Extracorporeal Carbon Dioxide Removal Enhanced by Lactic Acid Infusion in Spontaneously Breathing Conscious Sheep

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ABSTRACT

Background: The authors studied the effects on membrane lung carbon dioxide extraction (VCO_2ML), spontaneous ventilation, and energy expenditure (EE) of an innovative extracorporeal carbon dioxide removal ($ECCO_2R$) technique enhanced by acidification (acid load carbon dioxide removal [$ALCO_2R$]) *via* lactic acid.

Methods: Six spontaneously breathing healthy ewes were connected to an extracorporeal circuit with blood flow 250 ml/min and gas flow 10 l/min. Sheep underwent two randomly ordered experimental sequences, each consisting of two 12-h alternating phases of $ALCO_2R$ and $ECCO_2R$. During $ALCO_2R$, lactic acid (1.5 mEq/min) was infused before the membrane lung. Caloric intake was not controlled, and animals were freely fed. VCO_2ML, natural lung carbon dioxide extraction, total carbon dioxide production, and minute ventilation were recorded. Oxygen consumption and EE were calculated.

Results: $ALCO_2R$ enhanced VCO_2ML by 48% relative to $ECCO_2R$ (55.3 ± 3.1 *vs.* 37.2 ± 3.2 ml/min; *P* less than 0.001). During $ALCO_2R$, minute ventilation and natural lung carbon dioxide extraction were not affected (7.88 ± 2.00 *vs.* 7.51 ± 1.89 l/min, *P* = 0.146; 167.9 ± 41.6 *vs.* 159.6 ± 51.8 ml/min, *P* = 0.063), whereas total carbon dioxide production, oxygen consumption, and EE rose by 12% each (223.53 ± 42.68 *vs.* 196.64 ± 50.92 ml/min, 215.3 ± 96.9 *vs.* 189.1 ± 89.0 ml/min, 67.5 ± 24.0 *vs.* 60.3 ± 20.1 kcal/h; *P* less than 0.001).

Conclusions: $ALCO_2R$ was effective in enhancing VCO_2ML. However, lactic acid caused a rise in EE that made $ALCO_2R$ no different from standard $ECCO_2R$ with respect to ventilation. The authors suggest coupling lactic acid–enhanced $ALCO_2R$ with active measures to control metabolism. **(Anesthesiology 2016; 124:674-82)**

EXTRACORPOREAL carbon dioxide removal ($ECCO_2R$) is a low blood flow extracorporeal gas exchange technique used to remove carbon dioxide in patients affected by respiratory failure. It mitigates respiratory acidosis,[1] minimizes the ventilatory burden of patients at risk of ventilator-induced lung injury, and reduces the work of breathing.[2] Formerly, $ECCO_2R$ was carried out with older technology and, similar to full extracorporeal membrane oxygenation, was used as a rescue therapy for severe cases of acute respiratory distress syndrome.[3] Recent technological advances have reduced the complexity of $ECCO_2R$[4,5] and thus allowed this technique to be also used in acute exacerbation of chronic obstructive pulmonary disease,[6] as a bridge to lung transplant,[7] and to facilitate lung-protective ventilation.[8] Nonetheless, current $ECCO_2R$

What We Already Know about This Topic

- Extracorporeal carbon dioxide removal is used for lung protection in patients with hypercapnic respiratory failure. Current extracorporeal carbon dioxide removal technology has low efficiency and thus requires significant invasiveness to be clinically effective.

What This Article Tells Us That Is New

- In a study of six spontaneously breathing conscious sheep connected to a minimally invasive circuit, extracorporeal blood acidification with lactic acid (acid load carbon dioxide removal) increased extracorporeal carbon dioxide removal by 50% compared with standard extracorporeal carbon dioxide removal. Although lactic acid infusion increased the overall energy expenditure, feasibility safety and efficiency of acid load carbon dioxide removal were proved.

This article is featured in "This Month in Anesthesiology," page 1A. Corresponding article on page 532. 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).

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technology requires the use of blood flow (BF) of at least 0.5 to 1 l/min, and catheters of 16 to 19 F size, to remove a significant portion of the total carbon dioxide production of an adult patient.[9,10] If carbon dioxide removal could be further enhanced such that reduced BFs as low as 250 to 500 ml/min became possible, smaller cannulas could be used (*e.g.*, 13 to 15 F). Accordingly, $ECCO_2R$ could become a procedure with the same logistical footprint and invasiveness as continuous renal replacement therapy.

In pursuit of this goal, we developed a new $ECCO_2R$ technique consisting of regional blood acidification (acid load carbon dioxide removal [$ALCO_2R$]),[11] based on infusion of lactic acid (LA) extracorporeally into the blood entering the membrane lung (ML). Acidification converts bicarbonate ions into dissolved carbon dioxide. This increases the partial pressure of carbon dioxide (P_{CO_2}) in the blood entering the ML, raises carbon dioxide availability, and increases the transmembrane carbon dioxide partial pressure gradient. Because this is the driving force for carbon dioxide transfer across the membrane,[12] acidification enhances ML carbon dioxide removal (VCO_2ML). Previously, we demonstrated regional blood acidification to be capable of raising VCO_2ML by up to 70% (*i.e.*, to 170 ml/min), with an extracorporeal BF as low as 250 ml/min in a mechanically ventilated porcine model.[11,13]

Until now, $ALCO_2R$ has been performed by infusing a metabolizable acid, such as LA, citric acid, or acetic acid.[11,14] All three compounds are energy substrates, and therefore, their oxidation produces carbon dioxide, which may counterbalance the enhanced carbon dioxide removal effect provided by $ALCO_2R$. In this study, we examined the effects of $ALCO_2R$ on spontaneous ventilation and energy metabolism of LA in the absence of caloric control. We compared ventilation and energy expenditure (EE) during $ALCO_2R$ and standard $ECCO_2R$, in spontaneously breathing, freely fed, healthy ewes. We hypothesized that $ALCO_2R$ enhances carbon dioxide removal of the ML and reduces minute ventilation (MV). Moreover, we studied the safety profile of $ALCO_2R$, in terms of histological damage and signs of tissue inflammation and oxidative stress.

Materials and Methods

This study was approved by the U.S. Army Institute of Surgical Research Institutional Animal Care and Use Committee (Fort Sam Houston, San Antonio, Texas) and was conducted in compliance with the Animal Welfare Act, the Implementing Animal Welfare Regulations, and in accordance with the principles of the Guide for the Care and Use of Laboratory Animals.

Under general anesthesia, six healthy ewes (*Ovis aries*, David Josh Talley, Uvalde, Texas) (34.3 ± 1.5 kg) were instrumented as described in our previous article.[15] Tracheostomy was performed. Catheters were placed in the carotid and pulmonary arteries for pressure monitoring and sample withdrawal. A dual-lumen catheter (15.5 F; ALung; ALung Technologies, USA) was introduced in the right external jugular vein for connection to the extracorporeal circuit (for additional details on instrumentation, see Instrumentation, Additional Methods, Supplemental Digital Content 1, http://links.lww.com/ALN/B237).

A custom-made extracorporeal circuit optimized for $ALCO_2R$ was used for the study. Figure 1 shows the schematic representation of extracorporeal circuit. The circuit consisted of a Hemolung device (ALung; ALung Technologies), a standard polyethersulfone continuous renal replacement therapy hemofilter (Purema; NxStage Medical, USA) connected in series after the Hemolung ML, and a peristaltic pump for the recirculation of ultrafiltrate before the ML. BF was maintained constant at 250 ml/min, sweep gas flow was 10 l/min of ambient air, and ultrafiltrate flow was 100 ml/min. An acid injection port was located on the ultrafiltrate side of the circuit, as such avoiding direct contact between highly concentrated acids and the cellular components of the blood. This prevented hemolysis and unwarranted infusion of free water to dilute the acid. Six sampling outlets were arranged in the circuit: four on the blood side (inlet, postrecirculation, post-ML, and outlet) and two on the ultrafiltrate side (pre-acid, post-acid). Extracorporeal BF was measured by the Hemolung built-in flowmeter at the circuit outlet.

After surgery, anesthesia was discontinued and animals were moved into a cage. For the remainder of the study, continuous infusions of fentanyl and midazolam (0.5 $\mu g \cdot kg^{-1} \cdot h^{-1}$ and 0.01 $mg \cdot kg^{-1} \cdot h^{-1}$, respectively) were provided. Maintenance fluid (Plasma-Lyte A; Baxter International, USA) was infused at 1 $ml \cdot kg^{-1} \cdot h^{-1}$. During the experiment, animals were conscious and breathing spontaneously, connected to a mechanical ventilator (Dräger Evita XL; Dräger Medical, Germany) in continuous positive airway pressure mode at 5 cm H_2O with an F_{IO_2} of 21%. Hay and dry food pellets were provided *ad libitum* throughout the study.

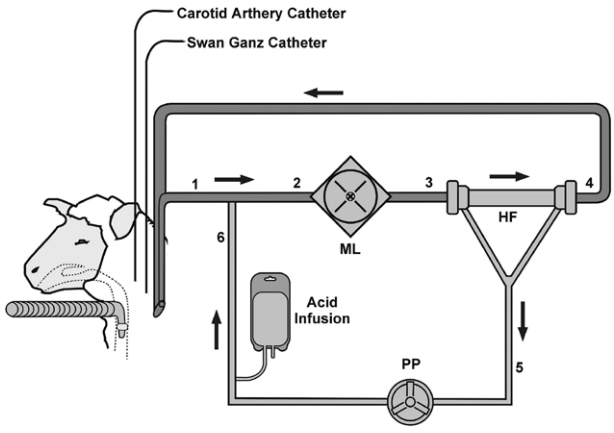

Fig. 1. Schematic representation of extracorporeal circuit. Membrane lung (ML), hemofilter (HF), and peristaltic pump (PP). 1–4 = circuit blood withdrawal sites: (1) inlet, (2) postrecirculation, (3) post-ML, and (4) outlet. 5–6 = ultrafiltrate withdrawal sites: (5) pre-acid, (6) post-acid. Flowmeter was positioned on the blood line after the outlet (4) withdrawal site.

After a recovery period of 6 h, each of the six animals was subjected to two repeated experimental sequences. Each of them lasted 24 h and consisted of a phase of $ALCO_2R$ and a phase of standard $ECCO_2R$. The $ALCO_2R$ and $ECCO_2R$ phases were alternated, ordered randomly, and lasted 12 consecutive hours each (for additional details on experimental setup, see Experimental Design and fig. S1, Additional Methods, Supplemental Digital Content 1, http://links.lww.com/ALN/B237). To counterbalance any order effect of the experimental sequences, three animals started the experiment with a standard $ECCO_2R$ phase, whereas three started with an $ALCO_2R$ phase. We did not perform a washout period after $ALCO_2R$ and before commencing $ECCO_2R$ because in a previous similar study[14] we observed LA (infused at 1.5 mEq/min) to be completely washed-out by sheep in less than 1 h. $ALCO_2R$ was achieved by infusing LA (4.4 M) in the ultrafiltrate side of the circuit, at a continuous fixed rate of 1.5 mEq/min.

A volumetric capnograph (CO_2SMO; Novametrix, USA) was used to measure respiratory rate, tidal volume (TV), MV, and natural lung carbon dioxide removal (VCO_2NL) at body temperature, ambient pressure, saturated (with water vapor) (BTPS) conditions. Alveolar ventilation (AV), physiologic dead space (V_d), and venous admixture (Qs/Qt) were calculated using standard equations (see equations A for Ventilatory Function Calculations, Supplemental Digital Content 1, http://links.lww.com/ALN/B237).[16,17] VCO_2ML at BTPS conditions was measured by a capnometer built into the Hemolung. Thus, the total carbon dioxide production (VCO_2tot) was calculated as the sum of VCO_2NL and VCO_2ML.

Arterial blood gas analyses (i-STAT; Abbott, USA) and activated clotting time (ACT) (Hemochron Jr. Signature; ITC, USA) were recorded hourly. Moreover, every 3 h, selected electrolytes (sodium, potassium, chloride, and ionized calcium) and glucose concentrations were measured in the arterial samples (i-STAT; Abbott). Mixed venous, extracorporeal circuit blood, and ultrafiltrate samples were collected every 3 h for blood gas analyses. Hematocrit was measured *via* centrifugation technique in mixed venous samples. Heart rate (HR), mean arterial pressure, central venous pressure, and pulmonary artery pressure were monitored continuously. Pulmonary artery occlusion pressure was recorded hourly. Cardiac output (CO) was measured every 3 h using intermittent bolus thermodilution technique.

ML carbon dioxide removal efficiency ratio was computed as described in our previous article (see equations B for ML CO_2 Removal Efficiency Calculation, Supplemental Digital Content 1, http://links.lww.com/ALN/B237).[15] ML oxygen delivery (VO_2ML), natural lung oxygen delivery, and total oxygen consumption (VO_2tot) were calculated using standard equations (see equations C for Oxygen Delivery and Consumption Calculation, Supplemental Digital Content 1, http://links.lww.com/ALN/B237) at BTPS conditions.

Respiratory quotient (RQ) was calculated as the ratio between VCO_2tot and VO_2tot. Thus, with VCO_2tot and VO_2tot, EE was calculated using the Weir equation, as previously described (see equations D for Energetic Expenditure Calculation, Supplemental Digital Content 1, http://links.lww.com/ALN/B237).[18]

Hemoglobin, complete blood count, prothrombin time, partial thromboplastin time, fibrinogen, D-dimers, blood urea nitrogen, creatinine, total bilirubin, alanine transaminase, aspartate transaminase, amylase, myoglobin, uric acid, blood glucose, and plasma-free hemoglobin were measured before instrumentation, at the end of the recovery period (*i.e.*, after 6 h of extracorporeal circulation [EC]), as well as at the end of any $ECCO_2R$ and $ALCO_2R$ phase. Plasma-free hemoglobin concentration was measured by spectrophotometric analysis (DU 800; Beckman Coulter Inc., USA).

Sheep were euthanized by an intravenous injection of 20 ml of Fatal Plus (Vortech Pharmaceuticals, USA) at the conclusion of the experiments. Lung, heart, liver, and renal tissue samples were collected for histological evaluation postmortem. Histological evaluation of injury was performed by a single pathologist blinded to the identity of the animal represented on the slide, as previously documented (see Postmortem Histological Evaluation, Additional Methods, Supplemental Digital Content 1, http://links.lww.com/ALN/B237).[19] Moreover, we studied the safety of $ALCO_2R$ technique in terms of tissue inflammatory responses, concentration of selected indices of oxidative stress, and biochemical markers of injury. Specifically, we measured thiobarbituric acid reactive substances (TBARs), interleukin-1β, interleukin-8, and nitric oxide concentration, as well as determined reduced glutathione and ferric reducing ability in the homogenate of lung, heart, and liver tissue. Myeloperoxidase activity was measured in lung homogenate. Indices of oxidant stress and inflammation were then compared with healthy time controls sham animals from another sheep study performed in our laboratory (see Oxidative Stress and Inflammation Analysis, Additional Methods, Supplemental Digital Content 1, http://links.lww.com/ALN/B237).[20]

Statistical Analysis

Data are expressed as means ± SD or median and interquartile range, when appropriate. The JMP 11 statistical program (SAS Institute Inc, USA) was used for statistical analysis. A paired difference model was used to gauge the sample size. Using an α error of 0.05 and power of 0.80, with an SD of 5 ml/min in VCO_2ML measurements expected from preliminary experiments, six paired matches were calculated to be needed to detect a difference of at least 7.5 ml/min in VCO_2ML between $ALCO_2R$ *versus* $ECCO_2R$ steps and six animals were sufficient. To further reduce the unnecessary use of animals, we performed two repeated experimental sequences on each of these animals. For systemic variables (*i.e.*, ventilation, hemodynamics, and energetic metabolism) and extracorporeal gas removal (VCO_2ML, VO_2ML, and ML efficiency), a two-way analysis of variance for repeated

measures was performed using a residual maximum likelihood method to fit a general linear model. Treatment (*i.e.*, $ALCO_2R$ *vs.* $ECCO_2R$, 2 levels) and time (*i.e.*, hours, 12 levels) were considered as fixed factor, whereas animals and sequence repetitions (nested within animals) were considered as random effects. Interactions between treatment and time were not analyzed. For extracorporeal gas analyses, a similar statistical model was used, with treatment (*i.e.*, $ALCO_2R$ *vs.* $ECCO_2R$, two levels) and circuit withdrawal port (*i.e.*, six levels) as fixed effects. Two-tailed values of P less than 0.05 were considered statistically significant. *Post hoc* Student's *t* test with Tukey adjustment was used for multiple comparisons.

Results

During LA infusion (*i.e.*, $ALCO_2R$ phases), VCO_2ML was enhanced by 48% relative to $ECCO_2R$ (55.3 ± 3.1 ml/min *vs.* 37.2 ± 3.2 ml/min; $P < 0.001$). Similarly, ML carbon dioxide removal efficiency was significantly increased (41.3 ± 5.3% *vs.* 24.3 ± 4.2%; $P < 0.001$). No reduction in VCO_2ML (fig. 2) and ML carbon dioxide removal efficiency was observed over time. VO_2ML was similar during $ALCO_2R$ and $ECCO_2R$ (9.8 ± 3.2 ml/min *vs.* 9.5 ± 3.2 ml/min; $P = 0.17$).

We did not observe alterations of ventilatory status during $ALCO_2R$ (see Table 1). MV was similar during $ALCO_2R$ and $ECCO_2R$, as was respiratory rate and VCO_2NL. TV and AV showed a 7% increase during $ALCO_2R$. V_d and pulmonary Qs/Qt were not influenced by $ALCO_2R$. No effect of time was observed on ventilatory variables.

Table 2 shows the metabolic parameters during experimental phases. EE increased by 12% during $ALCO_2R$.

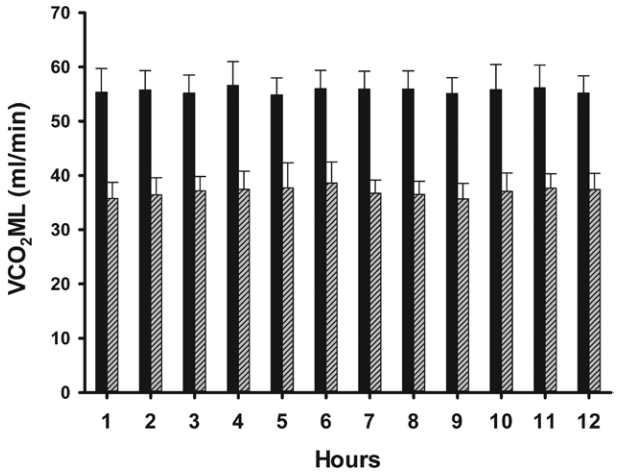

Fig. 2. Extracorporeal carbon dioxide removal (VCO_2ML) during acid load carbon dioxide removal ($ALCO_2R$) and extracorporeal carbon dioxide removal ($ECCO_2R$) phases. *Black bars* represent $ALCO_2R$ phase measurements, whereas *hatched bars* represent $ECCO_2R$ phase measurements. For any of the time-points, VCO_2ML was higher during $ALCO_2R$ compared with $ECCO_2R$ ($P < 0.001$). Data are expressed as means ± SDs.

Similarly, VCO_2tot, natural lung oxygen delivery, and VO_2tot were 13% higher during $ALCO_2R$ relative to $ECCO_2R$. RQ was not significantly influenced by $ALCO_2R$.

Table 3 presents arterial blood gas analyses and electrolyte concentrations measured during experimental phases. During $ALCO_2R$, pH, P_{CO_2}, bicarbonate ions (HCO_3^-), and base excess were lower relative to $ECCO_2R$. Oxygenation was not affected by $ALCO_2R$. Average lactate level was higher during the $ALCO_2R$ phase compared with $ECCO_2R$. During the first hour of $ALCO_2R$, lactate was significantly lower (3.31 ± 0.98 mmol/l; $P < 0.001$) compared with other time-points. After the first hour, lactate plateaued (see fig. S2, Additional Results, Supplemental Digital Content 1, http://links.lww.com/ALN/B237). Sodium, potassium, and ionized calcium were not influenced by $ALCO_2R$, whereas chloride was lower during $ALCO_2R$. Glucose was higher during $ALCO_2R$ compared with $ECCO_2R$. No effect of time was observed on arterial blood gas analyses.

The pH, P_{CO_2}, HCO_3^-, and lactate in the blood and ultrafiltrate samples from the circuit are shown in table 4. During $ALCO_2R$, the highest lactate was observed in the post-acid ultrafiltrate samples ($P < 0.001$). In postrecirculation, post-ML, and outlet blood samples, lactate was similarly higher relative to inlet blood ($P < 0.001$), which in turn had a lactate concentration similar to the arterial blood. During $ALCO_2R$, similar to arterial samples, inlet blood samples showed reduced pH, P_{CO_2}, and HCO_3^- compared with $ECCO_2R$. LA infusion significantly acidified post-acid ultrafiltrate and subsequently postrecirculation blood ($P < 0.001$). In turn, these samples showed higher P_{CO_2} and lower HCO_3^- ($P < 0.001$). Acid infusion buffered the extreme iatrogenic alkalosis observed in post-ML and outlet blood during $ECCO_2R$. Indeed, during $ALCO_2R$, post-ML and outlet blood samples had similarly lower pH and higher P_{CO_2} relative to $ECCO_2R$ ($P < 0.001$). Regardless of the experimental phase, post-ML and outlet blood, as well as pre-acid ultrafiltrate, had similar pH, P_{CO_2}, and HCO_3^- concentrations. Moreover, in these samples, pH was higher and P_{CO_2} was lower relative to postrecirculation blood independent of acid infusion ($P < 0.001$). pH, P_{CO_2}, HCO_3^-, and lactate values remained stable over time.

Hemodynamic parameters are shown in table S1 (Additional Results, Supplemental Digital Content 1, http://links.lww.com/ALN/B237). During $ALCO_2R$, a small increase in core temperature was observed (0.1°C). Moreover, $ALCO_2R$ was associated with a rise of about 5% in both HR and CO. Mean arterial pressure, central venous pressure, pulmonary artery occlusion pressure, mixed venous saturation, and hematocrit were similar during $ALCO_2R$ and $ECCO_2R$.

Changes observed in blood chemistries were associated with instrumentation and connection to the EC rather than with application of $ALCO_2R$ (see table S2, Additional Results, Supplemental Digital Content 1, http://links.lww.com/ALN/B237). No sign of hemolysis was observed because plasma-free hemoglobin concentration was always

Table 1. Ventilatory Parameters during Extracorporeal Carbon Dioxide Removal ($ECCO_2R$) and Acid Load Carbon Dioxide Removal ($ALCO_2R$)

	$ECCO_2R$	$ALCO_2R$	*P* Value
MV (l/min)	7.51 ± 1.89	7.88 ± 2.00	0.146
TV (ml)	292 ± 54	312 ± 51	<0.001
RR (bpm)	26.6 ± 8.9	26.1 ± 9.5	0.568
AV (l/min)	3.37 ± 1.05	3.72 ± 0.87	0.012
VCO_2NL (ml/min)	159.6 ± 51.8	167.9 ± 41.6	0.063
V_d (ml)	152 ± 23	156 ± 20	0.122
Qs/Qt (%)	8.8 (7.1–12.6)	9.4 (6.7–13.0)	0.920

AV = alveolar ventilation; MV = minute ventilation; Qs/Qt = venous admixture; RR = respiratory rate; TV = tidal volume; VCO_2NL = natural lung carbon dioxide removal; V_d = physiologic dead space.

Table 2. Metabolic Parameters during Extracorporeal Carbon Dioxide Removal ($ECCO_2R$) and Acid Load Carbon Dioxide Removal ($ALCO_2R$)

	$ECCO_2R$	$ALCO_2R$	*P* Value
VCO_2tot (ml/min)	196.6 ± 50.9	223.5 ± 42.7	<0.001
VO_2NL (ml/min)	234.1 ± 92.3	256.2 ± 110.0	0.041
VO_2tot (ml/min)	228.9 ± 107.8	260.7 ± 117.3	0.036
EE (kcal/h)	67.0 ± 26.8	76.1 ± 30.7	0.005
RQ	0.92 ± 0.33	0.98 ± 0.30	0.170

EE = energy expenditure; RQ = respiratory quotient; VCO_2tot = total carbon dioxide production; VO_2NL = natural lung oxygen delivery; VO_2tot = total oxygen consumption.

Table 3. Arterial Blood Gas Analyses and Electrolytes Concentration during Extracorporeal Carbon Dioxide Removal ($ECCO_2R$) and Acid Load Carbon Dioxide Removal ($ALCO_2R$)

	$ECCO_2R$	$ALCO_2R$	P Value
pH	7.449 ± 0.055	7.404 ± 0.051	<0.001
Pco_2 (mmHg)	35.5 ± 3.6	34.4 ± 3.5	<0.001
HCO_3– (mEq/L)	24.3 ± 3.7	21.2 ± 3.6	<0.001
BE (mEq/L)	0.9 ± 4.4	–2.9 ± 4.4	<0.001
HbO_2 (%)	97.0 ± 1.2	97.0 ± 1.0	0.545
Po_2 (mmHg)	100.2 ± 12.7	103.9 ± 9.5	0.002
Lactate (mmol/l)	0.66 ± 0.53	5.2 ± 2.0	<0.001
Sodium (mmol/l)	146.4 ± 3.1	145.6 ± 3.4	0.300
Chloride (mmol/l)	108.5 ± 4.3	106.8 ± 3.3	0.046
Potassium (mmol/l)	4.2 ± 0.5	4.1 ± 0.4	0.469
iCa^{++} (mmol/l)	1.3 ± 0.1	1.3 ± 0.1	0.234
Glucose (mg/dl)	67 ± 10	77 ± 12	<0.001

BE = base excess; HbO_2 = hemoglobin oxygen saturation; $HCO3^-$ = bicarbonate ions; iCa^{++} = ionized calcium.

lower than pathological thresholds[21] and bilirubin changes were negligible and lower during $ALCO_2R$ compared with $ECCO_2R$. We observed a slight elevation in white blood cells and in particular neutrophils after instrumentation. Hematocrit and platelets were lower after instrumentation compared with baseline, as an effect of hemodilution after connection to the extracorporeal circuit. As expected, due to heparin infusion, partial thromboplastin time was elevated after instrumentation and was stable during EC, $ALCO_2R$, and $ECCO_2R$. No alteration in prothrombin time, fibrinogen, or D-dimers was observed during the experiment. Alanine transaminase and aspartate transaminase were not different during $ECCO_2R$ and $ALCO_2R$, although they were higher than baseline. Creatinine, amylase, uric acid, and myoglobin were in normal ranges during the whole experiment.

During $ALCO_2R$, the heparin infusion rate was lower (38.2 ± 10.8 IU/kg*h *vs.* 40.0 ± 13.6 IU/kg*h; $P < 0.05$) and the ACT was higher (279 ± 37 *vs.* 262 ± 35; $P < 0.05$) compared with $ECCO_2R$.

Histological examination of lung, heart, liver, and kidney organs did not demonstrate tissue damage (see fig. S3 and table S3, Additional Results, Supplemental Digital Content 1, http://links.lww.com/ALN/B237). Biomarkers of inflammation, oxidant stress, and tissue injury are shown in table S4 (Additional Results, Supplemental Digital Content 1, http://links.lww.com/ALN/B237). Overall, no clinically meaningful changes were detected in these variables, but treated animals showed a statistically significant reduction in heart TBARs concentration, as well as a statistically significant increase in lung total antioxidant and glutathione, liver interleukin-1β, and lung interleukin-8.

Discussion

We investigated the effects of $ALCO_2R$ with LA on ventilation and metabolism of spontaneously breathing sheep. Feasibility, safety, and efficiency of $ALCO_2R$ technique was confirmed and independently validated in a different species adding to previous experiences in swine. To our knowledge, $ALCO_2R$ has never been attempted before in a conscious, spontaneously breathing animal model. For the first time, the effect of $ALCO_2R$ on energy metabolism was evaluated. Infusion of LA was associated with an increase in EE, such that $ALCO_2R$ use was not associated with a decrease in the animals' MV.

$ALCO_2R$ is an innovative, highly efficient $ECCO_2R$ technique based on extracorporeal blood acidification. It opens up the possibility that $ECCO_2R$ systems could be miniaturized, by allowing similar carbon dioxide removal efficiency at half of the currently used BFs. Previous experiments in swine studied the performance of an ML during $ALCO_2R$, verifying its effectiveness in raising VCO_2ML by up to 70%.[11,13,22] The current study was designed to evaluate the ventilatory and metabolic effects of $ALCO_2R$ *via* LA, in the absence of metabolic control, in a different species (*i.e.*, sheep) and in a different laboratory during awake, spontaneously breathing conditions. We confirmed the benefits of extracorporeal blood acidification on ML performance, providing independent validation of the $ALCO_2R$ concept.

Interestingly, we did not detect a significant reduction in MV in this model. This could be explained by the augmentation of caloric intake due to LA infusion. In a previous study involving mechanically ventilated anesthetized swine,[23] LA at 2.5 mEq/min increased VCO_2tot by 5% (*i.e.*, 13 ml/min) relative to an isocaloric glucose infusion, while stabilization

Table 4. Blood Gas Analyses in the Extracorporeal Circuitry during Extracorporeal Carbon Dioxide Removal ($ECCO_2R$) and Acid Load Carbon Dioxide Removal ($ALCO_2R$)

		Blood				Ultrafiltrate	
Phase	Variable	Inlet	Postrecirculation	Post-ML	Outlet	Pre-acid	Post-acid
$ECCO_2R$							
	pH	7.410 ± 0.042[A]	7.448 ± 0.075[B]	7.832 ± 0.138[C]	7.849 ± 0.076[C]	7.838 ± 0.075[C]	—
	P_{CO_2} (mmHg)	42.3 ± 2.4[A]	35.2 ± 3.1[B]	15.1 ± 2.4[C]	14.8 ± 2.0[C]	15.6 ± 1.5[C]	—
	HCO_3^- (mEq/L)	26.4 ± 3.0[A]	23.6 ± 3.4[B]	26.2 ± 4.9[A]	25.6 ± 4.8[A]	26.4 ± 4.6[A]	—
$ALCO_2R$							
	pH	7.370 ± 0.045*[A]	6.986 ± 0.138*[B]	7.489 ± 0.090*[A]	7.485 ± 0.095*[A]	7.492 ± 0.083*[A]	5.296 ± 0.677[C]
	P_{CO_2} (mmHg)	40.5 ± 3.2*[A]	77.8 ± 18.9*[B]	18.3 ± 1.7*[C]	18.3 ± 2.6*[C]	18.1 ± 1.6*[C]	382.1 ± 96.8[D]
	HCO_3^- (mEq/l)	22.9 ± 3.5*[A]	17.7 ± 3.2*[B]	14.0 ± 3.2*[C]	13.7 ± 3.2*[C]	13.8 ± 3.0*[C]	3.4 ± 2.9[D]
	LAC (mmol/l)	5.2 ± 2.0[A]	13.1 ± 2.5[B]	12.3 ± 2.0[B]	12.2 ± 1.9[B]	12.7 ± 1.9[B]	31.7 ± 5.9[C]

Two-way analysis of variance reporting differences in blood gas analyses due to treatment (*i.e.*, $ALCO_2R$ *vs.* $ECCO_2R$) or extracorporeal withdrawal site. $ALCO_2R$ *vs.* $ECCO_2R$ effect (*i.e.*, means on the same column): *$P < 0.05$ *vs.* $ECCO_2R$ (paired *t* test). Withdrawal site effect (*i.e.*, on the same row): samples from different withdrawal sites of the circuitry (*i.e.*, means on the same row) not sharing the same letter are significantly different ($P < 0.05$) (repeated measurements analysis of variance with *post hoc* Tukey honest significant test).

HCO_3^- = bicarbonate ions; LAC = lactate concentration; ML = membrane lung.

of the caloric intake by parenteral nutrition (*i.e.*, glucose infusion) and infusion of insulin was undertaken. The former avoids unwarranted overfeeding, which is known to augment ventilatory needs.[24] The latter is useful to overcome the insulin resistance associated with lactate infusion, and thus maintains euglycemia.[25,26] In previous experiments,[13] $ALCO_2R$ obtained *via* infusion of LA at 2.5 mEq/min yielded an augmentation in VCO_2ML to 45 ml/min. Taken together, these data suggest that $ALCO_2R$, despite increasing VCO_2tot slightly, produces a much higher increase in VCO_2ML and thus can potentially decrease patient ventilatory needs if associated with control of metabolism.

In this experiment, we let the animals eat freely and evaluated their ability to autoregulate their energy metabolism. LA infusion at 1.5 mEq/min was associated with a 10 kcal/h surge (*i.e.*, +12%) in EE, matched by an increase in VCO_2tot of 26.9 and in VO_2tot of 31.8 ml/min (*i.e.*, +13%). HR and CO rose 5% to support this increment in oxygen consumption. Such augmentation in carbon dioxide production increased ventilatory needs (*i.e.*, 7% increases in TV and AV). The sheep significantly compensated for the caloric input associated with LA, albeit not thoroughly. Indeed, because LA at 1.5 mEq/min corresponds to a caloric intake of 30 kcal/h, LA oxidation could have caused a much higher surge in EE (*i.e.*, +44%). This, added to the 67 kcal/h measured during the $ECCO_2R$ phases, may have led to an eventual EE of about 100 kcal/h during LA infusion. Thus, by the Weir equation, we can extrapolate a theoretical VCO_2tot and a VO_2tot during $ALCO_2R$ phases of 320 ml/min each. Such augmentation in VCO_2tot would have doubled AV, whereas it only increased by 7%. Thus, sheep spontaneously managed the LA caloric input to the point of avoiding metabolic and ventilatory derangements, although without intervention to control their metabolism we were not able to observe the effects of $ALCO_2R$ on ventilation. Notably, the caloric effects of LA infusion may differ in patients on controlled diet whose caloric metabolism and endocrine milieu may be deranged by underlying critical illness.[27] Thus, further studies targeting the metabolic and endocrine responses to $ALCO_2R$ are needed before translation of these results into clinical practice may be done.

The use of special techniques (*e.g.*, isotopic carbon-labeled glucose or lactate, direct calorimetry, and calculation of caloric intake from dietary consumption) would be necessary to investigate the metabolic fate of lactate and glucose during $ALCO_2R$. This goes beyond the scope of this study. We did observe that RQ shifted from 0.92 ± 0.33 to 0.98 ± 0.30 from $ECCO_2R$ to $ALCO_2R$, although not significantly. Insofar as the RQ of LA is 1, we may argue that this shift reflected oxidation of LA into carbon dioxide. Moreover, higher glucose levels were observed during LA infusion. This might be explained by the insulin resistance associated with infusion of LA.[25]

During $ALCO_2R$, we did not observe blood chemistry alterations, hemodynamic derangements, or hemolysis. Notably, this study is the first report of in-depth analysis of effects of $ALCO_2R$ on lung function, histopathology, and tissue inflammation. $ALCO_2R$ did not have any detrimental effects on V_d, Qs/Qt, and oxygenation. During LA infusion, we detected a slight increase in core temperature. A thermogenic effect is known to be associated with LA metabolism.[28] Interestingly, despite achieving target ACT levels, we noticed a reduction in anticoagulation requirements during $ALCO_2R$. Postmortem histological examination of the heart, liver, lung, and kidneys did not demonstrate major signs of tissue damage. Interestingly, a previous work[29] by our group showed similar lung histology in control animals subjected to multiday experiments. The indices of oxidative and nitrosative stress, as well as proinflammatory cytokines, suggest that $ALCO_2R$ does not invoke an inflammatory response in the lung or in other organs such as the heart or liver. This was also supported by the observation of no significant elevation in lung myeloperoxidase activity, lung total antioxidant status, or heart TBARs. Taken together

with the plasma biomarkers of tissue function and lack of histological evidence of tissue injury, these data suggest that $ALCO_2R$ is safe. Nevertheless, we cannot exclude the possibility that $ALCO_2R$ may have detrimental effects. The exposure of blood to reduced pH—even for a brief period—may have lasting consequences.[30] Indeed, acidosis is known to inhibit chemotaxis and bactericidal functions of polymorphonuclear leukocytes, as well as cytotoxicity and proliferation of lymphocytes. Similarly, platelet aggregation may be impaired by exposure to lowered pH.[31,32] In contrast, complement protein activation and antibody binding to leukocytes are enhanced during acidosis.[33] Thus, further studies including control animals are needed to determine whether these aspects of the safety profile would be preserved under conditions as a treatment for lung injury or in subjects experiencing infectious diseases.

Direct infusion of acids would have had deleterious effects on blood. To limit such consequences, we utilized an innovative approach to achieve extracorporeal acidification.[14] Briefly, a hemodiafilter is interposed in the extracorporeal circuit after the ML and ultrafiltrate was generated with a peristaltic pump. This ultrafiltrate is acidified and then recirculated before the ML, allowing highly concentrated hyperosmolar acids to be injected into the recirculating ultrafiltrate. Direct injection of concentrated acids into the blood would have caused hemolysis, while on the contrary, a high volume of free water would have been necessary to infuse isotonic acids, causing severe electrolyte derangements. Both these complication are limited by our approach. Notably, we detected plasma-free hemoglobin levels even lower than the ones measured during our previous experiment,[5] where the sole Hemolung device was used.

The impact of LA infusion on acid–base homeostasis and electrolyte equilibrium was minor. In effect, we observed a steady level of plasma lactate after the first hour of $ALCO_2R$, thus suggesting that LA was readily cleared from systemic circulation. Despite the fact that we provided LA at a lower dosage by weight compared with previous swine experiments (0.045 *vs.* 0.057 $mEq \cdot min^{-1} \cdot kg^{-1}$), we nevertheless observed higher lactate plasma levels. This can be ascribed to the lower lactate clearance of sheep (*i.e.*, about $10\,ml \cdot min^{-1} \cdot kg^{-1}$)[34] in contrast to swine (*i.e.*, about $20\,ml \cdot min^{-1} \cdot kg^{-1}$)[23] and to the small size of our experimental animals. Although human lactate clearance by weight is similar to that of sheep (9 to $12\,ml \cdot min^{-1} \cdot kg^{-1}$),[35–37] in an adult man (*i.e.*, weight 70 kg), LA infusion at 1.5 mEq/min (*i.e.*, 0.021 $mEq \cdot min^{-1} \cdot kg^{-1}$) would lead to an acceptable rise in arterial lactate up to 1.7 mEq/L.

We consider this experiment to be a "stress test" of the $ALCO_2R$ technique. In this study, animals with intrinsically limited clearance capacity for lactate and small weight underwent a high-dose $ALCO_2R$ technique without any corrective intervention to stabilize their metabolism. Despite this, in this challenging scenario, $ALCO_2R$ was safe with respect to organ function, electrolyte equilibrium, and acid–base homeostasis, and at least as effective as the standard $ECCO_2R$ technique. We suggest that $ALCO_2R$ should be coupled with metabolic control obtained by euglycemia, as well as with the reduction of caloric intake. This hypothesis will need to be tested in large animal models of lung injury. Moreover, future studies are warranted to evaluate the efficacy of $ALCO_2R$ on a clinically relevant model of hypercapnia and the safety profile of the technique with regard to impairment of immunologic function, platelet aggregation, and activation of complement.

Several solutions may be used to further ameliorate $ALCO_2R$ technique. Other metabolizable acids may be used (*e.g.*, citric and acetic). These compounds may provide advantages other than acidification, such as blood anticoagulation[14] or a more favorable metabolic profile.[38] During $ALCO_2R$, partial or total clearance of the infused acid may be desired. If so, the extracorporeal circuit we used may be easily supplemented with additional devices for ultrafiltrate removal and/or fluid replacement. Such a circuit would not only promote carbon dioxide removal but also provide blood purification and volume control, as well as clearance of the infused acid. Thus, in the setting of multiorgan failure (*e.g.*, renal and pulmonary), *modular* multiorgan support technology[15] can be envisioned. Last, nonmetabolizable acids may be used (*e.g.*, hydrochloric acid), thus avoiding the caloric effects due to infusion of metabolizable acids. However, infusion of a nonmetabolizable acid would result in progressive accumulation of the parent anion (*e.g.*, Cl^-),[39] thus requiring the development of further extracorporeal techniques to remove it. In this regard, we recently elaborated an $ECCO_2R$ technique based on electrodialysis of plasmatic water called respiratory electrodialysis capable of selectively modulating chloride concentration in the extracorporeal circuit.[40] In a mechanically ventilated swine model, respiratory electrodialysis proved efficient in enhancing VCO_2ML and controlling ventilation.

In conclusion, feasibility and effectiveness of extracorporeal blood acidification in enhancing carbon dioxide removal by a ML was confirmed in a different species and by an independent laboratory. Moreover, the $ALCO_2R$ technique was demonstrated to be safe. However, infusion of LA without metabolic control caused a rise in EE that made $ALCO_2R$ no different from standard $ECCO_2R$ with respect to ventilation. We suggest that LA-enhanced $ALCO_2R$ should be coupled with active measures to control metabolism. This technology is being further developed to permit its application in humans.

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Competing Interests

We acknowledge the following potential conflicts of interest. Dr. Pesenti received payment for lectures and service on speaker bureau from Maquet (Maquet Cardiopulmonary, Rastatt, Germany) and Novalung (Novalung, Heilbronn, Germany), received consulting honoraria from Maquet (Maquet Cardiopulmonary, Rastatt, Germany) and Novalung (Novalung, Heilbronn, Germany), and has patents pending or issued (WO2008EP03661, co-owned with Università Milano-Bicocca [Milano, Italy]; IT2012BO00405; IT2012BO00404). Dr. Batchinsky received support for travel and consulting honorarium from ALung Technologies (ALung, ALung Technologies, Pittsburgh, Pennsylvania) and Maquet (Maquet Cardiopulmonary, Rastatt, Germany). Dr. Cancio disclosed that this research was supported, in part, by his appointment to the Knowledge Preservation Program at the U.S. Army Institute of Surgical Research, administered by the Oak Ridge Institute for Science and Education, through an interagency agreement between the U.S. Department of Energy and the U.S. Army Medical Research and Materiel Command. The remaining authors stated that they do not have any potential conflicts of interest. 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.

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