Extracorporeal CO_2 removal enhanced by lactic acid infusion in spontaneously breathing conscious sheep

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SUPPLEMENTAL DIGITAL CONTENT

Additional Methods

Instrumentation

The animals were endotracheally intubated after an intramuscular injection (i.m.) of glycopyrrolate (100 µg/kg) and tiletamine-zolazepam (6 mg/kg). During surgical preparation, general inhalation anesthesia was maintained with isofluorane (MAC 1.0-1.5) and analgesia was obtained by administering an intravenous (i.v.) bolus of Fentanyl 5 µg/kg, followed by continuous infusion at 5 µg/kg/hr. A Foley catheter was inserted to monitor urinary output. Surgical preparations included the following. The left carotid artery was percutaneously cannulated with a 16G pediatric central venous catheter (Arrow International, Reading, PA) for pressure monitoring and blood gas analyses. An 8.5-F sheath introducer (Arrow International, Reading, PA) was placed into the left external jugular vein, for i.v. administration of medications and to facilitate the placement of a Swan-Ganz catheter (Edwards Lifescience, Irvine, CA) which was used to monitor central venous, pulmonary artery and wedge pressures, cardiac output, and core temperature and for sampling of mixed venous blood. Surgical tracheostomy was performed using a cuffed adult tracheostomy tube (10mm, Shiley, Covidien, Boulder, CO). Antibiotic prophylaxis (ceftriaxone 2 g) i.v. was administered. A dual-lumen catheter (Hemolung, Alung, Alung Technologies, Pittsburgh, PA) 15.5-F was percutaneously introduced in the right external jugular vein after i.v. bolus of unfractioned heparin (UFH, 100 UI/kg). Subsequently heparin was infused continuously to maintain activated clotting time (ACT) at approximately 150% of the baseline values. ACT was measured with Hemochron Signature Elite (ITC, Edison, NJ, USA).

Experimental Design

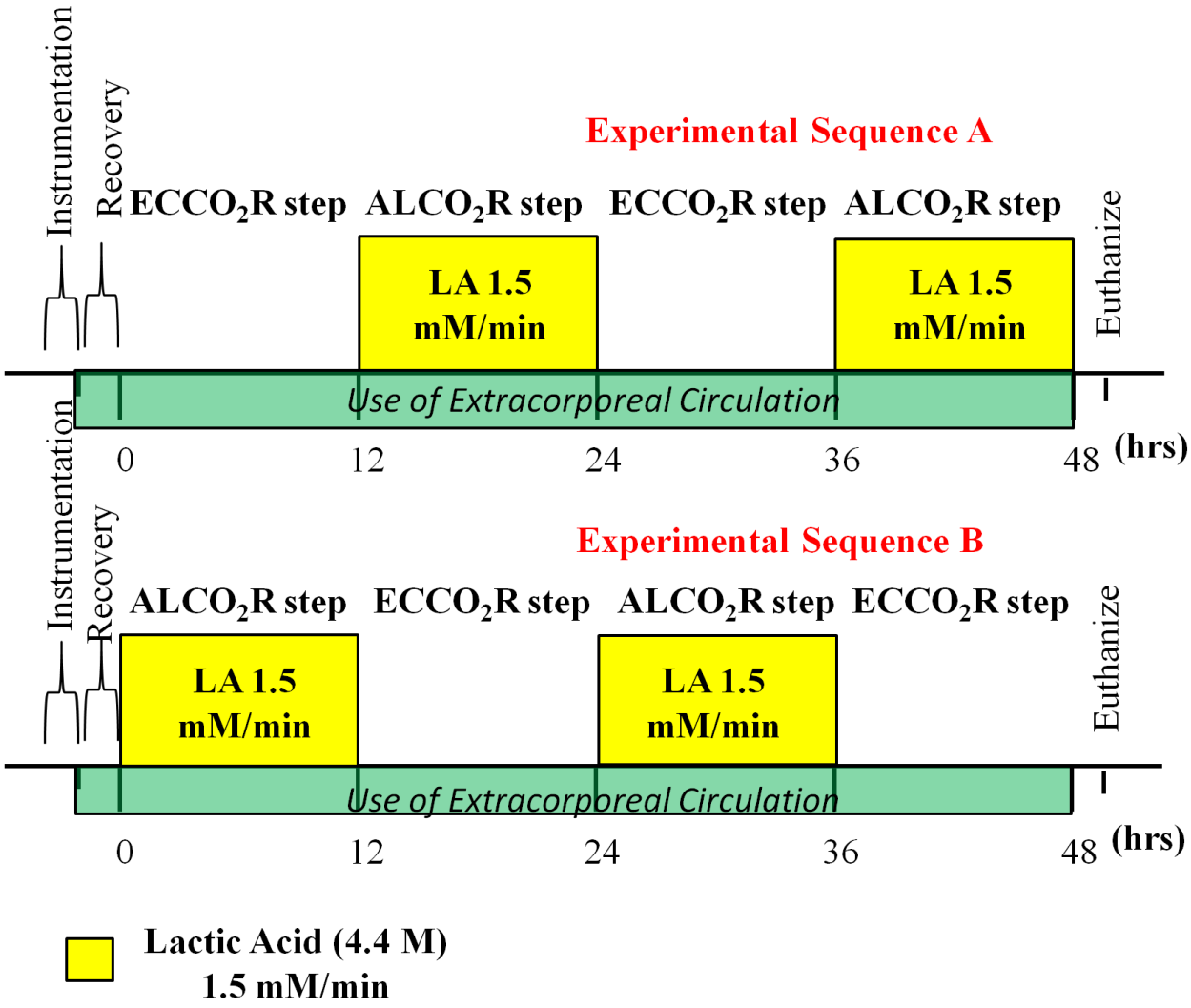


Figure S1. Experimental design. LA, lactic acid.

Each of the animals underwent 2 repetitions of the experimental sequences consisting of an $ALCO_2R$ phase and a standard $ECCO_2R$ phase. $ALCO_2R$ and $ECCO_2R$ phases were always alternated and lasted 12 hours each. To counterbalance any order effect of the experimental design, 3 animals started the experiment with a standard $ECCO_2R$ phase (i.e. experimental sequence A), while 3 started with an $ALCO_2R$ phase (i.e. experimental sequence B).

Equations A (Ventilatory Function calculation):

Knowing membrane lung (ML) CO_2 removal (VCO_2ML) at body temperature pressure saturated (BTPS) conditions, Alveolar ventilation (AV) as well as physiologic dead space (V_d) were calculated using standard equation,[7] as follows:

$$Expired\ CO_2\ fraction = FeCO_2 = \frac{VCO_2NL\ (L/min)}{MV(L/min)}$$

$$Partial\ Pressure\ of\ CO_2\ in\ expired\ gases = PeCO_2(mmHg) = FeCO_2 \times 760\ mmHg$$

$$Dead\ Space\ Fraction = \frac{Vd}{Vt} = \frac{(PaCO_2(mmHg) - PeCO_2(mmHg))}{PaCO_2(mmHg)}$$

$$Physiologic\ Dead\ Space\ (mL) = \frac{(PaCO_2(mmHg) - PeCO_2(mmHg))}{PaCO_2(mmHg)} \times TV\ (mL)$$

$$Alveolar\ Ventilation = AV(L/min) = MV(L/min) - MV(L/min) \times \frac{Vd}{Vt}$$

Once collected arterial and mixed venous arterial blood gas analyses as well as measured cardiac output (CO) by thermodilution technique, venous admixture (Qs/Qt) was calculated using standard equation,[7] as follows:

$$Venous\ Admixture = \frac{Qs}{Qt} = \frac{Cc\ (mL/dL) - Cv\ (mL/dL)}{Cc\ (mL/dL) - Ca\ (mL/dL)}$$

Where Ca is the arterial content of oxygen calculated as:

$$Ca\ (mL/dL) = \left(0.0031\ \left(\frac{mL/dL}{mmHg}\right) \cdot P_{art}O_2(mmHg) + 1.39\ (mL/gr) \cdot Hb\ (gr/dL) \cdot Sat_{art}O_2\ (\%)\right);$$

Cv is the mixed venous content of oxygen, calculated as follows:

$$Cv\ (mL/dL) = \left(0.0031\ \left(\frac{mL/dL}{mmHg}\right) \cdot P_{ven}O_2(mmHg) + 1.39\ (mL/gr) \cdot Hb\ (gr/dL) \cdot Sat_{ven}O_2(\%)\right);$$

and Cc is the pulmonary capillary content of oxygen, calculated as follows:

$$Cc\ (mL/dL) = \left(0.0031\left(\frac{mL/dL}{mmHg}\right) \cdot P_{ALV}O_2(mmHg) + 1.39\ (mL/gr) \cdot Hb(gr/dL)\right).$$

Where $P_{ALV}O_2$ is the alveolar partial pressure of oxygen, calculated as follows:

$$P_{ALV}O_2 = (713\ x\ FiO_2) - \frac{PaCO_2(mmHg)}{RQ}$$

Where 713 is the atmospheric pressure, 47 is water vapor pressure, $PaCO_2$ is the partial pressure of CO_2 in arterial blood and RQ is the respiratory quotient, calculated as the ratio of carbon dioxide production (VCO_2tot) and oxygen consumption (VO_2tot).

Equations B (Membrane Lung CO_2 removal efficiency calculation):

Once measured membrane lung CO_2 removal (VCO2ML, mL/min), extracorporeal blood flow (BF, L/min) and circuit inlet total content of CO_2 (Inlet *c*tCO_2, mL/dL), membrane lung CO_2 removal efficiency was computed as previously described,[8] as follows:

$$\text{MLCO}_2 \text{ Efficiency (\%)} = \frac{\text{VCO}_2\text{ML } (mL/min)}{\text{Inlet } c\text{tCO}_2 (mL/L) \times BF (L/min)}$$

Where Inlet *c*tCO_2 was total blood CO_2 content at the inlet of the ML and was calculated according to a simplified and standardized Henderson-Hesselbach equation formula, which is commonly used and approved for blood gas analyzers calculations,[9] as the sum of the bicarbonate ions concentration and dissolved CO_2 obtained by the *p*CO_2 and CO_2 solubility coefficient (αCO_2 = 0.03 mMol × L-1 × mmHg-1). Therefore, total CO_2 content is calculated as follows:

$$\text{Inlet } c\text{tCO}_2 = \text{Inlet cHCO}_3^- + (\alpha\text{CO}_2 \times \text{Inlet } p\text{CO}_2)$$

Equations C (Oxygen Delivery and Consumption calculation):

ML Oxygen delivery (VO_2ML) at BTPS conditions was calculated as follows:

$$VO_2ML\ (mL/min) = [(0.0031 \cdot P_{Outlet}O_2(mmHg) + 1.39 \cdot Hb(g/dL) \cdot Sat_{Outlet}O_2(\%)) - (0.0031 \cdot P_{Inlet}O_2(mmHg) + 1.39 \cdot Hb(g/dL) \cdot Sat_{Inlet}O_2(\%))] \times BF\ (L/min) \times 10$$

Natural lung Oxygen delivery (VO_2NL) at BTPS conditions was calculated as follows:

$$VO_2NL\ (mL/min) = [(0.0031 \cdot P_{Art}O_2(mmHg) + 1.39 \cdot Hb(g/dL) \cdot Sat_{Art}O_2(\%)) - (0.0031 cP_{MixedVenous}O_2(mmHg) + 1.39 \cdot Hb(g/dL) \cdot Sat_{MixedVenous}O_2(\%))] \times CO(L/min) \times 10$$

Accordingly, oxygen consumption (VO_2tot) at BTPS conditions was calculated as:

$$VO_2tot\ (mL/min) = VO_2ML(mL/min) + VO_2NL(mL/min)$$

Equations D (Energetic Expenditure calculation):

Knowing carbon dioxide production (VCO_2tot) and oxygen consumption (VO_2tot), energetic expenditure (EE) was calculated using Weir Equation, as previously described,[10] as follows:

$$Energy\ Expenditure\ (kCal/hr) = EE = (3.94 \times VO_2tot\ (mL/min) + 1.11 \times VCO_2tot\ (mL/min)) \times 0.06$$

Post-mortem histological evaluation

Lung, heart, liver and renal tissue samples were collected for histological evaluation post-mortem. The tissue samples were fixed in neutral-buffered 10% formalin for 24 hrs, trimmed, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (H&E). Histological images were recorded with 10, 20, and 40× objective under a light microscope (AX80; Olympus, Center Valley, Pa). Histological evaluation of injury was performed by a single pathologist blinded to the identity of the animal represented on the slide, as previously documented.[1]

Five parameters (alveolar fibrin edema, alveolar hemorrhage, septal thickening, intra-alveolar inflammation and vasculitis), four parameters (edema, degeneration, inflammatory cell infiltration, congestion), and four parameters (vascular congestion/thrombosis, cellular death, cellular degeneration, inflammation) were as criteria for pulmonary, cardiac, and hepatic as well as kidney injury respectively. Tissue injury was assessed on each H&E stained slide for: 1) severity of injury (0: absent; 1, 2, 3, and 4 for more severe changes), and 2) extent of injury.

Oxidative Stress and Inflammation Analysis

Lung, heart and liver tissues from the sheep were homogenized in 50 mM potassium phosphate buffer, pH 7.4. Malondialdehyde concentrations in these tissues, as an index of lipid peroxidation, were determined as thiobarbituric acid reactive substances (TBARs) in the butanol phase using 1,1,3,3-tetraethoxypropane as standard, as described by the method of Naito, et al.[2] Ferric reducing ability of lung, heart and liver, as an index of their overall antioxidant status, was determined spectrophotometrically by the method of Benzie and Strain.[3] Reduced glutathione (GSH) were determined enzymatically as described by Anderson.[4] Myeloperoxidase activity in lung was determined by a modification of the method of Trush, et al.[5] Briefly, tissues were homogenized in 50 mM potassium phosphate buffer pH 6.0 containing 0.5% hexadecyltrimethlammonium bromide. The homogenates then underwent 3 freeze-thaw cycles and sonification, followed by incubation at 60^{o} C in a water bath for 2 hr to extract myeloperoxidase and reduce interfering substances. Samples were then centrifuged at 10,000 x g for 30 min at 4^{o} C. Myeloperoxidase activity was determined in the resultant supernatant using o-dianisidine as substrate. Total nitric oxide concentrations in the tissues were determined by a commercial kit (Stressgen, Victoria, BC, Canada). Tissue levels of IL-1β and IL-8 were determined by ELISA using commercial kits (R&D Systems, Minneapolis, MN). Tissue protein concentrations were determined with a commercial kit (Pierce, Rockford, IL).

Healthy controls were obtained from a previous study performed in our laboratory.[11] In that study, 5 healthy neutered male sheep (*Ovis aeries*, Rambouillet X) with mean weight 30 kg, underwent similar instrumentation as in our study (i.e. general

anesthesia, tracheostomy, central venous cannulation, arterial cannulation, pulmonary artery cannulation). After a time period of 52 hours (i.e. as in our study), sham-animals were euthanatized with an i.v. injection of 20 mL of Fatal Plus (Vortech Pharmaceuticals, Dearborn, MI) and lung, heart, liver and renal tissue were collected for tissue inflammatory responses, concentration of selected indices of oxidative stress and biochemical markers of injury. Measurement of thiobarbituric acid reactive substances (TBARs), interleukin-1β (IL-1β), and nitric oxide concentration, determined reduced glutathione (GSH), ferric reducing ability in the homogenate of lung, heart and liver tissue were performed with the aforementioned techniques in the same laboratory. Myeloperoxidase activity was measured in lung homogenate. Interleukin-8 (IL-8) was measured only in homogenate of lung and liver.

Additional Results

Fig. E1

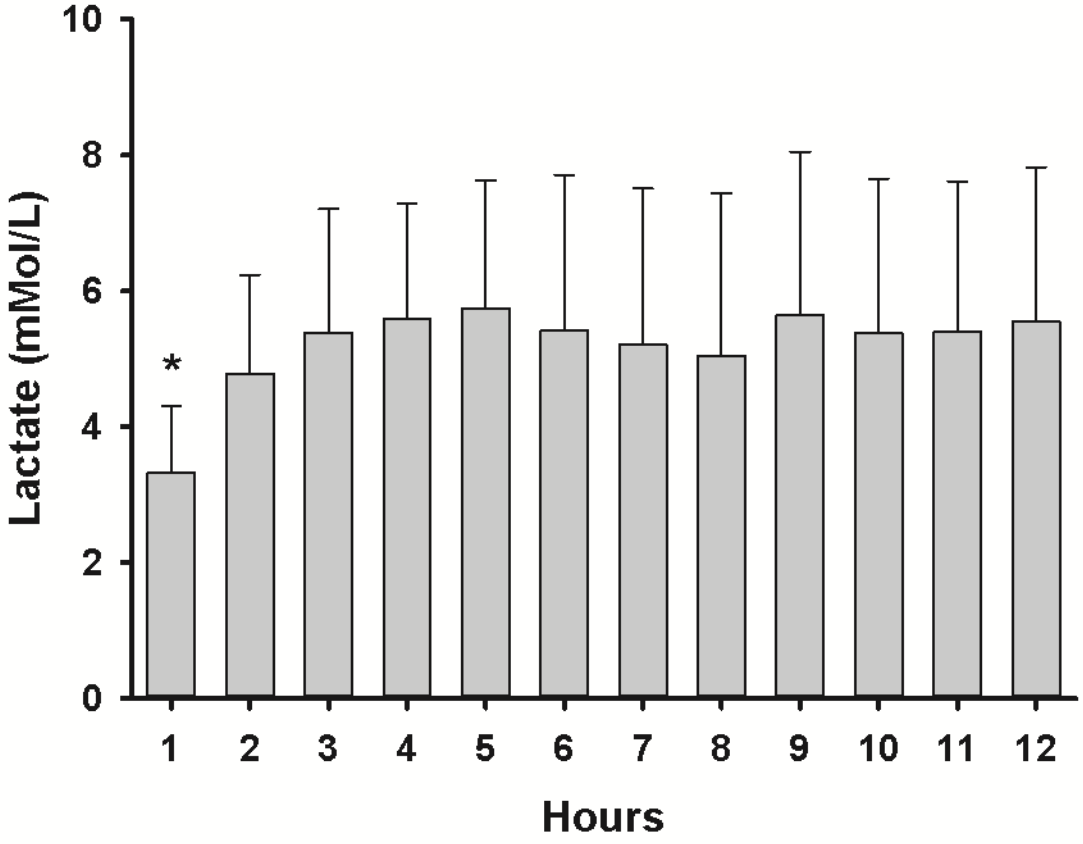


Figure S2. Time dependent variation of lactate concentration during $ALCO_2R$. *) $p < 0.001$ versus all the other time points.

Table S1. Hemodynamics parameters during $ECCO_2R$ and $ALCO_2R$.

	$ECCO_2R$	$ALCO_2R$	*P* value
T°c	39.7 ± 0.5	39.8 ± 0.6	< 0.001
HR (bpm)	118 ± 21	124 ± 22	0.006
MAP (mmHg)	98 ± 9	99 ± 10	0.206
CVP(mmHg)	8 ± 3	7 ± 3	0.065
PAOP (mmHg)	15 ± 4	15 ± 4	0.096
CO (L/min)	7.2 ± 1.5	7.6 ± 1.5	0.023
SvO_2 (%)	72.9 ± 5.1	71.7 ± 5.9	0.167
Hct (%)	26.7 ± 4.7	26.3 ± 4.5	0.067

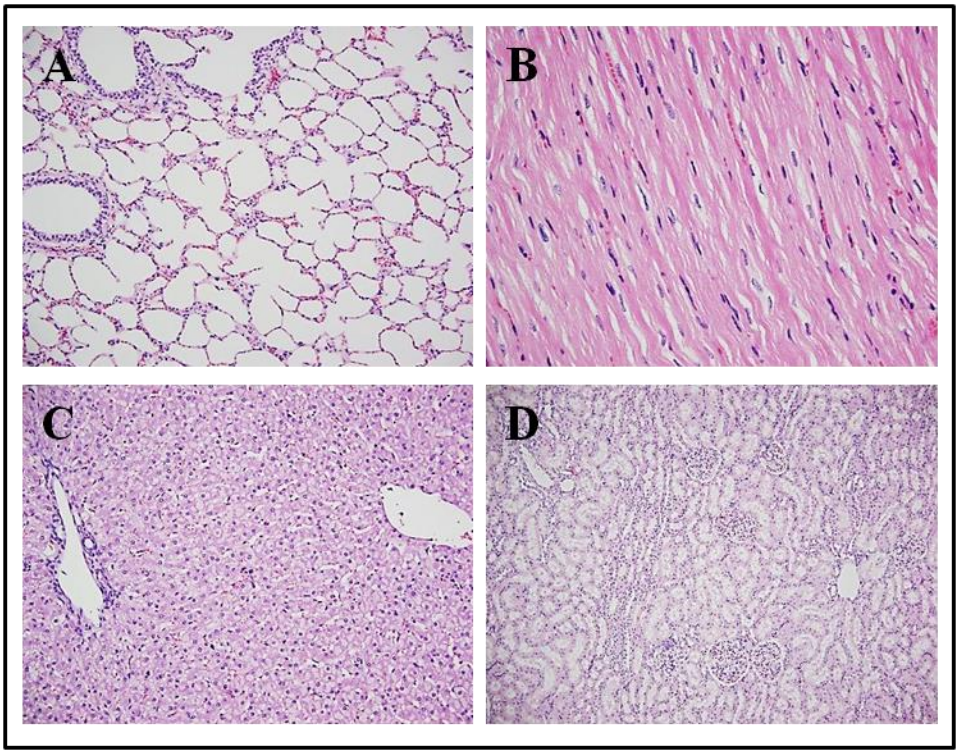
Core temperature (T°c), Heart Rate (HR), Mean Arterial Pressure (MAP), Central Venous Pressure (CVP), Pulmonary Artery Occlusion Pressure (PAOP), Cardiac Output (CO), Mixed Venous Saturation (SvO_2), Hematocrit (Hct).

Table S2. Blood chemistry during different experimental phases.

	BL	EC	$ECCO_2R$	$ALCO_2R$
PFHb (mg/dL)	9.6 (4.4 - 9.8)	3.4 (3.1 - 4.8)	7.0 (4.3 - 8.2)	6.7 (4.0 - 8.4)
WBC (K/µL)	3.8 (3.3 - 4.2)	6.2 (4.3 - 9.2)*	8.4 (5.2 - 9.2)*	7.7 (6.0 - 9.1)*
NEU (K/µL)	1.0 (0.5 - 2.2)	4.0 (2.5 - 7.7)*	5.4 (2.7 - 6.0)*	4.5 (3.1 - 6.0)*
LYM (K/µL)	2.6 (1.2 - 3.1)	1.1 (0.6 - 2.9)	2.1 (1.7 - 3.1)	2.6 (1.7 - 3.0)
RBC (M/µL)	8.6 (7.8 - 8.9)	7.7 (6.2 - 8.1)*	7.3 (6.9 - 8.0)*	7.2 (6.0 - 7.9)*
Hct (%)	29.6 (27.5 – 32.2)	26.9 (22.1 – 30.6)*	26.9 (23.4 – 30.2)*	26.7 (22.1 – 28.7)*
PLT (K/µL)	478 (163 - 694)	317 (124 - 398)*	182 (139 - 292)*	167 (116 - 257)*
PT (sec)	11.8 (11.4 - 14.0)	13.5 (12.6 - 13.9)	12.0 (11.7 - 12.7)	12.5 (11.8 - 13.6)
PTT (sec)	28.0 (23.7 - 45.7)	86.7 (70.4 - 103.6)*	64.6 (46.7 - 81.2)*	66.1 (62.5 - 105.1)*
FBG (mg/dL)	257 (134 - 378)	141 (112 - 211)	303 (223 - 510)	492 (227 - 567)
DD (mg/L)	0.27 (0.17 - 0.51)	0.23 (0.17 - 0.32)	0.19 (0.17 - 0.29)	0.21 (0.18 - 0.27)
CREA (mg/dL)	0.70 (0.65 - 0.85)	0.80 (0.70 - 0.95)	0.70 (0.60 - 0.80)	0.75 (0.63 - 0.90)
BIL (mg/dL)	0.02 (0.01 - 0.06)	0.09 (0.02 - 0.20)§	0.00 (0.00 - 0.00)	0.00 (0.00 - 0.00)†
ALT (IU/L)	25 (20 - 27)‡,§	24 (24 - 29)‡,§	34 (26 - 38)*,†	30 (26 - 38)*,†
AST (IU/L)	87 (72 - 111)	125 (108 - 156)*	140 (113 - 179)*	134 (118 - 166)*
AMYL (IU/L)	17 (9 - 21)	20 (14 - 23)	10 (5 - 17)	9 (7 - 13)
URCA (mg/dL)	0.1 (0.1 - 0.2)	0.1 (0.1 - 0.2)	0.1 (0.1 - 0.2)	0.1 (0.1 - 0.2)
MYOG (ng/mL)	1.0 (0.6 - 3.0)	1.0 (1.0 - 3.0)	1.0 (1.0 - 2.0)	1.5 (1.0 - 2.8)
GLU (mg/dL)	56 (47 - 85)§	73 (57 - 90)*	67 (61 - 69)§	75 (73 - 78)*, ‡

Baseline before instrumentation (BL), after 6 hours of extracorporeal circulation (EC), after 12 hours of $ECCO_2R$ ($ECCO_2R$), after 12 hours of $ALCO_2R$ ($ALCO_2R$). Data is describe as median and interquartile range. Plasma Free Hemoglobin (PFHb), White Blood Cells (WBC), Neutrophils (NEU), Lymphocytes (LYM), Red Blood Cells (RBC), Hematocrit (Hct), Platelets (PLT), Prothrombin Time (PT), Partial Thromboplastin Time (PTT), Fibrinogen (FBG), D-Dimers (DD), Creatinine (CREA), Total Bilirubin (BIL), Alanine Transaminase (ALT), Aspartate Transaminase (AST), Amylase (AMYL), Myoglobin (MYOG), Uric Acid (URCA). Tukey's post-hoc analysis: *) $p < 0.05$, vs. BL, †) $p < 0.05$, vs. EC, ‡) $p < 0.05$, vs. $ECCO_2R$, §) $p < 0.05$, vs. $ALCO_2R$.

Figure S3. Post-mortem histological evaluation.



Formalin-fixed tissues were embedded in paraffin, sectioned, and stained with hematoxylin-eosin. Histological images were obtained under a light microscope. A, Lung. Representative micrograph shows normal lung structure characterized by thin alveolar septa with occasional intra-alveolar macrophages and very few neutrophils, (magnification x200). B, Heart. Representative micrograph demonstrates normal myocardial structures, (magnification x400). C, Liver. Representative micrograph displays normal hepatic lobule, central vein, and portal triads without pathological alteration. (magnification x200). D, Kidney. Representative micrograph exhibits intact renal corpuscles and tubules without obvious vascular congestion, inflammation, and cellular death/degeneration (magnification x100).

Table S4. Histological evaluation of end organs.

Tissue	Animal										
Lung		Alveolar Fibrin Edema	Extent	Alveolar Hemorrhage	Extent	Septal Thickening	Extent	Alveolar Inflammation	Extent	Vasculitis	Extent
	1	0	0%	0	0%	0	0%	0	0%	0	0%
	2	0	0%	0	0%	0	0%	0	0%	0	0%
	3	0	0%	0	0%	0	0%	0	0%	0	0%
	4	0	0%	0	0%	2	25-50%	2	25-50%	1	<25%
	5	0	0%	0	0%	0	0%	0	0%	0	0%
	6	0	0%	0	0%	0	0%	0	0%	0	0%
Heart		Edema	Extent	Degeneration	Extent	Inflammatory cell infiltration	Extent	Congestion	Extent		
	1	0	0%	0	0%	0	0%	0	0%		
	2	1	<25%	1	<25%	0	0%	0	0%		
	3	1	<25%	1	<25%	0	0%	0	0%		
	4	0	0%	0	0%	0	0%	0	0%		
	5	0	0%	0	0%	0	0%	0	0%		
	6	1	<25%	1	<25%	0	0%	0	0%		
Liver		Vascular Congestion	Extent	Cellular Death	Extent	Cellular Degeneration	Extent	Inflammation	Extent		
	1	0	0%	0	0%	0	0%	0	0%		
	2	0	0%	0	0%	1	<25%	1	<25%		
	3	0	0%	0	0%	1	<25%	1	<25%		
	4	0	0%	0	0%	0	0%	0	0%		
	5	0	0%	0	0%	0	0%	0	0%		
	6	0	0%	0	0%	0	0%	0	0%		
Kidney		Vascular Congestion	Extent	Cellular Death	Extent	Cellular Degeneration	Extent	Inflammation	Extent		
	1	0	0%	0	0%	0	0%	0	0%		
	2	0	0%	0	0%	0	0%	0	0%		
	3	0	0%	0	0%	0	0%	0	0%		
	4	1	25%	0	0%	0	0%	1	<25%		
	5	0	0%	0	0%	0	0%	0	0%		
	6	0	0%	0	0%	0	0%	0	0%		

Tissue injury was assessed on each hematoxylin and eosin stained slide for: 1) severity of injury (0: absent; 1, 2, 3, and 4 for more severe changes), and 2) extent of injury.

Table S4. Indices of Oxidative Stress and Inflammation.

		$ALCO_2R$	**Control**	***P* value**
TBARs (nmol/mg protein)	Lung	1.69 ± 1.98	2.24 ± 0.52	0.567
	Heart	1.58 ± 0.29	3.90 ± 1.20	0.002
	Liver	6.84 ± 1.98	9.14 ± 0.64	0.037
Total Antioxidant (μmol/mg protein)	Lung	5.90 ± 0.73	4.50 ± 0.40	0.005
	Heart	4.60 ± 0.49	2.60 ± 2.00	0.060
	Liver	9.70 ± 1.22	8.60 ± 0.80	0.131
GSH (μmol/mg protein)	Lung	0.66 ± 0.20	0.35 ± 0.12	0.015
	Heart	1.43 ± 0.15	1.64 ± 0.42	0.323
	Liver	3.72 ± 1.74	2.80 ± 0.80	0.315
Nitric Oxide (nmol/mg protein)	Lung	6.50 ± 1.96	4.10 ± 2.00	0.090
	Heart	15.90 ± 4.41	11.20 ± 1.40	0.051
	Liver	24.80 ± 6.61	22.30 ± 2.00	0.443
IL-1β (pg/mg protein)	Lung	61.30 ± 15.92	51.70 ± 7.60	0.259
	Heart	72.70 ± 34.29	87.50 ± 36.60	0.530
	Liver	58.30 ± 15.92	32.90 ± 2.80	0.007
IL-8 (pg/mg protein)	Lung	56.70 ± 11.76	28.80 ± 9.60	0.003
	Heart	21.50 ± 9.06	---	---
	Liver	32.80 ± 2.45	27.80 ± 3.80	0.281
Myeloperoxidase (U/mg protein)	Lung	6.80 ± 4.90	8.80 ± 5.20	0.786

Thiobarbituric acid reactive substances (TBARs), reduced glutathione (GSH), interleukin-1β (IL-1β), interleukin-8 (IL-8).

*) $p < 0.05$ versus control sheep.

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