Extracorporeal Gas Exchange and Spontaneous Breathing for the Treatment of ARDS:

an Alternative to Mechanical Ventilation?

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SUPPLEMENTAL DIGITAL CONTENT

The opinions or assertions contained herein are the private views of the authors, and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.
ADDITIONAL METHODS

Sheep were housed under standard environmental conditions (air-conditioned room 20°C, 50% relative humidity) for at least 1 week prior to each study. Animals were fed standard chow and hay and fasted for 24 hours with free access to water before the experiments.

Anesthesia, instrumentation and ECGE connection

Anesthesia was induced with an intramuscular injection of 6 mg/kg of tiletamine-zolazepam (Telazol, Fort Dodge, IA) and 0.01 mg/kg of buprenorphine hydrochloride (Buprenex, Reckitt Benckiser, Richmond, VA) followed by Isoflurane 2-4% delivered via mask. Subsequently, sheep were orotracheally intubated in the prone position with an 8 mm ID endotracheal tube. A balloon catheter (Ackrad Labs, Cooper Surgical, CT) was placed through a nostril in the lower part of the esophagus and secured (E1). A Foley catheter (16 Ch) was placed transurethrally in the bladder. Sheep were thereafter moved to the Computed Tomography (CT) room for chest image acquisition (see below). Thereafter, sheep were instrumented in supine position. A tracheostomy was performed (Shiley, Single Cannula Low Pressure Cuffed, 10 mm ID). Next, the right carotid artery was surgically exposed and cannulated with a 5 Fr Catheter (Arrow International, Reading, PA). Sheath introducers (8.5 Fr) (Arrow International, Inc., Reading, PA) were percutaneously placed both in the right and left internal jugular veins. After a bolus (150 U/kg) of unfractionated heparin (APP Pharmaceuticals, LCC Schamburg, IL) a bicaval dual-lumen catheter (Avalon®, Maquet Cardiopulmonary, Rastatt, Germany) was placed as previously described (E2) and connected to the ECGE system which was primed with Lactated Ringer’s solution to avoid dilution related acid-base alterations (E3).

Surgical incisions were infiltrated with bupivacaine 2% (Hospira, Lake Forest, IL). Animals were then turned prone, moved to the metabolic cage and allowed to recover from anesthesia. Mechanical ventilatory support (Evita XL, Dräger Medical, Lübeck, Germany) was progressively
reduced. Sheep were kept on continuous positive airway pressure (CPAP) of 8 cmH\textsubscript{2}O with FiO\textsubscript{2} of 0.5. Blood flowing through the membrane lung was actively heated at a constant temperature of 38°C (Medi-Therm II, Gaymar Industries, Pittsburgh, PA). Unfractionated heparin was infused as needed to achieve an Activated Clotting Time (ACT) > 160 seconds (Hemochron Junior, International Technidyne, Piscataway, NJ). Sedation and analgesia were performed with i.m. buprenorphine 0.01 mg/kg (administered every 4-6 hours) and an infusion of 0.05-0.20 mg/kg/h of midazolam (Hospira, Lake Forest, IL). For each sheep, at the beginning of the study, an appropriate level of sedation was found. Thereafter the rate of infusion of midazolam was kept constant throughout the study.

*Management of sheep during the experiment*

Lactated Ringer’s solution was infused at a rate of 200 ml/h during instrumentation and between 150-200 ml/h during the study. During ARDS induction (see below), boluses of 250 ml of lactated Ringer’s (on average 750 ml) were administered to avoid hypotension and to assure an even distribution of the acid (see below). Moreover, after the injury, the rate of lactated Ringer’s was usually increased to 250-300 ml/min to avoid severe hypovolemia due to the massive plasma leakage caused by oleic acid.

*Pressure-Volume Curve measurement*

At baseline, i.e. with healthy lungs, static inspiratory respiratory system and lung pressure-volume (PV) curves were recorded and analyzed with a dedicated software (Pulmodyne, Hugo Sachs Elektronics, March-Hugstetten, Germany). Deeply anesthetized, non paralyzed sheep, placed in the prone position, were pre-oxygenated, shortly hyperventilated through a recruitment maneuver (20 breaths in Pressure-controlled mode with inspiratory pressure of 30 cmH\textsubscript{2}O, PEEP 0 cmH\textsubscript{2}O, respiratory rate of 10 and a 1:1 ratio of inspiration to expiration) and disconnected from the ventilator for complete expiration up to functional residual capacity (FRC). A glass syringe was
connected to the endotracheal tube and room air was inflated in steps of 100 ml. Airway pressure (Paw) and esophageal pressure (Pes) were recorded at the end of each step until Paw of ~40 cmH$_2$O. Animals were then disconnected from the syringe to permit complete exhalation. Thereafter mechanical ventilation was restarted.

Individual respiratory system and lung PV curves were constructed by plotting Paw variations and transpulmonary (Paw-Pes) pressure variations versus the volume of inflated gas. Individual curves were fitted with the most appropriate equation and a mean pressure-volume curve of the whole study population was obtained averaging the volumes computed at pressure intervals of 0.5 cmH$_2$O using individual fittings as previously described (E4).

**Measurements of esophageal pressure variations**

During the study, at each sweep gas flow setting, a measurement of esophageal pressure variation ($\Delta P_{es}$), (estimate of pleural pressure variation [$\Delta P_{pl}$]) was performed. The esophageal balloon, previously introduced through a nostril (correct position checked on CT-scan) was inflated with 1 ml of air and connected to a differential pressure transducer (MPX, 399/2, Hugo Sachs Elektroniks, March-Hugstetten, Germany). The waveform was recorded for 3 minutes and an average of 6 pressure variations was computed.

**Cytokine measurement**

Blood was drawn at baseline, right after induction of anesthesia and at the end of study before anesthesia was induced again. Samples were anticoagulated with citrate 3.2% (Vacutainer, BD, USA) and centrifuged at 3000 x g for 10 minutes at a controlled temperature of 4°C. Plasma was collected, aliquoted, flash frozen and stored at -80 °C for subsequent analysis. Prior to analysis samples were brought to room temperature. Plasma concentrations of IL-1 beta (MBS734810, Mybiosource, San Diego, CA), TNF-alpha (MBS735607, Mybiosource, San Diego, CA) IL-6 (MBS738671, Mybiosource, San Diego, CA), IL-8 (MBS743903, Mybiosource, San Diego, CA)
and IL-10 (MBS704435, Mybiosource, San Diego, CA) were measured with sheep specific enzyme-linked immunosorbent assay (ELISA) kits.

**Computed Tomography and lung quantitative analysis**

CT scans (Toshiba Aquilion 64-slice Medical System, Tustin, CA) were acquired with the following setting: tube current 60 mAs, voltage 120 kVp, pitch factor 0.85, collimation 0.5x32 mm, rotation time 0.5 seconds. The entire lung was imaged. Images were reconstructed at 5 mm thickness with an interval of 5 mm. Chest CT was performed at 0 and at 30 cmH2O of airway pressure by clamping the endotracheal tube during an expiratory and inspiratory hold, respectively (Servo 300, Siemens Elema, Solna, Sweden). Immediately before each CT scan acquisition a recruitment maneuver was performed (20 breaths in Pressure-controlled mode with inspiratory pressure of 30 cmH2O, PEEP 0 cmH2O, respiratory rate of 10 and a 1:1 ratio of inspiration to expiration).

Lung images were analyzed with a dedicated image-analysis software (Maluna, Göttingen, Germany), as previously described (E5). Briefly, lung boundaries were semi-automatically drawn on each baseline image and manually drawn on each CT image acquired at the end of the study. Hilar vessels and main bronchi were excluded from the analysis. The frequency distribution of CT numbers (expressed in Hounsfield Units (HU)) was thereafter analyzed by the software and lung compartments were quantified according to different degrees of aeration, applying usual thresholds adopted in literature (E6). Non-aerated lung tissue (density between -100 and +200 HU); poorly-aerated lung tissue (density between -101 and -500); normally-aerated lung tissue (density between -501 and -900) and hyper-inflated lung tissue (density between -901 and -1000) were therefore quantified.

The total lung volume was calculated as follows:

\[
\text{Lung volume}_{\text{tot}} = \text{Number of voxels} \times \text{Volume of voxel}
\]

Eq. 1
Where “Lung volume \([\text{tot}]\)” defines the total volume of the lung (expressed in milliliters), “Number of voxels” represents the total number of voxels included in the regions of interest and “Volume of voxel” represents the volume of the voxel (0.001953 ml).

The volumes of the differently aerated lung compartments (hyperinflated, normally aerated, poorly aerated and non aerated) were computed similarly:

\[
\text{Lung volume}_{\text{compartment}} = \text{Number of voxels}_{\text{compartment}} \times \text{Volume of voxel}
\]  
Eq. 2

Where “Lung volume \([\text{compartment}]\)” represents the volume of a specific compartment (expressed in milliliters) and “Number of voxels \([\text{compartment}]\)” represents the number of voxels included in the considered compartment.

The total lung tissue mass (Lung weight) was calculated as follows:

\[
\text{Lung tissue mass}_{\text{tot}} = \text{Lung volume}_{\text{tot}} \times \left(1 - \frac{\text{CT mean}}{-1000}\right)
\]  
Eq. 3

Where “Lung tissue mass \([\text{tot}]\)” represents the total mass of the lung (expressed in grams), “Lung volume \([\text{tot}]\)” represents the total lung volume (expressed in milliliters) (see Equation 1) and “CT mean” represents the mean CT number of the whole lung (expressed in Hounsfield Units).

The mass of tissue of the four different lung compartments was computed as:

\[
\text{Tissue mass}_{\text{compartment}} = \text{Lung volume}_{\text{compartment}} \times \left(1 - \frac{\text{CT mean}_{\text{compartment}}}{-1000}\right)
\]  
Eq. 4

Where “Tissue mass \([\text{compartment}]\)” represents the mass of tissue of a specific compartment (expressed in grams), “Lung volume \([\text{compartment}]\)” represents the lung volume of the considered compartment
(see Equation 2) and “CT\text{mean \ [compartment]}” represents the mean CT number of the considered compartment (expressed in Hounsfield Units).

**Calculation of physiological variables**

The following equations were used for the computation of physiological variables.

*Measurements of gas exchange across the membrane lung*

Throughout the study, gas concentrations of CO\textsubscript{2} entering and exiting the membrane lung were analyzed (VMax Encore 29 system, VIASYS Healthcare Inc, Yorba Linda, CA). Sweep Gas Flow (L/min) was set with a flowmeter/air-oxygen mixer (Model 3500 CP-G, Sechrist, Anaheim, CA); FiO\textsubscript{2} was set at 0.5 and kept constant throughout the study to allow accurate measurement of extracorporeal CO\textsubscript{2} (V\textsubscript{M}CO\textsubscript{2}) via indirect calorimetry (E7). The accuracy of the flowmeter was periodically checked (S-110 Flo Meter, McMillan Co., Georgetown, TX, USA). The additional gas outlet port of the respiratory membrane (HLS Module Advanced 7.0, Maquet, Cardiopulmonary AG, Hirrlingen, Germany) was sealed in order to avoid gas leakage.

The amount of CO\textsubscript{2} removed by the membrane lung (V\textsubscript{M}CO\textsubscript{2}) was calculated as reported in equation 5 and expressed in ml/min.

\[
V_MCO_2 = (F_ECO_2 - F_ICO_2) \times VE
\]

Eq. 5

Where \( F_ECO_2 \) and \( F_ICO_2 \) are respectively the fractions of CO\textsubscript{2} of the gas exiting and entering the membrane (expressed as percentages), VE is the Gas Flow ventilating the membrane expressed at standard temperature pressure dry (STPD) in L/min.
Oxygen delivery through the membrane lung ($V_{M}O_2$) was computed as described in equation 6 and expressed in ml/min.

$$V_{M}O_2 = (C_{postO_2} - C_{preO_2}) \times BF$$  \hspace{1cm} \text{Eq. 6}$$

Where $C_{postO_2}$ represents the oxygen content of the blood exiting the membrane lung (see equation 7); $C_{preO_2}$ represents the oxygen content of the blood entering the membrane lung (see equation 8) and BF represents the blood flow through the membrane lung.

$$C_{postO_2} = [Hb] \times 1.39 \times S_{postO_2} + [P_{postO_2} \times 0.003]$$  \hspace{1cm} \text{Eq. 7}$$

Where $[Hb]$ is concentration of hemoglobin expressed in g/dL, $S_{postO_2}$ is the oxygen Saturation of Hemoglobin in the blood exiting the membrane lung, $P_{postO_2}$ is the partial pressure of oxygen in the blood exiting the membrane lung.

$$C_{preO_2} = [Hb] \times 1.39 \times S_{preO_2} + [P_{preO_2} \times 0.003]$$  \hspace{1cm} \text{Eq. 8}$$

Where footnote \textit{pre} defines blood entering the membrane lung.

\textit{Measurements of gas exchange across the native lung}

The amount of CO$_2$ removed by the native lung ($V_{L}CO_2$) was measured with the CO$_2$SMO plus (CO$_2$SMO Model 8100, Novametrix Medical System Inc., Wallingford, CT) and expressed as ml/min.
The oxygen uptake through the native lung ($V_{L, O_2}$) was calculated from the Fick equation as described below:

$$V_{L, O_2} = Q \times (CaO_2 - CvO_2) \times 10$$  \hspace{1cm} \text{Eq. 9}

Where $V_{L, O_2}$ is expressed in ml/min; $Q$ represents cardiac output (L/min) measured via thermodilution (Vigilance II, Edwards Life Science, Irvine, CA); $CaO_2$ is the oxygen content of arterial blood (see equation 10); $CvO_2$ is the oxygen content of mixed venous blood (see equation 11) and 10 is the factor to convert oxygen content from ml/dL to ml/L. Of note, for cardiac output assessment (average of three consecutive measurements) BF pumped through the membrane lung was temporarily set to 0 L/min in order to avoid overestimation by indicator loss into the extracorporeal circuit (E8).

The oxygen content of arterial blood ($CaO_2$) was computed as:

$$CaO_2 = [Hb] \times 1.39 \times SaO_2 + [PaO_2 \times 0.003]$$  \hspace{1cm} \text{Eq. 10}

Where footnote $a$ represents arterial blood.

The oxygen content of mixed venous blood ($CvO_2$) was calculated as:

$$CvO_2 = [Hb] \times 1.39 \times SvO_2 + [PvO_2 \times 0.003]$$  \hspace{1cm} \text{Eq. 11}

Where footnote $v$ represents mixed venous blood.
The total amount of removed CO₂ was calculated as described in equation 12.

\[ V_{TOT} \, CO_2 = V_M \, CO_2 + V_L \, CO_2 \]  

Eq. 12

Where \( V_{TOT} \, CO_2 \) is the total amount of CO₂ removed expressed in ml/min. \( V_M \, CO_2 \) is the amount of CO₂ removed by the membrane lung (as described in equation 6) and \( V_L \, CO_2 \) is the amount of CO₂ removed by the native lung as measured by the CO2SMO.

Accordingly, the percentage amount of CO₂ removed by the membrane lung (\( V_M \, CO_2 \% \)) was calculated as follows:

\[ V_M \, CO_2 \% = \frac{V_M \, CO_2}{V_{TOT} \, CO_2} \times 100 \]  

Eq. 13

The total amount of oxygen uptake (\( V_{TOT} \, O_2 \)) was calculated as:

\[ V_{TOT} \, O_2 = V_M \, O_2 + V_L \, O_2 \]  

Eq. 14

As \( V_M \, O_2 \) was not measured for every step (see Table E1), but did not change significantly between different steps, an average was calculated for each sheep in each study phase and used for the computation of \( V_{TOT} \, O_2 \).

Physiologic dead space fraction (\( \frac{Vd}{V_t} \, phys \)) was calculated as reported in equation 15.

\[ \frac{Vd}{V_t} \, phys = \frac{PaCO_2 - PECO_2}{PaCO_2} \]  

Eq. 15
Where $\text{PaCO}_2$ is the arterial partial pressure of carbon dioxide and $\text{PECO}_2$ is the mixed expired partial pressure of carbon dioxide measured with the CO$_2$SMO (Novametrix Medical System Inc., Wallingford, CT). Physiologic dead space is expressed as a fraction of tidal volume.

Minute alveolar ventilation ($V_{alv}$) was calculated as expressed in equation 16:

$$V_{alv} = V_{tot} \times \left(1 - \frac{Vd}{Vt} \text{phys} \right)$$

Eq. 16

Where $V_{tot}$ is minute ventilation expressed in L/min measured by the ventilator (Evita XL, Dräger Medical, Lübeck, Germany) and $\frac{Vd}{Vt} \text{phys}$ is the physiologic dead space calculated as described in equation 15.

Percentage decrease in minute ventilation ($\Delta MV$) was computed as:

$$\Delta MV = \frac{MV \text{control} - MV \text{actual}}{MV \text{control}} \times 100$$

Eq. 17

Where $MV \text{ control}$ is the minute ventilation measured at 0 L/min of GF and $MV \text{ actual}$ is the minute ventilation measured at the time point, i.e. with various amounts of $V_M\text{CO}_2$. The $\Delta MV$ is expressed as percentage of control minute ventilation. Percentage decrease in alveolar ventilation ($\Delta V_{alv}$) was calculated similarly.

Pulmonary Shunt fraction ($\dot{Q}_s/\dot{Q}_t$) was calculated as described in equation 18.

$$\frac{\dot{Q}_s}{\dot{Q}_t} = \frac{CcO_2 - CaO_2}{CcO_2 - CvO_2}$$

Eq. 18
Where \( \text{CaO}_2 \) and \( \text{CvO}_2 \) are the contents of oxygen for arterial and mixed venous blood (Equations 10 and 11) and \( \text{CcO}_2 \) is the oxygen content of capillary blood, calculated as:

\[
\text{CcO}_2 = [\text{Hb}] \times 1.39 \times 1 + [\text{PAO}_2 \times 0.003]
\]

Eq. 19

Where \( \text{PAO}_2 \) is the alveolar partial pressure of oxygen expressed in mmHg and calculated as:

\[
\text{PAO}_2 = \text{FiO}_2 \times (\text{Patm} - 47) - \frac{\text{PaCO}_2}{\text{RQ}}
\]

Eq. 20

Where \( \text{Patm} \) is the atmospheric pressure, 47 is water vapor pressure, \( \text{PaCO}_2 \) is the partial pressure of \( \text{CO}_2 \) in arterial blood and \( \text{RQ} \) is the respiratory quotient as calculated in Equation 21.

\[
\text{RQ} = \frac{\dot{V}_L\text{CO}_2}{\dot{V}_L\text{O}_2}
\]

Eq. 21

**Induction of ARDS**

Buprenorphine 0.01 mg/kg was injected i.m. approximately 30 minutes before oleic acid injection; \( \text{FiO}_2 \) of inspiratory gas of the ventilator was increased to 1 and the infusion rate of Lactated Ringer’s was increased to 1000 ml/h in order to favor the distribution of oleic acid (E9) and to avoid the abrupt hypotension during the injury due to the pulmonary chemoreflex (E10, E11). Fifteen ml of blood were withdrawn from the arterial line into a 20 ml syringe and heparinized with 300 U of heparin. Oleic acid (0.1 ml/kg) (cis-9-Octadecenoic acid, Sigma-Aldrich) was added to the syringe and emulsified by vigorous shaking (E12). The emulsion was thereafter injected through the proximal port of the Swan-Ganz catheter into the right atrium in boluses of 2.5 ml. After each injection the catheter was flushed with 15 ml of normal saline. The total amount of 20 ml was injected over a period of 20-25 minutes. Blood flow of the ECGE was set at 0 L/min during the injection of the emulsion to avoid the entrance of undiluted, highly concentrated oleic acid into the membrane. If \( \text{PaO}_2 \) did not drop below 200 mmHg while on \( \text{FiO}_2 \) of 1 (with GF = 0 L/min) after 90
minutes from the end of injection, time at which edema formation was shown to be complete (E13), an additional dose of 0.05ml/kg was administered as described above. Measurements were started once PaO$_2$ dropped below 200 and 90 minutes had passed from the last oleic acid injection.

As oleic acid is known to bind to calcium and therefore reduce plasma calcium concentration (E14), calcium was replaced with calcium chloride (0.5 g) in case ionized calcium dropped below 0.95 mmol/L.

**Statistical Analysis**

Data are expressed as mean ± standard deviation unless otherwise specified.
ADDITIONAL FIGURES AND TABLES

Figure E1.

Figure E1. Schematic representation of the experimental timeline divided in the most relevant study phases. For more details see the Materials and Methods section. Definition of abbreviations: CT = Computed Tomography; PV = Pressure Volume Curve of the respiratory system; Lab exams = collection of blood samples for laboratory examination; Cytokines = collection of plasma samples for subsequent cytokine analysis.
**Figure E2.** Mean frequency distribution of CT numbers of scans performed on healthy sheep expressed as percentage of tissue mass and grouped into intervals of 50 HU. Data are presented as mean ± standard deviation. Black bars represent results obtained at 0 cmH₂O of airway pressure (functional residual capacity), while gray bars represent results obtained during an inspiratory hold performed at 30 cmH₂O of airway pressure (considered close to total lung capacity). Vertical dashed lines define different lung compartments as described in the “Computed Tomography and lung quantitative analysis” section in the additional methods.
**Figure E3.** Mean frequency distribution of CT numbers of scans performed on animals with ARDS expressed as percentage of tissue mass and grouped into intervals of 50 HU. Data are presented as mean ± standard deviation. Black bars represent results obtained at 0 cmH₂O of airway pressure (functional residual capacity), while gray bars represent results obtained during an inspiratory hold performed at 30 cmH₂O of airway pressure (considered close to total lung capacity). Vertical dashed lines define different lung compartments as described in the “Computed Tomography and lung quantitative analysis” section in the additional methods.
Figure E4. Relationship between esophageal pressure variations and tidal volume in healthy sheep (Panel A) and in sheep with ARDS (Panel B) during varying amounts of extracorporeal CO$_2$ removal.

Panel A: $V_T = 124.7 + 33.3 \Delta Pes$, $r^2 = 0.31$, $p<0.001$; Panel B: $V_T = 36.2 + 7.3 \Delta Pes$, $r^2 = 0.54$, $p<0.001$; The test for equality of slope and intercept showed a significant difference for slopes ($p<0.001$) and intercepts ($p=0.04$).
Table E1. Timeline of measurements

<table>
<thead>
<tr>
<th>Gas Flow$^+$ [L/min]</th>
<th>Respiratory Variables</th>
<th>Hemodynamic Variables</th>
<th>$V_M CO_2$, $V_L CO_2$, $V_L O_2$</th>
<th>$V_M O_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>*</td>
<td>*</td>
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<tr>
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<tr>
<td>10</td>
<td>*</td>
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</tbody>
</table>

Table E1. Table summarizes the timeline of performed measurements. Gas Flow was set between 1 and 10 L/min (FiO$_2$ = 0.5) in randomized order. Every two steps, i.e. approximately every 60-90 minutes gas flow was set at 0 L/min to perform control measurements, i.e., measurements without extracorporeal gas exchange. Respiratory variables included respiratory rate, tidal volume, minute ventilation and esophageal pressure variation; Hemodynamic variables included heart rate, systemic and pulmonary arterial pressure, central venous pressure, pulmonary wedge pressure and cardiac output. Gas exchange and metabolic data of the native lung ($V_L CO_2$, $V_L O_2$) + extracorporeal CO$_2$ removal ($V_M CO_2$) were measured or calculated at every timepoint (see Equations 5 and 9), while oxygen delivery thorough the membrane lung ($V_M O_2$) was calculated (as described in Equation 6) only at predefined timepoints. The same measurements in the same order were performed both at baseline, i.e. with healthy lungs and after the induction of ARDS. § The first 5 sheep performed only 5 steps of GF, i.e. GF of 1 L/min was not tested. † The sequence of GF settings was randomly decided for each sheep.
Table E2. Hemodynamic variables.

All data were recorded with stable, spontaneously breathing animals and no extracorporeal gas exchange. Comparison was performed with the paired t-test.

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>Healthy</th>
<th>ARDS</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure [mmHg]</td>
<td>107 ± 19</td>
<td>102 ± 16</td>
<td>0.33</td>
</tr>
<tr>
<td>Mean pulmonary pressure [mmHg]</td>
<td>16 ± 3</td>
<td>21 ± 6</td>
<td>0.003</td>
</tr>
<tr>
<td>Heart rate [bpm]</td>
<td>154 ± 37</td>
<td>148 ± 26</td>
<td>0.67</td>
</tr>
<tr>
<td>Central venous pressure [mmHg]</td>
<td>2 ± 5</td>
<td>-2 ± 5</td>
<td>0.03</td>
</tr>
<tr>
<td>Pulmonary wedge pressure [mmHg]</td>
<td>7 ± 3</td>
<td>4 ± 4</td>
<td>0.03</td>
</tr>
<tr>
<td>Cardiac output [L/min]</td>
<td>6.3 ± 1.1</td>
<td>4.6 ± 1.5</td>
<td>0.006</td>
</tr>
<tr>
<td>Systemic vascular resistance [dyn*s/cm⁵]</td>
<td>1410 ± 478</td>
<td>2093 ± 770</td>
<td>0.02</td>
</tr>
<tr>
<td>Pulmonary vascular resistance [dyn*s/cm⁵]</td>
<td>121 ± 42</td>
<td>300 ± 131</td>
<td>0.002</td>
</tr>
</tbody>
</table>
Table E3. Blood Laboratory Examinations

<table>
<thead>
<tr>
<th>Blood Laboratory Examinations</th>
<th>Baseline</th>
<th>ARDS</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin [g/dL]</td>
<td>9.2 ± 1.1</td>
<td>12.0 ± 1.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Haematocrit [%]</td>
<td>29 ± 4</td>
<td>37 ± 4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Platelets [10^3/μL]</td>
<td>492 ± 266</td>
<td>928 ± 1237</td>
<td>0.90</td>
</tr>
<tr>
<td>White Blood Cells [10^3/ml]</td>
<td>4.1 ± 1.3</td>
<td>1.0 ± 1.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Neutrophils [%]</td>
<td>47 ± 11</td>
<td>58 ± 18</td>
<td>0.08</td>
</tr>
<tr>
<td>Lymphocytes [%]</td>
<td>47 ± 9</td>
<td>37 ± 19</td>
<td>0.14</td>
</tr>
<tr>
<td>Monocytes [%]</td>
<td>2 ± 2</td>
<td>4 ± 5</td>
<td>0.08</td>
</tr>
<tr>
<td>Eosinophils [%]</td>
<td>6 ± 5</td>
<td>1 ± 1</td>
<td>0.002</td>
</tr>
<tr>
<td>Basophiles [%]</td>
<td>1 ± 1</td>
<td>3 ± 3</td>
<td>0.01</td>
</tr>
<tr>
<td>Sodium [mEq/L]</td>
<td>144 ± 4</td>
<td>155 ± 8</td>
<td>0.001</td>
</tr>
<tr>
<td>Potassium [mEq/L]</td>
<td>3.6 ± 0.3</td>
<td>4.4 ± 2.4</td>
<td>0.63</td>
</tr>
<tr>
<td>Chloride [mEq/L]</td>
<td>106 ± 3</td>
<td>117 ± 7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Calcium [mg/dL]</td>
<td>8.7 ± 0.5</td>
<td>8.0 ± 0.5</td>
<td>0.002</td>
</tr>
<tr>
<td>Phosphorus [mg/dL]</td>
<td>7.1 ± 1.2</td>
<td>7.9 ± 1.5</td>
<td>0.14</td>
</tr>
<tr>
<td>Blood Urea Nitrogen [mg/dL]</td>
<td>19 ± 4</td>
<td>15 ± 7</td>
<td>0.05</td>
</tr>
<tr>
<td>Creatinine [mg/dL]</td>
<td>0.9 ± 0.2</td>
<td>1.0 ± 0.3</td>
<td>0.12</td>
</tr>
<tr>
<td>Albumin [g/dL]</td>
<td>1.3 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose [mg/dL]</td>
<td>72 ± 17</td>
<td>93 ± 29</td>
<td>0.02</td>
</tr>
<tr>
<td>Total Bilirubin [mg/dL]</td>
<td>0.1 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.07</td>
</tr>
<tr>
<td>Gamma-Glutamyl Transferase [U/L]</td>
<td>75 ± 14</td>
<td>64 ± 13</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alkaline Phosphatase [U/L]</td>
<td>201 ± 64</td>
<td>149 ± 36</td>
<td>0.004</td>
</tr>
<tr>
<td>Alanine Aminotransferase [U/L]</td>
<td>25 ± 4</td>
<td>42 ± 17</td>
<td>0.002</td>
</tr>
<tr>
<td>Aspartate Aminotransferase [U/L]</td>
<td>97 ± 20</td>
<td>213 ± 110</td>
<td>0.03</td>
</tr>
<tr>
<td>Creatine Kinase [U/L]</td>
<td>432 ± 135</td>
<td>3135 ± 1542</td>
<td>0.006</td>
</tr>
<tr>
<td>Prothrombin time [sec]</td>
<td>11.6 ± 0.3</td>
<td>14.7 ± 2.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Activated partial thromboplastin time [sec]</td>
<td>30.4 ± 4.7</td>
<td>48.6 ± 14.9</td>
<td>0.004</td>
</tr>
<tr>
<td>D-dimer [ng/mL]</td>
<td>330 ± 260</td>
<td>420 ± 310</td>
<td>0.18</td>
</tr>
</tbody>
</table>

Table E3. Table summarizes the results of blood chemistry performed at baseline and the end of the study (after the induction of ARDS via intravenous oleic acid injection). All data are expressed as mean ± standard deviation. Comparison was performed via paired t-test.
ADDITIONAL REFERENCES


