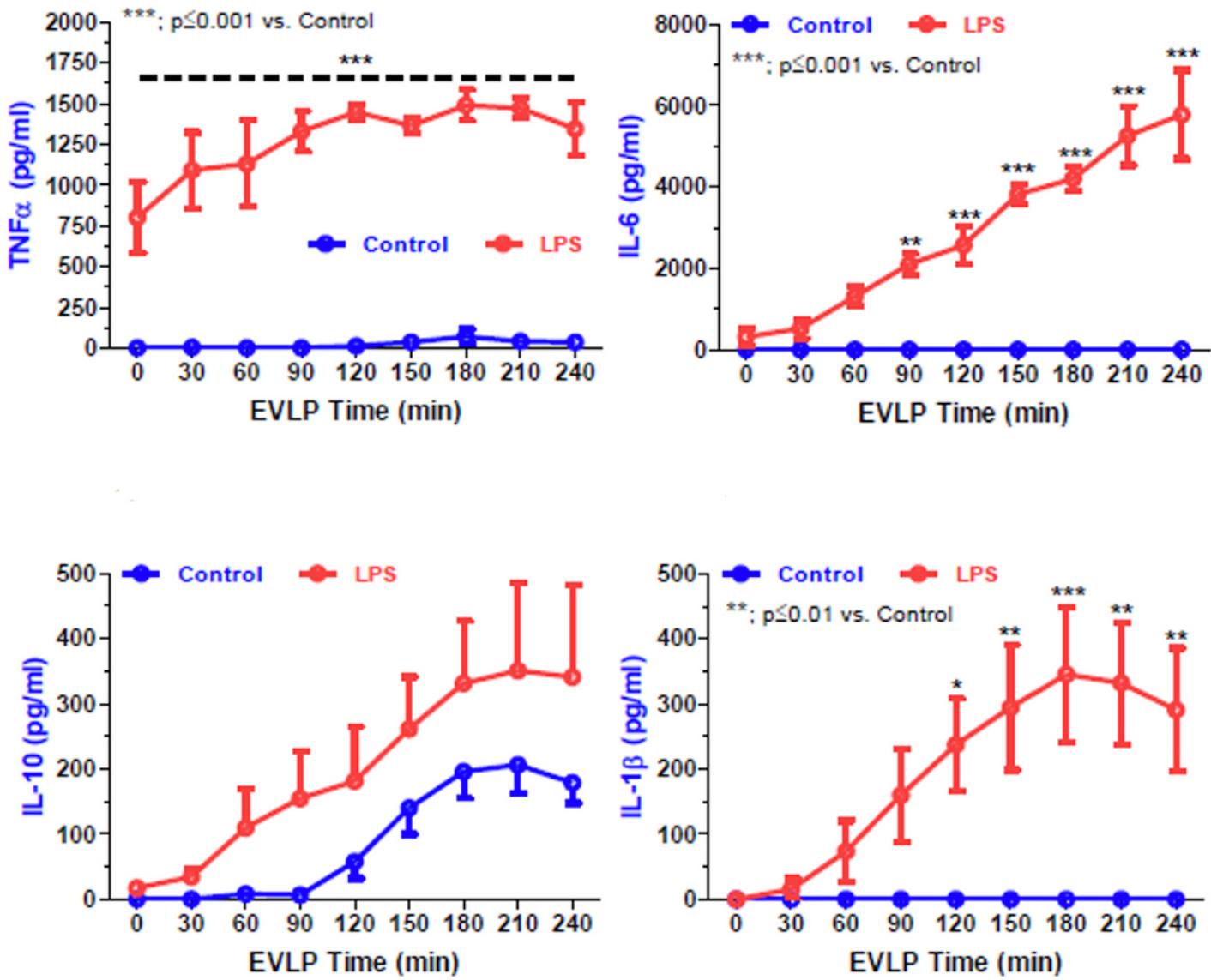


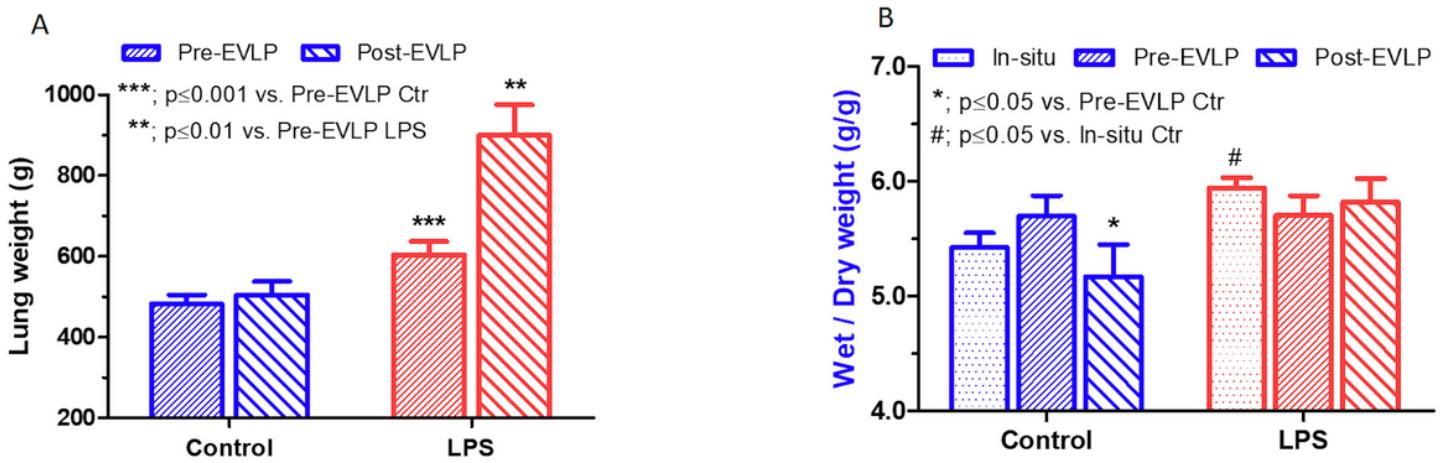
Figure 4

ABG's measured at the beginning of EVLP and then after 2, 3 and 4 hours. Oxygenation of the lungs is expressed as pO<sub>2</sub>:FiO<sub>2</sub> ratio.



**Figure 5**

Inflammatory profile during EVLP. Cytokines measured in the perfusate collected at the beginning and then every 30 minutes during 4 hours of EVLP.



**Figure 6**

Markers of acute lung injury after EVLP

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supp2.tif
- supp6.tif
- suppl5.tif
- supp4.tif
- suppFigure1.tif
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