

Regional Blood Acidification Enhances Extracorporeal Carbon Dioxide Removal

A 48-hour Animal Study

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ABSTRACT

Background: Extracorporeal carbon dioxide removal has been proposed to achieve protective ventilation in patients at risk for ventilator-induced lung injury. In an acute study, the authors previously described an extracorporeal carbon dioxide removal technique enhanced by regional extracorporeal blood acidification. The current study evaluates efficacy and feasibility of such technology applied for 48 h.

Methods: Ten pigs were connected to a low-flow veno-venous extracorporeal circuit (blood flow rate, 0.25 l/min) including a membrane lung. Blood acidification was achieved in eight pigs by continuous infusion of 2.5 mEq/min of lactic acid at the membrane lung inlet. The acid infusion was interrupted for 1 h at the 24 and 48 h. Two control pigs did not receive acidification. At baseline and every 8 h thereafter, the authors measured blood lactate, gases, chemistry, and the amount of carbon dioxide removed by the membrane lung (VCO_2 ML). The authors also measured erythrocyte metabolites and selected cytokines. Histological and metalloproteinases analyses were performed on selected organs.

Results: Blood acidification consistently increased VCO_2 ML by 62 to 78%, from 79 ± 13 to 128 ± 22 ml/min at baseline, from 60 ± 8 to 101 ± 16 ml/min at 24h, and from 54 ± 6 to 96 ± 16 ml/min at 48h. During regional acidification, arterial pH decreased slightly (average reduction, 0.04), whereas arterial lactate remained lower than 4 mEq/l. No sign of organ and erythrocyte damage was recorded.

Conclusion: Infusion of lactic acid at the membrane lung inlet consistently increased VCO_2 ML providing a safe removal of carbon dioxide from only 250 ml/min extracorporeal blood flow in amounts equivalent to 50% production of an adult man. (ANESTHESIOLOGY 2014; 120:416-24)

PATIENTS with acute respiratory distress syndrome and chronic obstructive pulmonary disease show a marked increase in ventilatory dead space,¹ which impairs the clearance of carbon dioxide. To maintain physiological blood pH and carbon dioxide partial pressure (p_{CO_2}) values, these patients require an increase in minute ventilation,² fostering ventilator-induced lung injury.³

Combined extracorporeal carbon dioxide removal (ECCO₂R) and low tidal volume ventilation have been proposed as a mean to reduce the risk of ventilator-induced lung injury.⁴⁻⁷ To achieve a significant ECCO₂R (*i.e.*, 50%

What We Already Know about This Topic

- Extracorporeal carbon dioxide removal may help decrease the need for mechanical ventilation

What This Article Tells Us That Is New

- Lactic acid infusion enhances low-flow extracorporeal carbon dioxide removal; this system, therefore, may be of great utility in critically ill patients

This article is featured in "This Month in Anesthesiology," page 1A. Corresponding article on page 266. Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are available in both the HTML and PDF versions of this article. Links to the digital files are provided in the HTML text of this article on the Journal's Web site (www.anesthesiology.org). Figure 3 was provided by Annemarie B. Johnson, C.M.I., Medical Illustrator, Vivo Visuals, Winston-Salem, North Carolina. Preliminary data were presented during the: 2012 American Thoracic Society International Conference; San Francisco, CA, May 18, 2012; XXV European Society of Intensive Care Medicine, Lisbon, Portugal, October 16, 2012; and XXIII Simposio Mostra Anestesia Rianimazione E Terapia Intensiva, Milan, Italy, May 9, 2012.

Submitted for publication May 2, 2013. Accepted for publication September 9, 2013. From the Dipartimento di Scienze della Salute, Università di Milano-Bicocca, Ospedale San Gerardo Nuovo dei Tintori, Monza, Italy (A.Z., P.M., S.R., V.S., M.G., D.F., N.P., and A.P.); Dipartimento di Patologia animale, Igiene e Sanità pubblica veterinaria, sez. di Biochimica e Fisiologia, Università degli studi di Milano, Milan, Italy (F.P. and M.A.); and Dipartimento di Scienze Farmacologiche e Biomolecolari (DiSFeB), Scuola di Scienze del Farmaco, Università degli studi di Milano, Milan, Italy (D.S.).

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of the mean body production), available technologies now require extracorporeal blood flow in the range of 0.5 to 1 l/min⁸⁻¹⁰ and large caliber vascular catheters. The sheer invasiveness of this technique and related complications limit its use to major medical centers and to the most severely ill patients. In contrast, the development of techniques that would allow relevant ECCO₂R at blood flows comparable with that commonly used for continuous renal-replacement therapies (0.2 to 0.4 l/min) would expand the ECCO₂R potential beyond solely preventing ventilator-induced lung injury in patients with severe acute respiratory distress syndrome¹¹: by reducing endotracheal intubation rates, it may help to reduce the incidence of ventilator-associated pneumonia, sedation requirements, and patient discomfort in the intensive care unit.

We recently developed and tested in an animal model¹² a technique designed to increase the carbon dioxide removal capability of the membrane lung (ML). This has been achieved by infusion of a metabolic acid into the blood entering the ML. Acidification converts bicarbonate ions into dissolved gaseous carbon dioxide, increasing pCO₂ transmembrane gradient and subsequent carbon dioxide transfer.

We report the effect of 48-h acid infusion on the ECCO₂R performance of a ML, in a swine model. In addition, we studied the major systemic physiological aspects of blood acidification in order to more fully explore the feasibility of this technique.

Materials and Methods

The study was approved and conducted according to the Institutional Guidelines for the Care and Use of Laboratory Animals (Università degli Studi di Milano, Milan, Italy).

We sedated, tracheostomized, and ventilated (Servo-Ventilator 900C; Siemens-Eléma, Solna, Sweden) 10 healthy female pigs (weight 44 ± 3 kg). Pigs were fasted for 12 h before the beginning of the experiment. Initial sedation was achieved by intramuscular administration of 0.03 mg/kg of medetomidine and 4 mg/kg of tiletamine-zolazepam. We induced anesthesia with propofol (2 to 2.5 mg/kg IV) and fentanyl (2 µg/kg IV), and maintained it with a continuous infusion of thiopental (5 to 20 mg kg⁻¹ h⁻¹), fentanyl (50 to 150 µg/h), and rocuronium (30 to 100 mg/h).

A femoral artery and the left internal jugular vein were cannulated for pressure monitoring, blood sampling, and medications infusion. A Swan-Ganz catheter was introduced through the left external jugular vein for hemodynamic and blood temperature monitoring. Urine flow was measured after introducing a Foley catheter into the bladder. Ceftriaxone (2 g) was administered prophylactically. After an IV injection of 200 U/kg of heparin, all 10 pigs were connected to a thermostated (38°C) veno-venous extracorporeal circuit by cannulation of the right internal jugular vein (14-French drainage; Medtronic, Minneapolis, MN) and of the left femoral vein (10- to 12-French, return; Medtronic). An open surgical technique was used to perform vessels cannulation, and standard dialysis dual-lumen catheters were not used to avoid the possible confounding factor of recirculation. The extracorporeal circuit included the drainage cannula, a Jostra centrifugal pump (Maquet, Hechingen, Germany), a dialyzer (1.8 m², F8HPS; Fresenius Medical Care, Bad Homburg, Germany), a Quadrox iD-Pediatric ML (Maquet), and the reinfusion cannula. The dialysate inlet and outlet ports of the dialyzer were connected to recirculate the dialysate countercurrent to blood flow. The dialysate recirculation circuit included a bubble trap and a peristaltic pump. Figure 1 outlines the experimental setup.

The extracorporeal blood flow was set at 250 ml/min, the ML oxygen flow was 5 l/min, and the dialysate flow 300 ml/min. The activated clotting time was kept at least 50% above baseline by continuous IV heparin infusion.

The animal was then turned in prone position. We set tidal volume at 10 ml/kg, and adjusted respiratory rate to obtain an arterial pCO₂ (PaCO₂) of 50 mmHg; we set positive end-expiratory pressure at 6 cm H₂O and the fraction of inspired oxygen to 40%: all these parameters were kept constant during the experiment. PaCO₂ was maintained constant at 50 mmHg throughout the experiment modifying the apparatus dead space. During the whole experiment, 2 ml kg⁻¹ h⁻¹ of an electrolyte solution and 35 ml/h of 33% glucose solution (approximately 1,000 kcal/day) were continuously infused.

Eight subjects received blood acidification, whereas two served as sham controls and did not received acidification; otherwise preparation and management were identical.

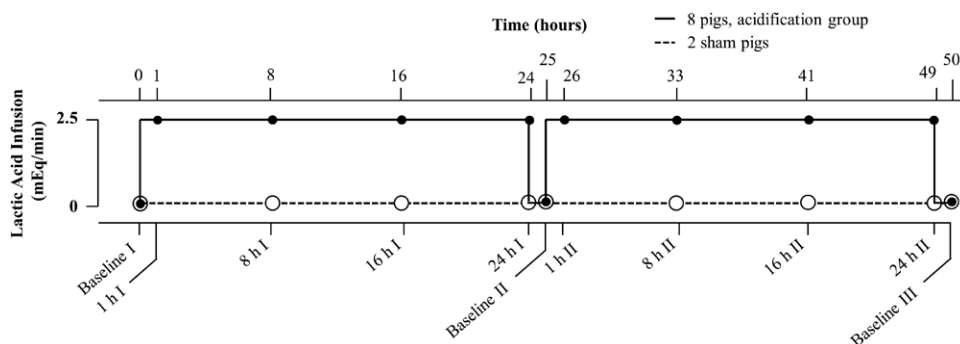


Fig. 1. Timeline of the experiment with lactic acid infusion rate and step names. *Solid dots* and *empty circles* mark the timing of the performed steps, respectively, in the eight pigs subjected to acidification and the two sham pigs.

The study lasted 50 h. In the eight pigs receiving acidification, lactic acid (40% solution, 4.4 N) was continuously infused at a rate of 2.5 mEq/min (34 ml/h) into the gas trap of the dialysis circuit for 24 h. After an interruption of 1 h, a second 24-h continuous acid infusion was repeated. The interruption of lactic acid infusion was performed to compare the ECCO₂R during acidification with the standard extracorporeal technique without acidification, 1 h was a reasonable interval to exclude that any residual effect of blood acidification was present; as the complete clearance of the infused lactate is normally achieved within 20 to 40 min.

We collected relevant data in 11 steps (as indicated in fig. 1), including three acid-free steps (baseline I, II, and III) plus four steps during each of the two acid trial. At each step, we measured: the amount of carbon dioxide removed by the ML (VCO₂ML, ml/min), hemodynamic parameters, blood lactate, and blood gas parameters (ABL 800 gas analyzer; Radiometer, Copenhagen, Denmark). Blood was sampled from the femoral artery, extracorporeal circuit before the dialyzer (predialyzer), after the dialyzer (postdialyzer), and after the ML (post-ML). Dialysate was sampled before (preacid) and after (postacid) the site of acid infusion (figs. 2 and 3).

In the two sham pigs, lactic acid was not infused, but the study structure was identical to that of pigs receiving acidification (except for steps 1 h I and 1 h II not performed). Data were therefore collected only on nine steps (fig. 1).

The VCO₂ML (body temperature and pressure, saturated) was calculated from the ML effluent gas flow (5 l/min) and carbon dioxide concentration, measured by an infrared carbon dioxide analyzer (WMA-4; GMR Strumenti SAS, Firenze, Italy).

VO₂ML (ml/min) was computed knowing the blood flow (l/min) and the blood oxygen content before and after the ML:

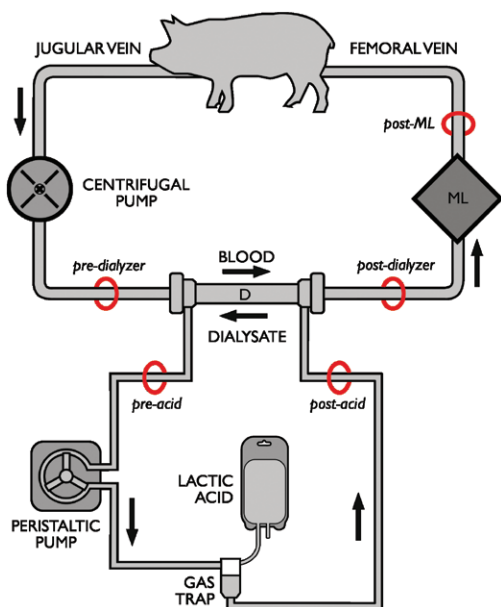


Fig. 2. Outline of the experimental setup. Circles represent the withdrawal sites. D = dialyzer; ML = membrane lung.

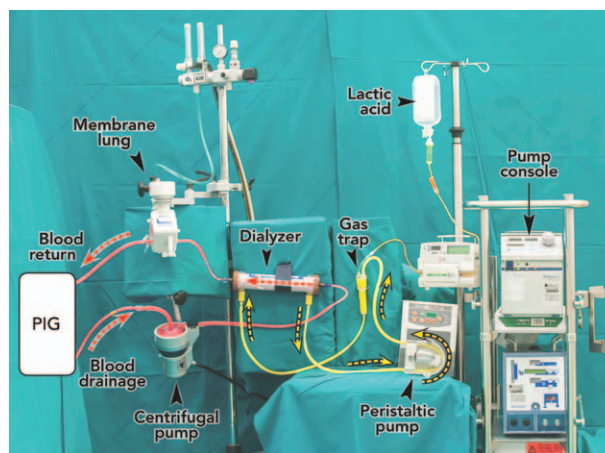


Fig. 3. Reconstruction of the experimental setup.

$$VO_2ML = [(0.0031 * P_{OUT}O_2 + 1.39 * Hb * HbO) - (0.0031 * P_{IN}O_2 + 1.39 * Hb * HbO_2)] * BF * 10$$

where: P_{OUT}O₂ = oxygen partial pressure post-ML (mmHg), P_{IN}O₂ = oxygen partial pressure pre-ML (mmHg), Hb = hemoglobin (g/dl), HbO₂ = hemoglobin oxygen saturation (%), BF = blood flow.

In all pigs, at steps: “baseline I,” “8 h I,” “16 h I,” “24 h I,” “8 h II,” “16 h II,” and “24 h II,” blood was sampled for measurement of hemoglobin, g/dl; leukocytes, 10³/μl; platelets, 10³/μl; blood glucose, mg/dl; blood urea nitrogen, mg/dl; creatinine, mg/dl; total plasma proteins, g/dl; total bilirubin, mg/dl; aspartate transaminase, U/l; alanine transaminase, U/l; and lactate dehydrogenase (LDH), U/l.

Blood cytokine samples were obtained at steps “baseline I,” “24 h I,” and “24 h II” and allowed to clot for 2 h at room temperature before centrifuging for 30 min at 1,000g. Serum was then removed and samples were aliquoted and stored at -70°C for subsequent analyses. Interleukin (IL)-1β, IL-10, and tumor necrosis factor-α were the acute-phase reactants chosen for this study. A quantitative analysis was performed in duplicate for each sample using porcine-specific standard Enzyme-Linked ImmunoSorbent Assay (ELISA) kits (R&D Systems, Inc., Minneapolis, MN) according to the manufacturer’s instructions.

At “baseline I,” “24 h I,” and “24 h II,” blood samples were obtained to evaluate erythrocyte metabolites (pyruvate, adenosine triphosphate, 2,3 diphosphoglycerate, and meta-hemoglobin), erythrocyte enzymatic activities (glyceraldehyde phosphate dehydrogenase, LDH, catalase, glutathione reductase, and acetyl cholinesterase), and plasma determinations (free hemoglobin and total antioxidant power), see Supplemental Digital Content 1, <http://links.lww.com/ALN/B19>, for methods for the determination of erythrocyte metabolites, erythrocyte enzymatic activities and plasma-free Hb, antioxidant power, and LDH.

At the end of the experiments, animals were euthanized by a central venous injection of 40 mEq of KCl, and lung, heart, and renal tissue samples were collected

for histological evaluation and zymographic analysis of the matrix metalloproteinases (MMPs) (see Supplemental Digital Content 1, <http://links.lww.com/ALN/B19>, for methods for MMPs analysis).

Statistical Analysis

Data are expressed as mean \pm SD; one-way ANOVA for repeated measurement with the post hoc Bonferroni correction (Sigmaplot 11.2; Systat Software Inc., Chicago, IL) was performed to compare data from the different steps. A *P* value less than 0.05 was considered statistically significant. A statistical comparison among the different steps was performed only in the eight pigs receiving blood acidification; the two sham pigs were used to discriminate the effect of acidification *versus* other confounding factors (surgery, sedation, and extracorporeal circulation). A linear regression, ignoring the repeated measures nature of the data, was performed to correlate variation of ECCO₂R and the increase on ML inlet pCO₂ due to acidification.

Results

In the eight pigs receiving acidification, lactic acid infusion increased the VCO₂ML at all experimental steps (fig. 4A). At baseline I, VCO₂ML was 79 \pm 13 ml/min and increased to 128 \pm 22 ml/min (62% increase; *P* < 0.001) at 1 h of acid infusion, to become 101 \pm 16 ml/min at “24 h I”; at “baseline II,” VCO₂ML decreased to 60 \pm 8 ml/min. At “24 h II” VCO₂ML was still 96 \pm 16 ml/min, 78% higher than “baseline III” (54 \pm 6 ml/min; *P* < 0.001).

During acidification, VCO₂ML was higher at 1 h I and slightly decreased within the first 8 h remaining fairly constant; the first VCO₂ML without acidification (baseline I) was also higher compared with the subsequent VCO₂ML without acidification (baseline II and baseline III).

In the two sham pigs, the VCO₂ML at baseline I, baseline II, and baseline III were comparable with the eight experimental subjects and showed a similar trend.

During infusion, arterial blood lactate increased significantly, from a baseline of 0.7 \pm 0.3 mEq/l to an almost constant level of approximately 3.3 mEq/l (fig. 4B). Within 1 h

from stopping the acid infusion, arterial blood lactate levels always returned to baseline levels, which were similar to the values of the two sham pigs.

The increase in arterial blood lactate, during the acid infusion, led to a slight decrease in systemic arterial pH, showing an average reduction of 0.04 units (corresponding to a 3.1 \times 10⁻⁹ Eq/l increase of [H⁺]), promptly returning to baseline values after discontinuation of acidification (fig. 4C). The arterial pH of the sham pigs remained unchanged during the study period.

Arterial pCO₂ was fairly constant (50 \pm 3 mmHg) throughout the study, as well as arterial PO₂ (221 \pm 43 mmHg). Arterial concentrations of Na⁺, K⁺, Cl⁻, and glucose remained constant throughout the whole study period, respectively at 137 \pm 3, 3.9 \pm 0.4, 103 \pm 3 mEq/l, and 112 \pm 25 mg/dl. Figure 4, D and E, shows that levels of adenosine triphosphate and acetyl cholinesterase of the erythrocytes were not affected by acid infusion.

The oxygen transfer of the artificial lung (VO₂ML) remained stable (13 \pm 3 ml/min) and was not affected by acidification.

Ventilator parameters, set before the beginning of extracorporeal circulation and kept constant through the study, were: tidal volume 495 \pm 81 ml (11 \pm 1 ml/kg), respiratory rate 16 \pm 3 breaths/min, fraction of inspired oxygen 44 \pm 6%, and positive end-expiratory pressure 6 \pm 3 cm H₂O.

Table 1 shows selected extracorporeal blood parameters of the eight pigs receiving acid infusion. Similar to arterial blood, “predialyzer” blood showed a pH reduction during acidification, a constant pCO₂ (approximately 58 mmHg) and an increase in lactate. “Post-dialyzer” blood pH decreased (*P* < 0.001) from 7.43 \pm 0.03 to 7.06 \pm 0.10 after 1 h of acidification and reached 6.99 \pm 0.09 and 6.95 \pm 0.11, respectively, at “24 h I” and “24 h II.” Post-dialyzer blood pCO₂ consequently increased (*P* < 0.001) from a baseline value of 58 \pm 6 mmHg to 110 \pm 25, 120 \pm 25, and 121 \pm 27 mmHg at “1 h I,” “24 h I,” and “24 h II.” During acidification, post-dialyzer blood lactate levels increased from a baseline value of 0.7 \pm 0.4 to 11.5 \pm 2.1 mEq/l after 1 h of acid infusion (*P* < 0.001) and then remained stable at “24 h I” and “24 h II” (11.7 \pm 2.6 and 12.0 \pm 3.9 mEq/l respectively).

Table 1. pH, pCO₂, and Lactate in Blood through the Extracorporeal Circuit in the Eight Pigs Subject to Acidification

		Baseline I	1 h I	24 h I	Baseline II	24 h II	Baseline III
pH	Predialyzer	7.43 \pm 0.03	7.39 \pm 0.04	7.36 \pm 0.08&	7.40 \pm 0.06	7.37 \pm 0.04	7.39 \pm 0.05
	Postdialyzer	7.43 \pm 0.03	7.06 \pm 0.10*	6.99 \pm 0.09*	7.40 \pm 0.06	6.95 \pm 0.11*	7.39 \pm 0.05
	Post-ML	7.92 \pm 0.25	7.56 \pm 0.09&	7.41 \pm 0.14*	7.75 \pm 0.22	7.40 \pm 0.13*	7.74 \pm 0.22
pCO ₂ (mmHg)	Predialyzer	58 \pm 6	58 \pm 5	58 \pm 5	58 \pm 3	59 \pm 4	60 \pm 8
	Postdialyzer	58 \pm 6	110 \pm 25*	120 \pm 25*	58 \pm 3	121 \pm 27*	60 \pm 8
	Post-ML	18 \pm 6	25 \pm 7	35 \pm 13#&	26 \pm 10	35 \pm 13#&	25 \pm 11
Lactate (mEq/l)	Predialyzer	0.70 \pm 0.39	3.16 \pm 0.48*	2.84 \pm 0.56*	0.44 \pm 0.11	3.50 \pm 1.43*	0.77 \pm 0.43
	Postdialyzer	0.70 \pm 0.39	11.54 \pm 2.10*	11.69 \pm 2.58*	0.44 \pm 0.11	11.96 \pm 3.85*	0.77 \pm 0.43
	Post-ML	0.80 \pm 0.35	11.13 \pm 2.04*	12.09 \pm 2.68*	0.74 \pm 0.44	12.30 \pm 3.84*	0.98 \pm 0.53

**P* < 0.001 vs. “baseline I,” “baseline II,” and “baseline III”; #*P* < 0.05 vs. “1 h I”; &*P* < 0.05 vs. “baseline I.”

ML = membrane lung; pCO₂ = carbon dioxide partial pressure.

The “post-ML” blood without acidification showed an extreme respiratory alkalosis, with a pH of 7.92 ± 0.25 and a pCO_2 of 18 ± 6 mmHg at “baseline I,” which decreased during time to a minimum pH of 7.74 ± 0.22 and pCO_2 of 25 ± 11 mmHg at “baseline III.” When infused, lactic acid buffered the respiratory alkalosis due to ML carbon dioxide removal: the same trend in pCO_2 increase and pH reduction was observed, but in a more physiological range: from 7.56 ± 0.09 with a pCO_2 of 25 ± 7 mmHg after 1 h of acidification to 7.40 ± 0.13 with a pCO_2 of 35 ± 13 mmHg at “24 h II.”

In sham pigs, the main parameters of blood at the inlet of the extracorporeal circuit were comparable with baseline values of pigs receiving acidification. The “post-ML” blood showed a similar trend but with a more alkaline pH, which reached a maximum of 8.16 and 8.20 at “baseline I” with a pCO_2 of 10 and 13 mmHg and then decreased to pH 7.70 and 7.98 at “baseline III.”

The correlation between variation of ECCO₂R and the increase in ML inlet pCO_2 due to acidification is reported in figure 1, Supplemental Digital Content 2, <http://links.lww.com/ALN/B20>.

Table 1, Supplemental Digital Content 3, <http://links.lww.com/ALN/B21>, shows pH, pCO_2 , and lactate concentrations in the dialysis circuit.

Electrolytes (Na^+ , K^+ , and Cl^-) of predialyzer and postdialyzer blood as well as of preacid and postacid are reported in tables 2 and 3, Supplemental Digital Content 3, <http://links.lww.com/ALN/B21>. Hemodynamic data are shown in tables 4 and 5, Supplemental Digital Content 3, <http://links.lww.com/ALN/B21>. In the eight pigs subjected to

acidification, all recorded parameters remained constant over time, with the only exception of systemic blood pressure that was significantly higher at “baseline I” and “1 h I.” The same trend was observed in the two sham pigs. The internal temperature of the animals did not change significantly during the experiment and was maintained between 38° and 38.5°C, which is the normal temperature in swine.

Blood chemistry of the eight pigs subjected to acidification and that of the two sham pigs showed similar trends (table 2). Hemoglobin and platelets significantly decreased over time, whereas leukocytes did not change. Total plasma protein significantly decreased over time. Creatinine showed a slightly increase at “24 h II.” Transaminases (alanine transaminase and aspartate transaminase) significantly increased after the 8 h II step. LDH statistically increased at “24 h II.”

Blood tumor necrosis factor- α levels, in animals receiving acidification, remained stable over time (75 ± 18 , 75 ± 28 , and 76 ± 32 pg/ml, respectively, at “baseline I,” “24 h I,” and “24 h II”) and were slightly lower compared with the sham group (102 to 118, 157 to 118, and 91 to 97 pg/ml, respectively, at “baseline I,” “24 h I,” and “24 h II”); IL-1 β did not reach the detection limit (<13.6 pg/ml) in any of the blood samples analyzed. IL-10 was not detectable (<5.5 pg/ml) at the “baseline I,” but significantly increased at “24 h I” to 24 ± 33 pg/ml ($P < 0.001$) in the eight pigs receiving acidification and to 118 to less than 5.5 pg/ml in the two sham pigs and then decreased again to undetectable values at “24 h II.”

The metabolic activity of the erythrocytes was minimally affected by acid infusion: the levels of pyruvic

Table 2. Blood Chemistry of the Eight Pigs Subjected to Acidification (Acid) and the Two Pigs Used as Control (Sham)

Step	Group	Baseline I	8 h I	16 h I	24 h I	8 h II	16 h II	24 h II
Hemoglobin (g/dl)	Acid	8.8 ± 1.6	8.1 ± 0.9	8.1 ± 0.8	7.7 ± 1.1*	7.5 ± 1.3*	7.8 ± 1.7*	7.3 ± 1.4*
	Sham	8.8, 9.4	7.4, 8.9	7.9, 8.9	8.5, 6.9	8.2, 6.9	7.4, 6.8	6.7, 6.8
Platelets ($10^3/\mu$ l)	Acid	314 ± 133	214 ± 87	178 ± 77*	159 ± 77*	134 ± 71*	121 ± 80*	125 ± 68*
	Sham	Not measured	Not measured	Not measured	Not measured	Not measured	Not measured	Not measured
Leukocytes ($10^3/\mu$ l)	Acid	17.3 ± 7.2	19.7 ± 9.2	21.3 ± 9	21.0 ± 8.1	20.4 ± 8.9	20.6 ± 8.6	20.5 ± 7.6
	Sham	Not measured	Not measured	Not measured	Not measured	Not measured	Not measured	Not measured
Total plasma protein (g/dl)	Acid	5.1 ± 0.6	4.3 ± 0.6*	4.05 ± 0.6*	3.9 ± 0.6*	3.6 ± 0.2*§	3.5 ± 0.3*§	3.4 ± 0.2*§
	Sham	5.3, 4.8	4.5, 4.5	3.8, 4	3.5, 3.7	4.1, 3.2	3.5, 2.9	3.6, 3.5
Urea (mg/dl)	Acid	23 ± 10	36 ± 10	34 ± 12	31 ± 17	31 ± 24	37 ± 24	36 ± 22
	Sham	20, 8	22, 14	23, 18	32, 20	42, 29	47, 38	50, 46
Creatinine (mg/dl)	Acid	1.1 ± 0.3	1.4 ± 0.6	1.5 ± 0.8	1.6 ± 0.9	1.5 ± 0.8	1.5 ± 0.6	1.4 ± 0.4*
	Sham	1.2, 1.1	1.2, 1.3	1.3, 1.5	1.4, 1.3	1.5, 1.5	1.6, 1.5	1.6, 1.6
Total bilirubin (mg/dl)	Acid	0.1 ± 0.04	0.09 ± 0.04	0.06 ± 0.04	0.07 ± 0.03	0.08 ± 0.02	0.05 ± 0.03*	0.05 ± 0.03*
	Sham	0.11, 0.16	0.06, 0.18	0.05, 0.18	0.06, 0.15	0.05, 0.11	0.03, 0.08	0.1, 0.15
ALT (U/l)	Acid	60 ± 9	66 ± 12	77 ± 22	90 ± 30	101 ± 37*	110 ± 48*§	118 ± 59*§&
	Sham	60, 40	62, 46	62, 58	75, 56	87, 64	85, 94	88, 126
AST (U/l)	Acid	34 ± 8	48 ± 14	71 ± 29	84 ± 45	110 ± 54*§	126 ± 64*§	127 ± 68*§&
	Sham	43, 36	45, 35	64, 63	75, 60	98, 80	88, 130	90, 164
LDH (U/l)	Acid	707 ± 113	607 ± 52#	664 ± 95#	686 ± 111	688 ± 108	782 ± 174	862 ± 297
	Sham	820, 649	631, 588	635, 640	683, 609	786, 638	871, 714	938, 818

Statistical analysis was performed only on the eight pigs subjected to acidification.

* $P < 0.05$ vs. “baseline I,” # $P < 0.05$ vs. “24 h II,” § $P < 0.05$ vs. “8 h I,” & $P < 0.05$ vs. “16 h I.”

ALT = alanine transaminase; AST = aspartate transaminase; LDH = lactate dehydrogenase.

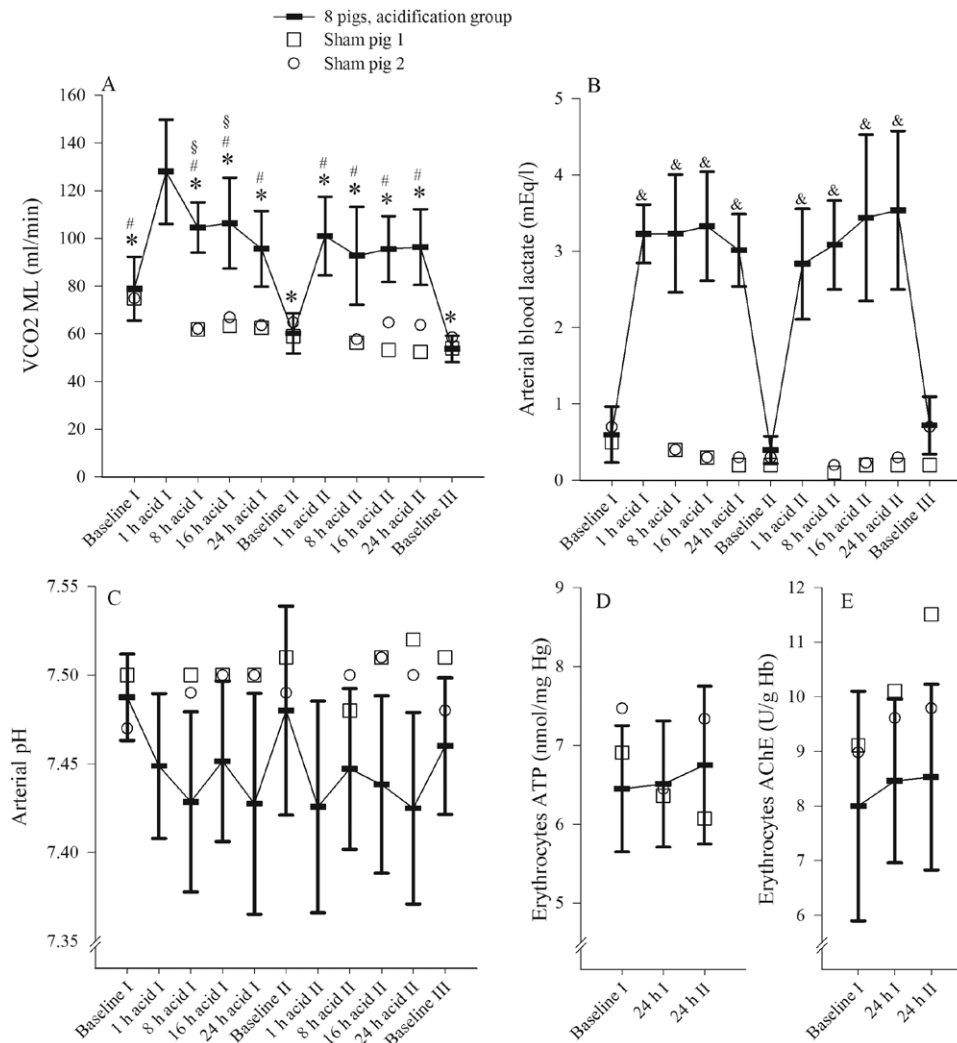


Fig. 4. Time course of membrane lung carbon dioxide removal (VCO₂ML, A), arterial blood lactate (B), arterial pH (C), erythrocyte adenosine triphosphate (erythrocytes ATP, D), and erythrocyte acetyl cholinesterase (erythrocytes AChE, E). Statistical analysis was performed only on the eight pigs subjected to acidification. **P* < 0.001 versus “1 h I”; #*P* < 0.001 versus “baseline II” and “baseline III”; \$*P* < 0.001 versus “baseline I”; &*P* < 0.001 versus “baseline I,” “baseline II,” and “baseline III.”

acid, adenosine triphosphate, glyceraldehyde phosphate dehydrogenase, catalase, acetyl cholinesterase, and LDH remained close to baseline I values. 2,3 Diphosphoglycerate slightly decreased at “24 h I” and “24 h II,” a similar reduction was present in sham pigs. Glutathione reductase activity showed a slight reduction at “24 h II” only in the acidification group. Meta-hemoglobin, free hemoglobin, and total antioxidant power did not change over time (tables 6–8, Supplemental Digital Content 3, <http://links.lww.com/ALN/B21>).

Histological examination of tissues showed no alterations attributable to the prolonged infusion of lactic acid. The MMPs analysis showed that MMP-2 was expressed only in its inactive form in heart specimens, whereas it was undetectable in either the lung or kidney. MMP-9 was not detected in heart and kidney but was present only in the inactivated form in the lungs.

Discussion

This study’s primary objective was to evaluate the effect of 48-h of regional extracorporeal blood acidification on ML carbon dioxide removal. The infusion of 2.5 mEq/min of lactic acid consistently increased (+62 to 78%) VCO₂ML, attaining values between 96 ± 16 ml and 128 ± 22 ml/min at an extracorporeal blood flow of 250 ml/min, well in the range of clinical continuous renal-replacement therapy.

The pH reduction raises blood pCO₂ and consequently pCO₂ transmembrane gradient (fig. 1, Supplemental Digital Content 2, <http://links.lww.com/ALN/B20>). A similar improvement in carbon dioxide removal by blood acidification was reported in our previous “acute” study,¹² where we studied the relationship between acid infusion (three incremental lactic acid infusion rates were tested for 15 min each), blood acidification and ECCO₂R. The long-term effects of this technique were not evaluated. In the current study,

VCO₂ML remained substantially stable over time, showing a slight decrease during the first hour possibly due to an initial loss of ML performance. A similar early decrease in ML efficiency was observed in the two sham swine. However, we were able to maintain for up to 48 h an ECCO₂R approximately 100 ml/min (corresponding to approximately 50% of the total carbon dioxide production of an adult man) from a blood flow of only 250 ml/min.

Currently, there are several ECCO₂R systems available for clinical use.^{9,13,14} These decrease the ventilatory needs of mechanically ventilated patients with acute respiratory distress syndrome, in whom, as shown by Terragni et al.,¹⁵ even a tidal volume of 6 ml/kg may be injurious.

Three decades ago, blood acidification was shown experimentally to increase VCO₂ML.¹⁶ Currently, modern technology has substantially improved the feasibility and the practical application of the acidification principle, as discussed in the current article.

It follows that a very low-flow (250 ml/min), high-efficiency carbon dioxide removal system could make an ultra-protective ventilation, a procedure of limited complexity, comparable with continuous renal-replacement therapy.¹² It could be applied to limit endotracheal intubation in patients with acute respiratory distress syndrome, with decompensated chronic obstructive pulmonary disease,^{9,17} or pending lung transplant.^{18–20}

We recognize a blood flow of 250 ml/min is not suitable to treat severe hypoxemia as only a very small proportion (approximately 5%) of the body oxygen consumption of an adult man can be supplied through such a low blood flow. Indeed, carbon dioxide removal, in its purest form, cannot provide rescue for the extremely hypoxemic patient, but is rather a technique to provide support to enable lung healing and prevent ventilator-induced lung injury.

Arterial lactate, at 24 h II, reached a maximum level of 3.5 ± 1.0 mEq/l such mild hyperlactatemia should not be deleterious because it is now universally accepted that lactic acid *per se* is not toxic.^{21–23} Furthermore, hypertonic lactate infusions have been used in postcoronary artery bypass grafting and in brain-injured patients with favorable results.^{24,25} Sodium lactate infusions, up to plasma lactate levels higher than those reached in the current study, have been performed in human without any complications.^{26,27} Finally, values of arterial pH and lactate concentration consistently returned to basal levels within 1 h after the interruption of acid infusion. It has been demonstrated that lactate clearance is not impaired even in patients with severe sepsis and cardiogenic shock.²⁸

In this experiment, extracorporeal blood was exposed to acute pH changes; as an example, at 24 h I, the pH of the blood entering the extracorporeal system was 7.36 ± 0.08, decreased to 6.99 ± 0.09 immediately after the point of infusion of lactic acid and then increased again to 7.41 ± 0.14 after the ML. However, similar levels of acidosis are normally observed in blood effluent from exercising muscles and other

physiological conditions.^{26,27} Indeed, we observed no hemolysis at any time of the study.

It is also important to point out that at such a low flow, the ML substantially hyperventilates venous blood. In absence of acid infusion, the pH of blood exiting the ML was extremely alkaline (7.92 ± 0.25), whereas during acid infusion, it remained closer to 7.4, in spite of a substantially increased carbon dioxide removal. Hence, blood acidification not only enhances the efficiency of ECCO₂R but also buffers the extreme respiratory alkalosis, which is nonphysiological and more iatrogenic than acidosis.

Pigs were hemodynamically stable during the whole experiment; the mild decrease in systemic arterial pressure observed both in pigs receiving acidification and in controls was probably due to accumulation of sodium thiopental resulting in a deeper sedation level. Importantly, mean pulmonary pressure remained unchanged during acid infusion.

Because blood chemistry could be influenced by several factors other than blood acidification (medications, surgery, activation of coagulation due to blood-circuit surface interaction), we repeated the experiment with the same settings except for lactic acid infusion in two sham swine. In these two pigs, the same trends in alanine transaminase, aspartate transaminase, LDH, total plasma protein, urea, and creatinine were observed. Because blood cells were exposed to significant pH shifts, we performed several specific tests to assess erythrocyte function during acidification: we observed no signs of hemolysis and plasma-free hemoglobin remained below detection limits. Erythrocyte stability is also confirmed by the normal activity of acetyl cholinesterase, an integral erythrocyte membrane-bound enzyme strictly dependent on the physico-chemical properties of the membrane. The metabolic activity of erythrocytes was unaffected by acid treatment, as indicated by pyruvic acid and adenosine triphosphate levels. The activity of the glycolytic enzymes glyceraldehyde phosphate dehydrogenase and LDH are unchanged as well. The stability of glyceraldehyde phosphate dehydrogenase, a thiolic enzyme particularly sensitive to oxidative stress, suggests that erythrocytes remain normally able to cope with peroxidative damage. Consistent with this finding are the normal activity of the main antioxidant enzymes catalase and glutathione reductase and the normal value of erythrocytes meta-hemoglobin. Analogously, the acid infusion does not affect the total antioxidant power of plasma. MMPs levels were analyzed as a marker of tissue inflammation, specifically, MMP-2 activation is traditionally considered an early event that triggers the cascade of proteolytic enzyme activation, whereas MMP-9 expression is induced by activation of macrophages and neutrophils during inflammation.²⁹ Active forms of MMPs were not detected among our histological samples, and histological analyses showed no evidence of organ damage, thus infusion of lactic acid did not induce acute inflammation or organ damage. These data were confirmed by cytokine measurements that remained comparable between sham and experimental pigs.

In contrast to our previously published work,¹² to avoid the direct injection of acid into the blood stream and to limit the volume of injected solution, we administered a highly concentrated lactic acid solution (4.4 N, 34 ml/h) through a dialyzer placed *ad hoc* in the extracorporeal circuit. This technique also prevented abrupt pH decreases, which are known to be associated with pulmonary vasoconstriction.³⁰

Because the primary aim of the current study was to evaluate the effect of blood acidification on ECCO₂R over time, we tried to maintain a constant pCO₂ in the blood entering the extracorporeal circuit. Indeed, we set the ventilatory parameters before starting the extracorporeal circulation to achieve a PaCO₂ of 50 mmHg, then, during the trials, the dead space could be modified to maintain a constant PaCO₂. Certainly, the metabolic effects of lactic acid infusion need to be investigated in further studies. Finally, in our experiment, the lactic acid infusion accounted for approximately 1,150 kcal/day, which should be taken into account in the total caloric intake.³¹

Conclusions

Forty-eight hours of regional extracorporeal blood acidification with lactic acid infusion is feasible and remove approximately 50% of the total carbon dioxide production of an adult man from a blood flow as low as 250 ml/min. This can be achieved much less invasively than with the currently available methods. Clinical application of regional blood acidification to enhance ECCO₂R through a ML seems a realistic perspective, but further studies are required, in particular, to evaluate the metabolic impact of lactic acid infusion.

Indications for future research include blood acidification through different means and acid load clearances. Such developments appear plausible and may prevent some untoward effects of lactic acid infusion.

Acknowledgments

The authors thank Marco Costanzi (Dipartimento di Patologia animale, Igiene e Sanità pubblica veterinaria, sez. di Biochimica e Fisiologia, Università degli studi di Milano, Milan, Italy) for his valuable technical assistance. The authors wish to express their gratitude to Manuela Viola, M.Sc. (Dipartimento di Scienze Biomediche Sperimentali e Cliniche, Università degli Studi dell'Insubria, Varese, Italy), for performing tissue zimography. The authors are indebted to Giacomo Grasselli, M.D. (Dipartimento di Scienze della Salute, Università di Milano-Bicocca, Ospedale San Gerardo Nuovo dei Tintori, Monza, Italy), for his critical review of this article. The authors are grateful to Lucia Cortelezzi, M.Sc., and Maria Fausta Omodeo Salè, Ph.D. (both from Dipartimento di Scienze Farmacologiche e Biomolecolari [DiSFeB], Scuola di Scienze del Farmaco, Università degli studi di Milano, Milan, Italy), for performing erythrocyte analysis. The authors also thank the valuable support of Luigi Castagna, M.D., Salva Deab, M.D., Federico Magni, M.D., Daniele Bonacina, M.D., Stefano Arrigoni, M.D., and Emanuele Vassena, M.D. (all from Dipartimento di Scienze della Salute, Università di Milano-Bicocca, Ospedale San Gerardo Nuovo dei Tintori, Monza, Italy). The authors are grateful to Bryan S. Jordan,

M.S., R.N. (U.S. Army Institute of Surgical Research, San Antonio, Texas), for his critical review of the article's English.

Support was provided solely from institutional and/or departmental sources.

Competing Interests

Patents: Some procedures described here are part of a blood processing technique covered by patents or for which patents are pending:

1. Pesenti A, Patroniti N, Inventors; Università degli Studi Milano Bicocca, assignee: Blood treatment method adapted to at least eliminate the carbon dioxide content and the related device. WO 2008/135282 A2; Nov 13, 2008.
2. Patroniti N, Pesenti A, Inventors; Circuito extracorporeo per la rimozione della CO₂ dal sangue. BO2012A000404; Italy, July 26, 2012.
3. Patroniti N, Pesenti A, Inventors; Circuito extracorporeo per la rimozione della CO₂ dal sangue. BO2012A000405; Italy, July 26, 2012.

The authors declare no competing interests.

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References

1. Nuckton TJ, Alonso JA, Kallet RH, Daniel BM, Pittet JF, Eisner MD, Matthay MA: Pulmonary dead-space fraction as a risk factor for death in the acute respiratory distress syndrome. *N Engl J Med* 2002; 346:1281–6
2. The Acute Respiratory Distress Syndrome Network: Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. *N Engl J Med* 2000; 342:1301–8
3. Mascheroni D, Kolobow T, Fumagalli R, Moretti MP, Chen V, Buckhold D: Acute respiratory failure following pharmacologically induced hyperventilation: An experimental animal study. *Intensive Care Med* 1988; 15:8–14
4. Gattinoni L, Kolobow T, Tomlinson T, White D, Pierce J: Control of intermittent positive pressure breathing (IPPB) by extracorporeal removal of carbon dioxide. *Br J Anaesth* 1978; 50:753–8
5. Pesenti A, Pelizzola A, Mascheroni D, Uziel L, Pirovano E, Fox U, Gattinoni L, Kolobow T: Low frequency positive pressure ventilation with extracorporeal CO₂ removal (LEPPV-ECCO2R) in acute respiratory failure (ARF): Technique. *Trans Am Soc Artif Intern Organs* 1981; 27:263–6
6. Terragni PP, Del Sorbo L, Mascia L, Urbino R, Martin EL, Birocco A, Faggiano C, Quintel M, Gattinoni L, Ranieri VM: Tidal volume lower than 6 ml/kg enhances lung protection: Role of extracorporeal carbon dioxide removal. *ANESTHESIOLOGY* 2009; 111:826–35
7. Bein T, Weber-Carstens S, Goldmann A, Müller T, Staudinger T, Brederlau J, Muellenbach R, Dembinski R, Graf BM, Wewalka M, Philipp A, Wernecke KD, Lubnow M, Slutsky AS: Lower tidal volume strategy (≈3 ml/kg) combined with extracorporeal CO₂ removal *versus* 'conventional' protective ventilation (6 ml/kg) in severe ARDS: The prospective randomized Xtravent-study. *Intensive Care Med* 2013; 39:847–56

8. Gattinoni L, Pesenti A, Kolobow T, Damia G: A new look at therapy of the adult respiratory distress syndrome: Motionless lungs. *Int Anesthesiol Clin* 1983; 21:97-7
9. Crotti S, Lissoni A, Tubiolo D, Azzari S, Tarsia P, Caspani L, Gattinoni L: Artificial lung as an alternative to mechanical ventilation in COPD exacerbation. *Eur Respir J* 2012; 39:212-5
10. Pesenti A, Patroniti N, Fumagalli R: Carbon dioxide dialysis will save the lung. *Crit Care Med* 2010; 38(10 suppl): S549-54
11. Patroniti N, Bellani G, Pesenti A: Nonconventional support of respiration. *Curr Opin Crit Care* 2011; 17:527-32
12. Zanella A, Patroniti N, Isgrò S, Albertini M, Costanzi M, Pirrone F, Scaravilli V, Vergnano B, Pesenti A: Blood acidification enhances carbon dioxide removal of membrane lung: An experimental study. *Intensive Care Med* 2009; 35:1484-7
13. Cressoni M, Zanella A, Epp M, Corti I, Patroniti N, Kolobow T, Pesenti A: Decreasing pulmonary ventilation through bicarbonate ultrafiltration: An experimental study. *Crit Care Med* 2009; 37:2612-8
14. Batchinsky AI, Jordan BS, Regn D, Necsoiu C, Federspiel WJ, Morris MJ, Cancio LC: Respiratory dialysis: Reduction in dependence on mechanical ventilation by venovenous extracorporeal CO₂ removal. *Crit Care Med* 2011; 39:1382-7
15. Terragni PP, Rosboch G, Tealdi A, Corno E, Menaldo E, Davini O, Gandini G, Herrmann P, Mascia L, Quintel M, Slutsky AS, Gattinoni L, Ranieri VM: Tidal hyperinflation during low tidal volume ventilation in acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2007; 175:160-6
16. Snider MT, Chaudhari SN, Richard RB, Whitcomb DR, Russell GB: Augmentation of CO₂ transfer in membrane lungs by the infusion of a metabolizable organic acid. *ASAIO Trans* 1987; 33:345-51
17. Cardenas VJ Jr, Lynch JE, Ates R, Miller L, Zwischenberger JB: Venovenous carbon dioxide removal in chronic obstructive pulmonary disease: Experience in one patient. *ASAIO J* 2009; 55:420-2
18. Fuehner T, Kuehn C, Hadem J, Wiesner O, Gottlieb J, Tudorache I, Olsson KM, Greer M, Sommer W, Welte T, Haverich A, Hoepfer MM, Warnecke G: Extracorporeal membrane oxygenation in awake patients as bridge to lung transplantation. *Am J Respir Crit Care Med* 2012; 185:763-8
19. Javidfar J, Brodie D, Iribarne A, Jurado J, Lavelle M, Brenner K, Arcasoy S, Sonett J, Bacchetta M: Extracorporeal membrane oxygenation as a bridge to lung transplantation and recovery. *J Thorac Cardiovasc Surg* 2012; 144:716-21
20. Javidfar J, Bacchetta M: Bridge to lung transplantation with extracorporeal membrane oxygenation support. *Curr Opin Organ Transplant* 2012; 17:496-2
21. Leverve XM: Lactate in the intensive care unit: Pyromaniac, sentinel or fireman? *Crit Care* 2005; 9:622-3
22. Figley CR: Lactate transport and metabolism in the human brain: Implications for the astrocyte-neuron lactate shuttle hypothesis. *J Neurosci* 2011; 31:4768-70
23. Cruz RS, de Aguiar RA, Turnes T, Penteado Dos Santos R, de Oliveira MF, Caputo F: Intracellular shuttle: The lactate aerobic metabolism. *ScientificWorldJournal* 2012; 2012:420984
24. Leverve XM, Boon C, Hakim T, Anwar M, Siregar E, Mustafa I: Half-molar sodium-lactate solution has a beneficial effect in patients after coronary artery bypass grafting. *Intensive Care Med* 2008; 34:1796-803
25. Ichai C, Armando G, Orban JC, Berthier F, Rami L, Samat-Long C, Grimaud D, Leverve X: Sodium lactate *versus* mannitol in the treatment of intracranial hypertensive episodes in severe traumatic brain-injured patients. *Intensive Care Med* 2009; 35:471-9
26. Cavelliers V, De Geeter F, Pansar I, Dendale P, Bossuyt A, Franken PR: Effect of exercise induced hyperlactatemia on the biodistribution and metabolism of iodine-123-15(p-iodophenyl)-3-R,S-methyl pentadecanoic acid in normal volunteers. *Eur J Nucl Med* 2000; 27:33-40
27. Martin NA, Zoeller RF, Robertson RJ, Lephart SM: The comparative effects of sports massage, active recovery, and rest in promoting blood lactate clearance after supramaximal leg exercise. *J Athl Train* 1998; 33:30-5
28. Revelly JP, Tappy L, Martinez A, Bollmann M, Cayeux MC, Berger MM, Chioléro RL: Lactate and glucose metabolism in severe sepsis and cardiogenic shock. *Crit Care Med* 2005; 33:2235-40
29. Ferry G, Lonchamp M, Pennel L, de Nanteuil G, Canet E, Tucker GC: Activation of MMP-9 by neutrophil elastase in an *in vivo* model of acute lung injury. *FEBS Lett* 1997; 402:111-5
30. Wideman RF Jr, Kochera Kirby Y, Forman MF, Marson N, McNew RW, Owen RL: The infusion rate dependent influence of acute metabolic acidosis on pulmonary vascular resistance in broilers. *Poult Sci* 1998; 77:309-21
31. Zanella A, Giani M, Redaelli S, Mangili P, Scaravilli V, Ormas V, Costanzi M, Albertini M, Bellani G, Patroniti N, Pesenti A: Infusion of 2.5 meq/min of lactic acid minimally increases CO₂ production compared to an isocaloric glucose infusion in healthy anesthetized, mechanically ventilated pigs. *Crit Care* 2013; 11:17:R268