

Extracorporeal Chloride Removal by Electrodialysis (CRe-ED): A Novel Approach to Correct Acidemia.

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A.Z. conceived the study, collected, interpreted and analyzed data, searched literature, and wrote the manuscript; A.Z., D.S. and A.P. developed the CRe-ED prototype; P.C. conceived the study, interpreted data and revised the manuscript; L.C., E.R. and E.S. collected, interpreted and analyzed data, searched literature, and wrote the manuscript; D.S. and T.L. interpreted data and revised the manuscript; V.S., S. A. D., T.M., F.M., M.C., F.Z collected data and revised the manuscript; M.F.

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All authors gave final approval of the version to be published and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy and integrity of any part of the work are appropriately investigated and resolved.

Conflict of interest statement:

A.Z., P.C., D.S., L.G. and A.M.P are inventors of a patent owned with the “Università degli Studi di Milano-Bicocca”, with the “Università degli Studi di Milano” and with the Fondazione IRCCS Ca’ Granda, Ospedale Maggiore Policlinico, Milano” titled: “Extracorporeal circuit system for the treatment of hydroelectrolyte and acid-base blood imbalances”. Application number: WO2016024217.

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At a Glance Commentary

Scientific Knowledge on the Subject:

Acidemia is a severe complication among critically ill patients. Despite being controversial, sodium bicarbonate is frequently used to increase blood pH. This treatment increases the strong ion difference (SID) by elevating sodium concentration, and raises blood pH. However, sodium bicarbonate administration can lead to several side effects including hypernatremia, hyperosmolality and intracellular acidosis.

What This Study Adds to the Field:

This proof of concept study shows the feasibility, safety, and effectiveness of extracorporeal chloride removal through electro dialysis to correct acidemia. Plasma chloride reduction mimics the physiologic renal response to acidosis. Extracorporeal chloride removal by electro dialysis quickly corrects blood pH without altering plasma osmolarity and maintains its effects up to several hours following treatment suspension.

This article has an online data supplement, which is accessible from this issue's table of content online at www.atsjournals.org.

Abstract

Rationale: Acidemia is a severe condition among critically ill patients. Despite lack of evidence, sodium bicarbonate is frequently used to correct pH. However, its administration is burdened by several side effects. We hypothesized that the reduction of plasma chloride concentration could be an alternative strategy to correct acidemia.

Objectives: To evaluate feasibility, safety, and effectiveness of a novel strategy to correct acidemia through Extracorporeal Chloride Removal by Electrodialysis (CRE-ED).

Methods: Ten swine (6 treatments, 4 controls) were sedated, mechanically ventilated and connected to an electrodialysis extracorporeal device capable of removing selectively chloride. In random order, an arterial pH of 7.15 was induced either through reduction of ventilation (respiratory acidosis) or through lactic acid infusion (metabolic acidosis). Acidosis was subsequently sustained for 12-14 hours. In treatment pigs, soon after reaching target acidemia, electrodialysis was started in order to restore pH.

Measurements and Main Results: During respiratory acidosis, electrodialysis reduced plasma chloride concentration by 26 ± 5 mEq/L within 6 hours (final pH= 7.36 ± 0.04). Control animals exhibited incomplete and slower compensatory response to respiratory acidosis (final pH= 7.29 ± 0.03 , $p < 0.001$). During metabolic acidosis, electrodialysis reduced plasma chloride concentration by 15 ± 3 mEq/L within 4 hours (final pH= 7.34 ± 0.07). No effective compensatory response occurred in controls (final pH= 7.11 ± 0.08 ; $p < 0.001$). No complications occurred.

Conclusions: We described the first in-vivo application of an extracorporeal system targeted to correct severe acidemia by lowering plasma chloride concentration. The

CRe-ED proved to be feasible, safe, and effective. Further studies are warranted to assess its performance in presence of impaired respiratory and renal functions.

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Keywords: Acidosis; Electrolytes; Chloride; Extracorporeal Circulation; Electrolysis

INTRODUCTION

Metabolic and respiratory acidosis are pathological conditions frequently occurring in critically ill patients, and are associated with increased morbidity and mortality (1-4). Cardiac arrhythmias, organ failure, suppression of the immune response, release of pro-inflammatory cytokines and bacterial proliferation are severe complications associated with acidemia (5-7).

The treatment of acidosis is primarily aimed at correction of the underlying cause. In case of persistent severe acidemia (blood pH<7.15), sodium bicarbonate is frequently administered in order to correct blood pH (6,8,9), although its efficacy to improve survival has been observed only in a selected subgroup of critically ill patients (10) and patients with sepsis (11). Indeed, intravenous infusion of sodium bicarbonate can cause side effects such as hypernatremia and hyperosmolarity (12). In addition, being bicarbonate hydrated carbon dioxide, its intravenous infusion causes a transient rise in plasma partial pressure of carbon dioxide, potentially leading to paradoxical intracellular acidosis (13). Furthermore, in respiratory acidosis the correction of the underlying cause may not be effectively achieved, especially when lung parenchyma is acutely (or chronically) responsible for the insufficient carbon dioxide elimination (14).

According to Stewart's acid-base approach, the Strong Ion Difference (SID), i.e., the difference between the concentrations of strong cations ($[Na^+]+[K^+]+[Ca^{2+}]+[Mg^{2+}]$) and strong anions ($[Cl^-]+[Lac^-]$), is one of the three independent variables determining blood pH (7,15,16). Sodium bicarbonate infusion causes an increase in plasma sodium concentration leading to SID increase and, consequently, of pH. We hypothesized that the reduction of plasma chloride concentration, mimicking the

physiologic renal response occurring during chronic respiratory acidosis, could be an alternative strategy to normalize blood pH by increasing plasma SID during experimental acidemia (16-18).

Many studies focused on the role of chloride in acid-base equilibrium (19,20). Chloride is the most represented anion in the extracellular fluid, and variations in chloride levels significantly affect the acid-base status (19). Hypo- or hyper-chloremia are frequently observed in acid-base derangements, especially in the ICU setting (20). Of note, the infusion of 0.9% NaCl-containing solutions is known to induce hyperchloremic acidosis through SID reduction (21,22). Conversely, hypochloremic alkalosis is a frequent consequence of loop-diuretic administration, because of urinary chloride loss through $\text{Na}^+\text{-K}^+\text{-2Cl}^-$ pump inhibition (23).

We developed an extracorporeal device to remove chloride in order to decrease its plasma concentration (Chloride Removal Electrodialysis, CRe-ED), thereby increasing SID and pH. This novel technique features an electrodialysis cell, as we previously described (24,25). Here we report the first *in-vivo* application of CRe-ED. In a swine randomized controlled crossover model of metabolic and respiratory acidosis, we tested feasibility, safety and effectiveness of this novel approach to correct severe acidemia.

Some of the results of these studies have been previously reported in the form of an abstract (26).

Methods

The study protocol was approved by the Italian Ministry of Health. Ten healthy piglets (24 ± 2 kg, mean \pm SD) were anesthetized, surgically instrumented, mechanically

ventilated (tidal volume (Vt): 10 mL/kg; respiratory rate (RR): 10 breaths per minute; PEEP: 5 cmH₂O) and connected to an extracorporeal circuit through a double-lumen 14Fr catheter positioned in the external jugular vein. The extracorporeal circuit consisted of a blood circuit, composed of two in-series hemofilters, an electro dialysis circuit and a circuit for lactic acid infusion (acid infusion circuit), connected to the first and the second hemofilter, respectively (**Figure 1** and online data supplement). Anticoagulation was performed with continuous intravenous heparin infusion.

Study design

Six piglets were assigned to the CRe-ED treatment (*treatment group*) and four were controls (*control group*) (**Figure 2**). Regardless of treatment allocation, each swine randomly underwent both respiratory and metabolic acidosis (crossover randomization).

At baseline, respiratory rate was adjusted in order to achieve an arterial pH between 7.38 and 7.42. Thereafter, pH was lowered to reach a target of 7.15 ± 0.02 through either a decrease in minute ventilation by respiratory rate reduction (respiratory acidosis), or by a continuous infusion of 1.5 M lactic acid into the acid infusion circuit (metabolic acidosis).

In the *treatment* group, once the target pH was reached, CRe-ED was started and continued until normalization of arterial pH. Thereafter, CRe-ED was stopped and animals were monitored for 8 hours. In contrast, in the control group, CRe-ED was not applied, and, after induction of acidosis, animals were monitored for a total period comparable with the CRe-ED duration plus 8-hr monitoring period after CRe-ED treatment (14 hours of respiratory acidosis and 12 hours of metabolic acidosis). Arterial pCO₂ (± 3 mmHg) and lactate (± 1 mEq/L) were kept constant throughout all

the experiments until the end of observation period, by modulating respiratory rate and lactic acid infusion, respectively.

Before the second phase of the assigned acidosis, plasma chloride concentrations were restored to baseline values (± 2 mEq/L) by the infusion of 1.5 M hydrochloric acid through the acid infusion circuit (re-chloration phase).

Data collection

At the end of each step (i.e., baseline, start and end of CRe-ED treatment, 4h and end (8h) of observation period), every hour during CRe-ED treatment and every two hours during the control observation period, we collected hemodynamic and ventilator parameters; laboratory biomarkers, and blood gas analyses and electrolytes from arterial line and extracorporeal circuit; urinary output, pH and urinary electrolytes (27); glomerular filtration rate.

Statistical Analysis

Data are presented as mean \pm standard deviation unless otherwise specified. Differences among groups were tested by a 2-way analysis of variance for repeated measurements over time. Multiple comparisons analyses were tested among different time points versus baseline, start and end of CRe-ED treatment using Dunnet's test. A p-value <0.05 (two-tailed) was deemed statistically significant.

Please refer to the online data supplement for detailed methods.

Results

Respiratory acidosis

During respiratory acidosis, targeted pH was achieved by decreasing minute ventilation from 4.0 ± 0.8 L/min to 1.8 ± 0.2 ($p < 0.001$) in the treatment group, and from 3.7 ± 0.7 to 1.6 ± 0.2 L/min ($p < 0.001$) in the control group (**Table 1**). An increase in $p\text{CO}_2$ from 45 ± 6 mmHg to 91 ± 12 mmHg ($p < 0.001$) and from 49 ± 4 mmHg to 95 ± 6 mmHg ($p < 0.001$) was recorded in the treatment and control groups, respectively. Subsequently, $p\text{CO}_2$ was kept constant during the whole experiment in both groups (**Figure 3A**).

In the treatment group, after the application of CRe-ED, plasma chloride concentration decreased from 103.7 ± 3.4 mEq/L to 78.2 ± 8.0 mEq/L ($p < 0.001$) (**Figure 3B**), thereby leading to an increase in plasma SID from 41.4 ± 4.4 mEq/L to 64.2 ± 9.5 mEq/L, ($p < 0.001$), and an increase in HCO_3^- from 30.7 ± 3.6 mEq/L to 54.3 ± 6.6 mEq/L, ($p < 0.001$) (**Table 1**). As plasma chloride concentration decreased and SID increased, arterial pH increased from 7.16 ± 0.01 to 7.40 ± 0.01 ($p < 0.001$). Correction of pH during CRe-ED treatment was achieved in 333 ± 59 minutes (range, 255-430 minutes) (**Figure 3C**). During the 8-h observation period, pH slightly decreased from 7.40 ± 0.01 to 7.36 ± 0.04 ($p < 0.001$), as plasma chloride concentration increased from 78.2 ± 8.0 mEq/L to 84.8 ± 4.6 mEq/L ($p < 0.001$) (**Figure 3B,C**).

In control animals, we observed a slower compensatory response to respiratory acidosis: SID and HCO_3^- progressively increased from 41.7 ± 3.5 to 53.3 ± 3.9 mEq/L (acidosis induction to end of observation, $p < 0.001$), and from 32.6 ± 2.8 to 43.6 ± 2.3 mEq/L ($p < 0.001$), respectively (**Table 1**). Accordingly, over the whole 14 hours of respiratory acidosis, pH increased in the control group from 7.16 ± 0.01 to 7.29 ± 0.03 ,

though up to a lower level as compared to the treatment group ($p < 0.001$) (**Figure 3C**).

Table 1 summarizes respiratory, hemodynamic, plasma electrolytes, acid-base status and renal function changes over time among the groups during respiratory acidosis. Plasma biochemistry and respiratory variables in both groups, as well as electrolytes concentrations in the treatment group along the withdrawal port of the extracorporeal circuit are reported in Table E1-E4.

Urinary response

At acidosis induction, urinary pH (pH_u) decreased in the treatment (acidosis induction versus baseline, 4.9 ± 0.3 versus 5.8 ± 0.5 , $p = 0.013$) and control group (4.7 ± 0.2 versus 5.5 ± 0.5 , $p = 0.118$), although only in the treatment group pH reduction reached statistical significance (**Figure 4A**). No significant change of urinary chloride and ammonium concentration was observed in both treatment and control groups (**Table 2** and **Figure 4 A-C**).

In the treatment group, during CRe-ED treatment, urinary chloride concentration significantly decreased from 179.3 ± 29.2 to 34.3 ± 14.1 mEq/L ($p < 0.001$) and urinary anion gap (AG) increased accordingly from -79.6 ± 32.6 to 79.9 ± 65.3 ($p < 0.001$) (**Table 2**). Consequently, pH_u increased from 4.9 ± 0.3 up to 6.9 ± 1.0 ($p < 0.001$) and remained stable during the 8h observation (**Figure 4A**, **Table 2**). Overall, the restoration of baseline blood pH required the removal of 392 ± 65 mEq of chloride ions, of which 336 ± 54 mEq were removed by CRe-ED and 56 ± 37 mEq by the renal system, mainly excreted during the acidosis induction phase (**Figure 4B**).

In the control group, a total of 248 ± 65 mEq of chloride were removed by the kidneys during the entire 14-h period of respiratory acidosis, causing constantly negative values of urinary anion gap (**Table 2**), and leading to a decrease in plasma chloride concentration from 105 ± 4 (acidosis induction) to 92 ± 5 mEq/L (end of observation) ($p < 0.001$) (**Figure 4B**). Of note, after the induction of respiratory acidosis, creatinine clearance increased from 69.1 ± 13.7 (baseline) to a maximum value of 141.4 ± 29.9 mL/min ($p = 0.002$) (**Table 1**).

Metabolic acidosis

Metabolic acidosis was induced and maintained by infusing 2.2 ± 0.4 mL/kg/h of 40% lactic acid (3.5 ± 1.3 mmol/min) on the acid infusion circuit. From baseline to the end of lactic acidosis induction, plasma lactate concentration increased from 0.6 ± 0.2 to 13.1 ± 1.5 mmol/L ($p < 0.001$) in the treatment group and from 0.6 ± 0.2 to 12.9 ± 1.5 mmol/L ($p < 0.001$) in the control group (**Figure 5A**). As a consequence, plasma SID decreased from 38.7 ± 5.4 to 26.5 ± 2.7 mEq/L in the treatment group ($p < 0.001$) and from 42.6 ± 6.1 to 28.6 ± 5.2 mEq/L in the control group ($p = 0.001$), while HCO_3^- decreased from 28.5 ± 5 to 16.0 ± 2.4 mEq/L in the treatment group ($p < 0.001$), and from 29.1 ± 1.4 to 16.6 ± 1.4 mEq/L in the control group ($p < 0.001$) (**Table 3**). Targeted arterial pH was similarly achieved in both treatment and control groups (7.16 ± 0.02 and 7.16 ± 0.02 respectively, $p < 0.001$ versus baseline) (**Figure 5B**).

In the treatment group, plasma chloride was removed by CRe-ED, and its concentration decreased from 106 ± 4 to 91 ± 6 mEq/L, from the beginning to the end of CRe-ED treatment, respectively ($p < 0.001$) (**Figure 5C**). Consequently, plasma SID and HCO_3^- significantly increased (**Table 3**) and arterial pH returned to baseline after 199 ± 43 minutes of CRe-ED treatment (range, 135-255 minutes) (**Figure 5C**). Of

note, arterial pH remained stable from the end of the CRe-ED treatment to the end of the observation period (**Figure 5C**). In contrast, after infusion of lactic acid in the control group, no spontaneous correction of acidosis was observed (**Figures 5C**).

Table 3 summarizes **respiratory**, hemodynamic, plasma electrolytes, **acid-base status and renal function** changes over time among the groups during lactic acidosis. Plasma biochemistry and respiratory variables in both groups, as well as electrolyte concentrations in the treatment group along the withdrawal port of the extracorporeal circuit are reported in Table E5-E8.

Urinary response

During acidosis, in both treatment and control groups, urinary pH (pH_u) decreased (**Figure 6A**) while urinary ammonium and chloride concentration increased only in the control group (**Table 2** and **Figures 6 A-C**).

In the treatment group, during CRe-ED treatment, pH_u remained significantly lower compared to baseline (from 5.0 ± 0.4 versus 5.7 ± 0.7 , $p=0.016$), did not differ compared to acidosis induction (versus 4.7 ± 0.3 , $p=0.569$) and remained stable during the 8h of observation (**Figure 6A** and **Table 2**). Furthermore, urinary chloride concentration decreased from 85.8 ± 51.6 (acidosis induction) to 34.5 ± 16.7 mEq/L (end of treatment) ($p=0.160$) (**Figure 6B**) and urinary AG increased accordingly from -1.4 ± 54.5 to 46.8 ± 45.4 ($p=0.123$), although these differences did not reach statistical significance (**Table 2**). Overall, normal arterial pH was restored by the removal of 185 ± 56 mEq of chloride, of which 159 ± 55 mEq were removed by CRe-ED and 26 ± 9 mEq by urinary output, mainly excreted during the acidosis induction phase (**Figure 6B**).

In the control group, cumulative urinary chloride excretion was 77 ± 46 mEq (**Figure 6B**).

In all the treated animals by CRe-ED, no treatment was withdrawn due to the development of complications, including cardiac arrhythmias, pulmonary hypertension, or visible signs of hemolysis.

Discussion

In this experimental proof of concept study, we were able to reduce plasma chloride concentration *in vivo* through an extracorporeal device characterized by an electro dialysis cell (CRe-ED), aimed at selective chloride removal. The removal of plasma chloride rapidly corrected the acidemia regardless of the respiratory or metabolic origin of the acidosis. Indeed, during respiratory acidosis, six hours of CRe-ED treatment increased arterial pH from 7.16 to 7.40 by decreasing chloride concentration from 104 to 78 mEq/L. During metabolic acidosis, less than four hours of CRe-ED treatment were necessary to increase arterial pH from 7.16 to 7.40, through a reduction of chloride from 106 to 92 mEq/L. Within the same time frame, six and four hours, control swine subjected to comparable acidosis were able to increase arterial pH up to 7.29 ± 0.03 only during respiratory acidosis while no modifications of pH were recorded during metabolic acidosis.

This is the first *in vivo* application of an extracorporeal electro dialytic device aimed at restoring physiologic pH during severe acidosis through selective plasma chloride removal. Chloride is the main anion in the extracellular fluid, and it has a key role in the regulation of acid-base equilibrium. As an example, because of SID reduction, hyperchloremia is a well-known cause of metabolic acidosis, as reported after administration of normal saline (19,20). Conversely, gastrointestinal, renal or sweat

chloride loss may lead to hypochloremia, which, by increasing SID, causes metabolic alkalosis (28). Hypochloremia is likewise found in hypercapnic patients suffering from chronic obstructive pulmonary disease as a response to chronic respiratory acidosis (29). Selective binding and removal of hydrochloric acid from the gastrointestinal tract has been recently introduced as a novel treatment for chronic metabolic acidosis in patients suffering from chronic kidney disease. In a recently published randomized clinical trial, veverimer was able to achieve in 12 weeks a least squares mean change from baseline of blood bicarbonate of +4.4 mEq/L compared to +1.8 mEq/L of the placebo group (30).

In our experimental setting, investigating acute acidosis, CRe-ED normalized pH from 7.15 to 7.40 extremely rapidly. This was achieved through an average reduction of about 26 and 15 mEq/L in plasma chloride concentration, leading to an increase in blood bicarbonate of about 24 and 12 mEq/L, during respiratory and metabolic acidosis, respectively. According to Stewart's model, these decreases in chloride concentrations lead to an equivalent increase in SID, provided that strong cation concentrations remain constant. Sodium bicarbonate is a known therapeutic option to treat metabolic acidosis (6,12). However, if identical pH corrections were performed by administering sodium bicarbonate, a similar increase in SID would have been achieved at the cost of a marked increase of sodium concentration, with an increase in plasma osmolarity, and the consequent water shifts from the intracellular toward the extracellular fluid compartment. Indeed, sodium bicarbonate is burdened by side effects, such as increased plasma osmolarity, intracellular acidosis, and even risk of myelinolysis, when rapidly administered (6,12,13,31).

As mentioned above, the rationale of CRe-ED arises from the physiological response of the renal system to acidosis, and, in parallel, from the physico-chemical approach to acid-base by Peter Stewart. According to traditional knowledge of renal physiology, the kidney reacts to acid load by enhancing net acid excretion (mainly through an increased NH_4^+ excretion), and HCO_3^- reabsorption / regeneration (32). The net result will be an increase in plasma HCO_3^- concentration. However, due to electroneutrality, an increased HCO_3^- concentration will inevitably be associated with a reduction of strong anions (Cl^-), provided that osmolality remains constant. In control animals, during respiratory acidosis urinary pH decreased, paralleled by a significant increase in urinary NH_4^+ excretion. Such modifications were, however, also associated with a parallel increase in urinary Cl^- excretion, likely due to an increase in Cl^- voltage-dependent channel secretion to preserve electroneutrality after the increased activity of distal H^+ -ATPase pumps (33-34). Indeed, CRe-ED treatment switched-off such compensatory mechanism, possibly as a consequence of a very rapid reduction of the available Cl^- content in the peritubular capillary, and of the increase in urinary pH. During metabolic acidosis, we observed a similar, although markedly blunted, course of urinary Cl^- and NH_4^+ concentration, while in contrast, urinary pH remains acidotic also during CRe-ED treatment. Although a full understanding of this mechanism cannot be achieved, we may speculate that a significant urinary excretion of another strong anion, i.e., lactate, was present, thereby limiting urinary pH correction despite the selective plasma Cl^- removal and the associated progressive pH correction.

In order to selectively reduce the concentration of plasma chloride while maintaining a constant plasma sodium concentration, we have modified the electro dialysis circuit previously used to enhance extracorporeal CO_2 removal (23,25). Treatment of

metabolic acidosis by conventional continuous renal replacement therapy (CRRT) would have partially increased blood pH by removing fixed acids with little, if any, change in plasma chloride concentration (35-37), depending on the composition of the dialytic bath. Treatment of respiratory acidosis by conventional CRRT might be potentially effective at increasing blood pH through a chloride reduction exclusively in conjunction with the use of replacement fluids with low or zero chloride concentration. Unfortunately, the use of such fluids are not commercially available today. However, CRRT would have also changed plasma sodium concentration towards the concentration of the replacement solution. Therefore, the final effect on blood pH and SID would have resulted by both the modifications of chloride and sodium, thus making it impossible to assess the sole effect of chloride reduction. Indeed, CRe-ED provided an effective treatment of acidemia through a selective and controlled plasma chloride removal.

After pH correction by CRe-ED, plasma chloride concentration was lowered to 91 ± 6 and 78 ± 8 mEq/L during metabolic and respiratory acidosis, respectively. We recorded no major adverse events of either hemodynamic, blood gas or biochemical nature. Furthermore, no visible signs of haemolysis were detected despite the concentration of plasma chloride in the post-filter blood was as low as 85 ± 7 mEq/L and 71 ± 8 mEq/L after metabolic and respiratory acidosis correction, respectively, with a chloride shift across the first hemofilter of 5.0 ± 2.1 mEq/L and 7.4 ± 1.9 mEq/L, and an associated pH of 7.29 ± 0.09 and 7.37 ± 0.02 , respectively. However, despite maintaining a steady plasma osmolarity, we do not know to what extent chloride removal can be forced before causing harmful side effects *in vivo*, and this issue will require future investigation. Indeed, both hyper- and hypochloremia have been associated with adverse outcomes (38-40), when associated with pathological

conditions. Hyperchloremia was associated with immune activation, clotting impairment, decreased splanchnic perfusion, renal vasoconstriction and the consequent reduction in glomerular filtration (19,20,41). In the recent SALT randomized trial, ICU patients exposed to higher volumes of saline infusion – which was associated with higher levels of serum chloride – presented a higher incidence of the composite outcome of major adverse kidney events at 30 day follow up, compared to larger volumes of balanced crystalloid infusions. This finding suggested a potential dose-response relationship between intravenous fluid-related hyperchloremia and outcome (42). On the other hand, hypochloremia is considered a marker of illness severity (43), even though no specific threshold appears to be independently associated to unfavourable outcomes and a definite causal connection is lacking. To the best of our knowledge, no complications attributable to low chloride levels have been reported even at very low levels of chloremia (44).

As a secondary aim, our study was also designed to evaluate the hypothesis that the increment in pH achieved with a single CRe-ED treatment, would have been sustained even after the suspension of the treatment despite maintaining the underlying acidosis. This hypothesis has been partially refuted by the experimental data observed. Indeed, shortly following CRe-ED suspension during both respiratory and metabolic acidosis, pH slightly dropped to 7.36 and 7.33 at 4h, but it remained subsequently stable until the end of the observation period (7.36 and 7.34). The drop in pH was associated to an increase of plasma chloride concentration by about 7 and 4 mEq/L during the observation period of the respiratory and metabolic acidosis, respectively. Indeed, such increase in chloremia was not associated with a renal-induced chloride retaining state occurring after the achievement of very low level of hypochloremia, since urinary excretion of chloride remained active even after the end

of CRe-ED treatment (see Figure 4c and 6c). We may speculate that plasma chloride increase may be at least partially associated with a chloride shift back into the plasma from other connecting fluid compartments, most likely from red blood cells (45,46), during a very rapid, and likely non-physiological reduction of plasma chloride concentration. In addition, intravenous administration of fluid maintenance during the observation period, which included chloride, may have played a role. Further data are warranted to evaluate the potential stability of low levels of hypochloremia over a longer period of time.

The application of CRe-ED, although still as a prototype in a pilot animal study, opens up the possibility of promising future applications, that may have an important impact in clinical settings.

First, CRe-ED may represent the only possibility of rapidly correcting acidosis when the renal system is failing. In the present study, we applied CRe-ED to healthy animals. However, patients admitted to ICU, often develop impaired renal function, a further hurdle which affects the physiologic response to acidosis (47). It is therefore conceivable that critically ill patients, such as patients with septic shock, acute respiratory failure, and other pathological processes, may benefit from CRe-ED even at greater extent.

Second, CRe-ED may represent a unique method facilitating the tolerance of hypercapnia, when related to the necessity of reducing minute ventilation during mechanical ventilation, such as during ARDS. In the present experimental setting of respiratory acidosis, chloride removal of about 400 mEq allowed to reduce minute ventilation down to 50% while keeping a constant $p\text{CO}_2$ (as high as about 90 mmHg). Extracorporeal chloride removal could be applied to patients with respiratory failure

(i.e. ARDS patients) to buffer acidemia generated by permissive hypercapnia (7,48). This could be beneficial in severe ARDS patients, enabling a low tidal volume protective mechanical ventilation without the drawback of acidemia consequent to permissive hypercapnia induction. Furthermore, preclinical evidence supports the association of severe hypercapnic acidosis with immunosuppression, bacterial proliferation in sepsis and increased injury in lung cells (49-51). Caples SM et al. reported that buffering hypercapnic acidosis can protect alveolar cells and enhance the repair of wounded cells after exposure to injurious ventilation (51). The application of CRe-ED in ARDS patients could easily support even the use of ultra-low tidal volume ventilation (52).

Third, CRe-ED might be suitable as an intermittent treatment to enhance the tolerance of chronic hypercapnia, such as in the case of patients with chronic respiratory failure. Patients with chronic obstructive pulmonary disease often develop hypochloremia, which compensates for hypercapnia and a high plasma HCO_3^- concentration. However, this compensation is hardly achievable in the presence of concomitant chronic kidney disease, and may be limited by the maximal chloride excretive capability of the renal system. In this scenario, patients with end-stage respiratory failure are not only challenged by hypoxemia, but they are also unable to handle further minimal loads of CO_2 . We speculate that the application of an intermittent chloride removal may increase the tolerance to higher levels of CO_2 , thus extending the life span and improving the quality of life for those patients.

Fourth, therapeutic modulation of plasma chloride concentration could have some advantages in the treatment of acid-base and hydro-electrolytic imbalances. Chloride removal proved to be effective in increasing blood pH, and, in case of hyperchloremic

acidosis, such as following administration of conspicuous amounts of normal saline, it would also effectively treat the cause of acidosis. On the opposite side, the administration of highly concentrated hydrochloric acid through a dialysis circuit, as performed during the re-chloration phases, could be a feasible and safe alternative to the historical treatment of systemic alkalosis based on direct infusion of diluted hydrochloric acid (53,54).

Our study presents also some limitations. First, this experimental investigation was a proof-of-concept study in healthy animals. Therefore, no information about the effect of normalization of respiratory or metabolic acidosis in critically ill patients, such as during respiratory failure or renal impairment can be determined. Second, we did not evaluate higher safety limits of chloride removal, both in terms of total amount of chloride to be removed and velocity of chloride shift from plasma. Third, we cannot establish the time of chloride removal by CRe-ED in humans, but we assume it may be longer relative to the time needed to remove chloride in this preclinical investigation model, based on estimated total body chloride. Fourth, during the metabolic acidosis experiment, compensatory respiratory response to acidosis was not permitted, as arterial PCO_2 was maintained constant from baseline until the end of the observation period.

In conclusion, we documented the first *in vivo* application of an extracorporeal system targeted to correction of severe acidemia by lowering plasma concentration of chloride. In two experimental models of metabolic and respiratory acidosis, CRe-ED proved to be superior in normalizing arterial pH compared to the physiological compensatory response in healthy controls. Further studies are warranted to access

the feasibility and safety profile of CRe-ED, especially with regard of the amount and the shift velocity of plasma chloride modulation.

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Figure 1.

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Figure legends

Figure 1. Schematic of extracorporeal circuit and electro dialysis (ED) unit. In the blood circuit, blood flowed (150 and 200 mL/min, during respiratory and metabolic acidosis experiments, respectively) through two serially connected hemofilters. In the ED circuit, ultrafiltrate was generated by the first hemofilter (30 mL/min), flowed through a calcium filter, the ED base chamber, a membrane lung (ML), and after a safety filter it was reinfused between the two hemofilters. To prevent calcium precipitation inside the ED cell, 30 mL/min of ultrafiltrate was recirculated from the outlet of the ED base chamber prior to the calcium filter, in order to alkalize the ultrafiltrate and to allow calcium precipitation and entrapment into the calcium filter. A gas mixture of CO₂ was delivered into the gas inlet port of the ML to target the pH of ultrafiltration fluid after the ML between 7.50 and 8.50. Finally, 30 mL/min of normal saline solution was driven towards the ED acid chamber and then wasted. The ED circuit was powered only during the chloride removal ED (CRe-ED) treatment. In the acid infusion circuit, outlet and inlet hemodiafiltration ports of the second hemofilter were connected to create a closed loop circuit, with the dialysate flowing countercurrent to the blood flow. Lactic acid 1.5 M and hydrochloric acid 1.5 M were infused into the acid infusion circuit during the experimental phases of the metabolic acidosis and re-chloration phases, respectively. Nine different withdrawal ports were arranged in the circuit: 1 blood pre-filter, 2 blood post-filter, 3 ultrafiltrated fluid, 4 ED base inlet pre-filter, 5 ED base inlet post-filter, 6 ED base outlet pre-ML, 7 ED base outlet post-ML, 8 ED acid inlet and 9 ED acid outlet (**Figure 1**). Enlarged figure in **Figure 1** represents the details of the ED unit. The ED unit was customized with two bipolar membranes (bpm) and one anionic membrane (am) creating four chambers: anode, acid chamber, base chamber and cathode. Electric current forces the

movement of anions (mainly chloride) from the base to the acid chamber (**Figure 1**, online data supplement). In red, the blood circuit; in light and dark green, the ED circuit; in light blue, the acid infusion circuit.

Figure 2. Study design. First, the animals were allocated in two groups, the *Treatment group* (Chloride Removal Electrodialysis (CRe-ED) group), on the left, and the *Control group*, on the right. Regardless of treatment assignment, each piglet was subjected to two randomly ordered experiments (metabolic and respiratory acidosis). In the CRe-ED group, both experiments consisted of: 1) a *baseline* step; 2) an *acidosis induction* phase (either metabolic with infusion of lactic acid by the acid infusion circuit or respiratory by reducing the respiratory rate); 3) the restoration of the baseline arterial pH (pH_a) by *CRe-ED treatment*; and 4) finally 8 hours of observation (*Observation Treatment*). In control subjects, CRe-ED was not applied and thus, after the 1) *baseline* and 2) *acidosis induction* phase, 3) the physiological response to acidemia was observed for 12 and 14 hours during metabolic and respiratory acidosis, respectively (*Observation Control*). Before starting the second randomized experiment, plasma chloride was restored to the baseline value (± 2 mEq/L) of the first experiment.

Figure 3. Induction of respiratory acidosis and effects of CRe-ED treatment on blood acid-base balance. Black circles with dashed line represent the treatment group. White squares with dotted line represent the control group. Time 0 corresponds to the start of the CRe-ED or of the observation in the treatment and control group, respectively. Data are reported as mean \pm SD. p-value of difference between groups is reported by a 2-way ANOVA for repeated measurements over

time. * $p < 0.05$ vs baseline step in treatment group; ‡ $p < 0.05$ vs baseline step in control group.

Figure 4. Urinary electrolytes and pH changes over time after induction of respiratory acidosis and CRe-ED treatment. Black circles with dashed line represent the treatment group. White squares with dotted line represent the control group. Red circles with dashed-dotted line represent the total amount of chloride removed in the treatment group by CRe-ED and urine output. Time 0 corresponds to the start of the CRe-ED or of the observation in the treatment and control group, respectively. Data are reported as mean \pm SD. p-value of difference between groups is reported by a 2-way ANOVA for repeated measurements over time. * $p < 0.05$ vs baseline step in treatment group; ‡ $p < 0.05$ vs baseline step in control group.

Figure 5. Induction of metabolic lactic acidosis and effects of CRe-ED treatment on blood acid-base balance. Black circles with dashed line represent the treatment group. White squares with dotted line represent the control group. Time 0 corresponds to the start of the CRe-ED or of the observation in the treatment and control group, respectively. Data are reported as mean \pm SD. p-value of difference between groups is reported by a 2-way ANOVA for repeated measurements over time. * $p < 0.05$ vs baseline step in treatment group; ‡ $p < 0.05$ vs baseline step in control group.

Figure 6. Urinary electrolytes and pH changes over time after induction of metabolic lactic acidosis and CRe-ED treatment. Black circles with dashed line represent the treatment group. White squares with dotted line represent the control group. Red circles with dashed-dotted line represent the total amount of chloride removed in the treatment group by CRe-ED and urine output. Time 0 corresponds to

the start of the CRe-ED or of the observation in the treatment and control group, respectively. Data are reported as mean \pm SD. p-value of difference between groups is reported by a 2-way ANOVA for repeated measurements over time. * p<0.05 vs baseline step in treatment group; ‡ p<0.05 vs baseline step in control group.

Table 1. Physiological variables and electrolytes changes during and after induction of respiratory acidosis

Variables	Acidosis Induction										p-value of difference between groups
	Baseline		CRe-ED treatment				Observation				
	Treatment (n=6)	Control (n=4)	Start		End		4 hours		8 hours		
	Treatment (n=6)	Control (n=4)	Treatment (n=6)	Control (n=4)	Treatment (n=6)	Control (n=4)	Treatment (n=6)	Control (n=4)	Treatment (n=6)	Control (n=4)	
Respiratory variables											
Vt/kg [mL]	10.1 ± 0.2	10.0 ± 0.3	10.2 ± 0.4	10.2 ± 0.6	10.0 ± 0.4	9.8 ± 0.4	9.8 ± 0.5	9.8 ± 0.5	9.7 ± 0.5	10.3 ± 1.1	0.806
RR [breaths/min]	17 ± 3	15 ± 3	8 ± 1*	7 ± 1‡	8 ± 1*	8 ± 1‡	8 ± 1*	8 ± 1‡	9 ± 2*	8 ± 1‡	0.255
Minute Ventilation [L/min]	4.0 ± 0.8	3.7 ± 0.7	1.8 ± 0.2*	1.6 ± 0.2‡	1.9 ± 0.2*	1.9 ± 0.3‡	1.9 ± 0.3*	1.8 ± 0.3‡	1.9 ± 0.4*	1.9 ± 0.3‡	0.648
Electrolytes and acid-base status											
Na ⁺ [mmol/L]	136.7 ± 2.6	137.3 ± 3.0	137.0 ± 2.5	138.3 ± 4.1	136.0 ± 3.2	137.5 ± 4.4	137.0 ± 3.2	136.5 ± 5.5	136.8 ± 3.3	137.8 ± 5.6	0.716
K ⁺ [mmol/L]	3.9 ± 0.3	4.0 ± 0.7	4.1 ± 0.4	4 ± 0.6	3.3 ± 0.2*	3.8 ± 0.3	3.4 ± 0.3	3.7 ± 0.3	3.4 ± 0.5	3.7 ± 0.3	0.259
Ca ²⁺ [mmol/L]	1.4 ± 0.1	1.3 ± 0.2	1.4 ± 0.1	1.3 ± 0.2	1.2 ± 0.1*	1.2 ± 0.1	1.1 ± 0.1*	1.2 ± 0.1	1.1 ± 0.1*	1.3 ± 0.1	0.718
Mg ²⁺ [mg/dL]	1.8 ± 0.3	1.9 ± 0.2	1.9 ± 0.4	2.1 ± 0.4	1.4 ± 0.3*	1.8 ± 0.2	1.7 ± 0.3	2.0 ± 0.3	1.8 ± 0.2	1.8 ± 0.2	0.297
HCO ₃ ⁻ [mEq/L]	27.3 ± 3.6	30.6 ± 2.2	30.7 ± 3.6*	32.6 ± 2.8	54.3 ± 6.6*	37.4 ± 1.5‡	49.5 ± 4.9*	40.6 ± 2.2‡	49.3 ± 2.8*	43.6 ± 2.3‡	0.043

BE [mmol/L]	2.4 ± 3.4	5.5 ± 2.0	2.8 ± 3.4	4.1 ± 2.3	27.3 ± 5.9*	9.8 ± 1.3 [‡]	22.3 ± 4.4*	13.1 ± 2.1 [‡]	22.1 ± 2.3*	16.2 ± 2.1 [‡]	0.020
Lactate [mmol/L]	0.7 ± 0.2	0.6 ± 0.4	0.3 ± 0.1*	0.4 ± 0.2 [‡]	0.5 ± 0.1*	0.3 ± 0.1 [‡]	0.4 ± 0.1*	0.3 ± 0.1 [‡]	0.5 ± 0.1*	0.3 ± 0.1 [‡]	0.839
SID [mEq/L]	38.8 ± 4.5	41.0 ± 2.4	41.4 ± 4.4	41.7 ± 3.5	64.2 ± 9.5*	47.6 ± 3.1	60.6 ± 6.6*	49.1 ± 4.3	58.6 ± 4.2*	53.3 ± 3.9 [‡]	< 0.001
Hemodynamics											
HR [bpm]	107 ± 25	116 ± 36	120 ± 21	137 ± 34	123 ± 16	131 ± 26	127 ± 25	126 ± 16	121 ± 28	112 ± 22	0.717
MAP [mmHg]	96 ± 22	111 ± 18	102 ± 17	98 ± 17	86 ± 13	93 ± 10	96 ± 8	91 ± 13	87 ± 10	88 ± 11	0.679
CVP [mmHg]	8 ± 3	12 ± 2	8 ± 3	11 ± 2	7 ± 3	10 ± 3	8 ± 2	11 ± 3	9 ± 2	11 ± 4	0.093
PMAP [mmHg]	26 ± 4	30 ± 9	34 ± 7*	36 ± 7	27 ± 5	31 ± 4	29 ± 3	31 ± 3	28 ± 4	32 ± 3	0.230
WP [mmHg]	10 ± 3	14 ± 4	10 ± 4	13 ± 2	9 ± 3	12 ± 4	10 ± 2	13 ± 3	11 ± 2	13 ± 4	0.146
CO [L/min]	3.4 ± 0.6	3.5 ± 0.3	4.0 ± 0.5	4.6 ± 1.1	3.1 ± 0.8	4.7 ± 0.8	3.8 ± 0.9	5.0 ± 1.2 [‡]	3.8 ± 0.5	4.2 ± 0.6	0.055
Renal function											
Urea [mg/dL]	14.7 ± 3.2	13.3 ± 7.5	14.8 ± 4.0	14.0 ± 8.1	20.2 ± 5.2	17.8 ± 8.7	23.3 ± 6.0*	22.8 ± 5.0 [‡]	23.5 ± 5.4*	19.5 ± 8.4	0.565
Creatinine [mg/dL]	0.7 ± 0.1	0.7 ± 0.2	0.7 ± 0.1	0.6 ± 0.2	0.8 ± 0.1	0.6 ± 0.2	0.7 ± 0.1	0.7 ± 0.2	0.7 ± 0.1	0.6 ± 0.2	0.167
Creatinine Clearance [mL/min]	57.9 ± 17.0	69.1 ± 13.7	104.3 ± 57.7*	108.4 ± 26.7	81.0 ± 21.2	141.4 ± 29.9 [‡]	64.2 ± 23.6	103.1 ± 4.4	80.7 ± 33.2	109.6 ± 35.6	0.060

Data are reported as mean \pm SD. p-value of difference between groups is reported by a 2-way ANOVA for repeated measurements over time. * p<0.05 vs baseline step in treatment group; ‡ p<0.05 vs baseline step in control group.

Table 2. Urinary sodium, potassium and anion gap changes during and after induction of respiratory and metabolic acidosis

Variables	Acidosis Induction										p-value of difference between groups
	Baseline		CRe-ED treatment				Observation				
			Start		End		4 hours		8 hours		
	Treatment (n=6)	Control (n=4)	Treatment (n=6)	Control (n=4)	Treatment (n=6)	Control (n=4)	Treatment (n=6)	Control (n=4)	Treatment (n=6)	Control (n=4)	
Respiratory acidosis											
Na⁺_U [mEq/L]	48.2 ± 44.7	67.0 ± 54.8	61.1 ± 37.1	72.1 ± 39.5	76.4 ± 61.9	115.8 ± 34.3	74.6 ± 30.6	105.9 ± 76.7	49.0 ± 36.0	88.0 ± 27.6	0.122
K⁺_U [mEq/L]	30.1 ± 19.6	48.5 ± 38.0	38.7 ± 5.3	45.8 ± 41.9	37.9 ± 16.4	42.1 ± 24.2	31.3 ± 8.7	41.5 ± 29.4	29.9 ± 14.9	30.7 ± 22.5	0.527
Cl_U [mEq/L]	127.2 ± 87.2	151.2 ± 86.0	179.3 ± 29.2	133.9 ± 110.4	34.3 ± 14.1*	168.7 ± 129.9	54.0 ± 31.8*	186.5 ± 126.3	60.3 ± 51.6	156.8 ± 114.0	0.159
AG_U [mEq/L]	-48.9 ± 36.9	-35.6 ± 33.4	-79.6 ± 32.6	-16.0 ± 109.2	79.9 ± 65.3*	-10.8 ± 132.2	52.0 ± 54.9*	-39.1 ± 116.7	18.6 ± 62.0	-38.2 ± 93.9	0.399
Metabolic acidosis											
Na⁺_U [mEq/L]	37.5 ± 37.7	100.5 ± 98.4	47.0 ± 28.9	70.4 ± 70.6	32.3 ± 26.4	43.6 ± 43.6‡	28.3 ± 26.3	42.7 ± 30.6‡	25.2 ± 22.1	32.1 ± 39.0‡	0.364
K⁺_U [mEq/L]	33.2 ± 21.6	22.7 ± 3.4	37.4 ± 11.2	32.2 ± 10.5	49.0 ± 25.5	28.0 ± 19.2	30.6 ± 13.2	20.4 ± 11.7	27.1 ± 16.9	14.9 ± 10.1	0.162
Cl_U [mEq/L]	102.1 ± 72.6	185.7 ± 67.8	85.8 ± 51.6	63.4 ± 47.9‡	34.5 ± 16.7*	49.3 ± 32.1‡	34.8 ± 25.5*	93.9 ± 64.7‡	35.3 ± 29.0*	91.1 ± 62.0‡	0.077
AG_U [mEq/L]	-31.5 ± 39.8	-62.5 ± 53.3	-1.4 ± 54.5	39.2 ± 31.9‡	46.8 ± 45.4*	22.3 ± 45.5‡	24.1 ± 36.6	-30.9 ± 103.3	17.0 ± 34.4	-44.1 ± 79.2	0.347

Data are reported as mean \pm SD. p-value of difference between groups is reported by a 2-way ANOVA for repeated measurements over time. * p<0.05 vs baseline step in treatment group; ‡ p<0.05 vs baseline step in control group.

Table 3. Physiological variables and electrolytes changes during and after induction of metabolic lactic acidosis

Variables	Baseline		Acidosis Induction				CRe-ED treatment				Observation		p-value of difference between groups
			Start		End		4 hours		8 hours				
	Treatment (n=6)	Control (n=4)	Treatment (n=6)	Control (n=4)	Treatment (n=6)	Control (n=4)	Treatment (n=6)	Control (n=4)	Treatment (n=6)	Control (n=4)			
Respiratory variables													
Vt/kg [mL]	10.2 ± 0.2	9.7 ± 0.4	10.2 ± 0.2	9.5 ± 0.9	10.2 ± 0.2	9.5 ± 0.9	10.1 ± 0.2	9.4 ± 0.9	10.0 ± 0.3	9.6 ± 1.0	0.112		
RR [breaths/min]	16 ± 3	16 ± 3	23 ± 2*	23 ± 3‡	22 ± 2*	24 ± 3‡	23 ± 3*	25 ± 3‡	23 ± 5*	26 ± 4‡	0.469		
Minute ventilation [L/min]	3.7 ± 0.7	3.7 ± 0.5	5.3 ± 0.8*	5.3 ± 0.9‡	5.1 ± 0.6*	5.6 ± 1.0‡	5.2 ± 0.8*	5.7 ± 0.9‡	5.2 ± 1.3*	6.1 ± 1.0‡	0.505		
pCO ₂ [mmHg]	46.9 ± 7.6	47.2 ± 2.6	46.8 ± 6.6	48.6 ± 2.4	46.7 ± 7.0	47.9 ± 3.0	47.1 ± 6.8	47.9 ± 1.8	46.6 ± 7.2	46.5 ± 3.0	0.831		
Electrolytes and acid-base status													
Na ⁺ [mmol/L]	135.8 ± 2.6	138.0 ± 1.4	137.3 ± 2.1	136.8 ± 1.3	136.0 ± 2.4	133.0 ± 5.9‡	136.5 ± 2.4	135.3 ± 3.0	136.5 ± 2.9	134.3 ± 3.6	0.476		
K ⁺ [mmol/L]	4.1 ± 0.6	3.7 ± 0.4	3.6 ± 0.4*	3.5 ± 0.3	3.0 ± 0.5*	3.4 ± 0.2	2.6 ± 0.4*	3.3 ± 0.3	2.7 ± 0.4*	3.5 ± 0.8	0.352		
Ca ²⁺ [mmol/L]	1.4 ± 0.1	1.4 ± 0.0	1.5 ± 0.1*	1.5 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	1.4 ± 0.0	1.3 ± 0.1	1.4 ± 0.0	0.049		
Mg ²⁺ [mg/dL]	1.8 ± 0.1	2.1 ± 0.4	1.8 ± 0.1	2.1 ± 0.4	1.4 ± 0.1*	1.9 ± 0.2	1.7 ± 0.1	2.1 ± 0.4	1.7 ± 0.2	2.0 ± 0.3	0.020		

BE [mmol/L]	0.9 ± 5.4	4.1 ± 1.3	-10.2 ± 3.6*	-10.5 ± 1.6‡	3.2 ± 4.4	-11.7 ± 3.1‡	-1.1 ± 3.5	-12.5 ± 4.4‡	-0.4 ± 6.3	-13.4 ± 3.4‡	0.007
HCO₃⁻ [mEq/L]	28.5 ± 5.0	29.1 ± 1.4	16.0 ± 2.4*	16.6 ± 1.4‡	28.2 ± 4.7	15.6 ± 2.5‡	24.3 ± 3.6*	15.0 ± 3.2‡	25.0 ± 6.2*	14.2 ± 2.6‡	0.023
SID [mEq/L]	38.7 ± 5.4	42.6 ± 6.1	26.5 ± 2.7*	28.6 ± 5.2‡	38.6 ± 4.7	22.1 ± 8.9‡	35.2 ± 3.4	25.0 ± 1.7‡	35.6 ± 5.8	23.0 ± 3.2‡	<0.001
Hemodynamics											
HR [bpm]	109 ± 15	103 ± 20	115 ± 18	96 ± 18	111 ± 19	115 ± 24	101 ± 14	119 ± 26	103 ± 14	127 ± 26	0.700
MAP [mmHg]	98 ± 23	99 ± 20	98 ± 13	100 ± 16	87 ± 6	93 ± 12	89 ± 11	88 ± 9	86 ± 12	90 ± 9	0.687
CVP [mmHg]	8 ± 2	9 ± 3	8 ± 2	10 ± 3	7 ± 2	10 ± 3	7 ± 2	9 ± 3	7 ± 3	10 ± 3	0.214
PMAP [mmHg]	27 ± 4	24 ± 4	32 ± 4	34 ± 6	26 ± 4	37 ± 9‡	30 ± 5	30 ± 8	28 ± 5	36 ± 11‡	0.248
WP [mmHg]	10 ± 1	12 ± 4	10 ± 2	12 ± 4	9 ± 2	11 ± 4	10 ± 3	12 ± 5	10 ± 2	13 ± 4	0.304
CO [L/min]	3.3 ± 0.4	3.4 ± 0.6	4.2 ± 0.3*	3.4 ± 0.3	3.2 ± 0.3	3.8 ± 0.4	3.7 ± 0.4	3.9 ± 0.1	3.5 ± 0.2	3.8 ± 0.1	0.573
Renal function											
Urea [mg/dL]	18.0 ± 7.0	15.3 ± 6.4	16.3 ± 4.3	16.5 ± 8.2	15.7 ± 3.4	15.0 ± 7.5	16.8 ± 4.2	16.3 ± 4.7	17.3 ± 6.1	14.5 ± 4.4	0.684
Creatinine [mg/dL]	0.7 ± 0.1	0.5 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.6 ± 0.2	0.8 ± 0.2	0.7 ± 0.1	0.8 ± 0.2	0.6 ± 0.1	0.009
Creatinine Clearance [mL/min]	77.4 ± 28.2	112.4 ± 40.6	60.3 ± 27.7	83.3 ± 31.4	64.1 ± 16.2	140.8 ± 66.9	79.2 ± 25.2	84.0 ± 12.2	71.0 ± 27.0	96.9 ± 15.0	0.023

Data are reported as mean \pm SD. p-value of difference between groups is reported by a 2-way ANOVA for repeated measurements over time. * p<0.05 vs baseline step in treatment group; ‡ p<0.05 vs baseline step in control group.

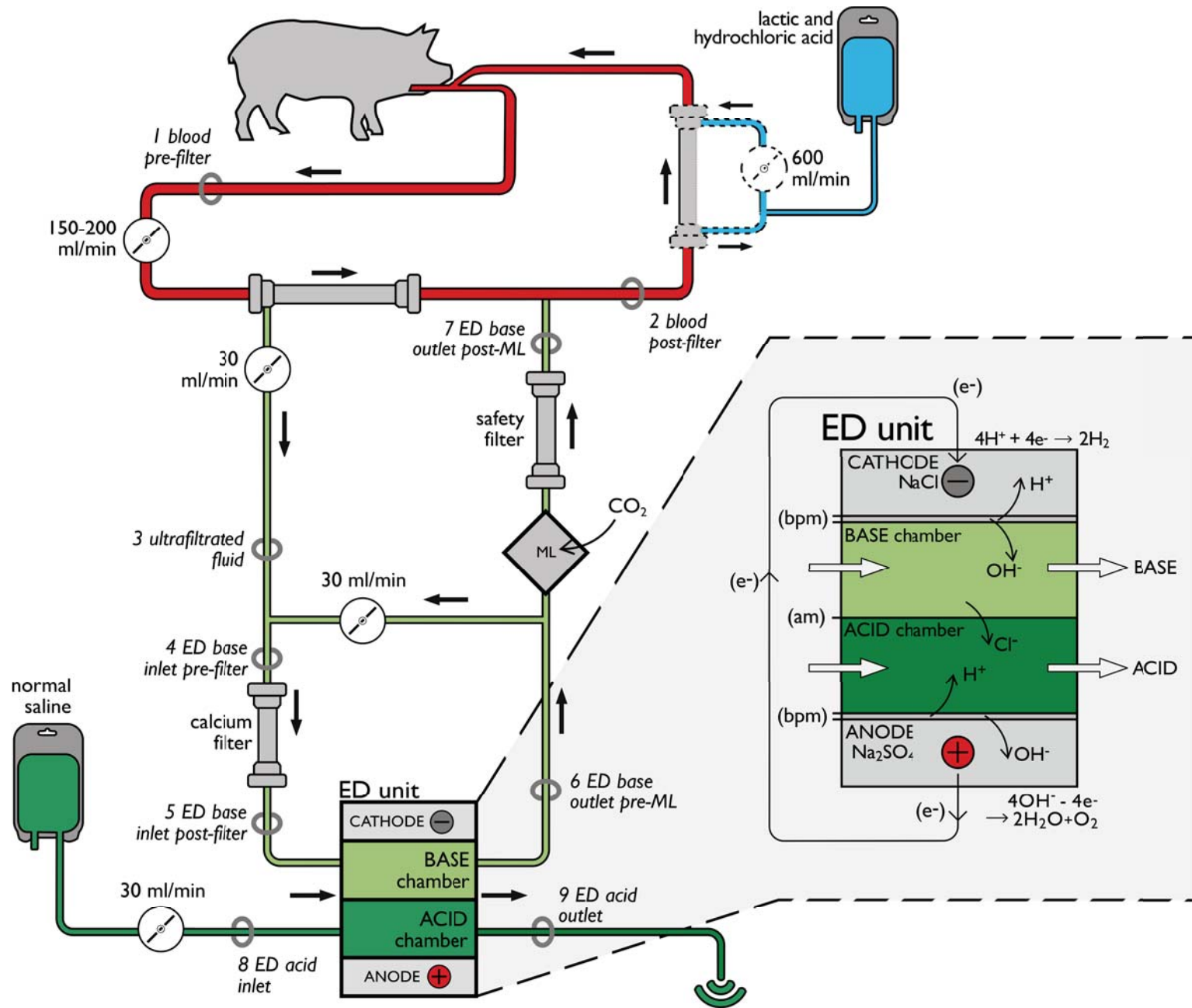


Figure 1

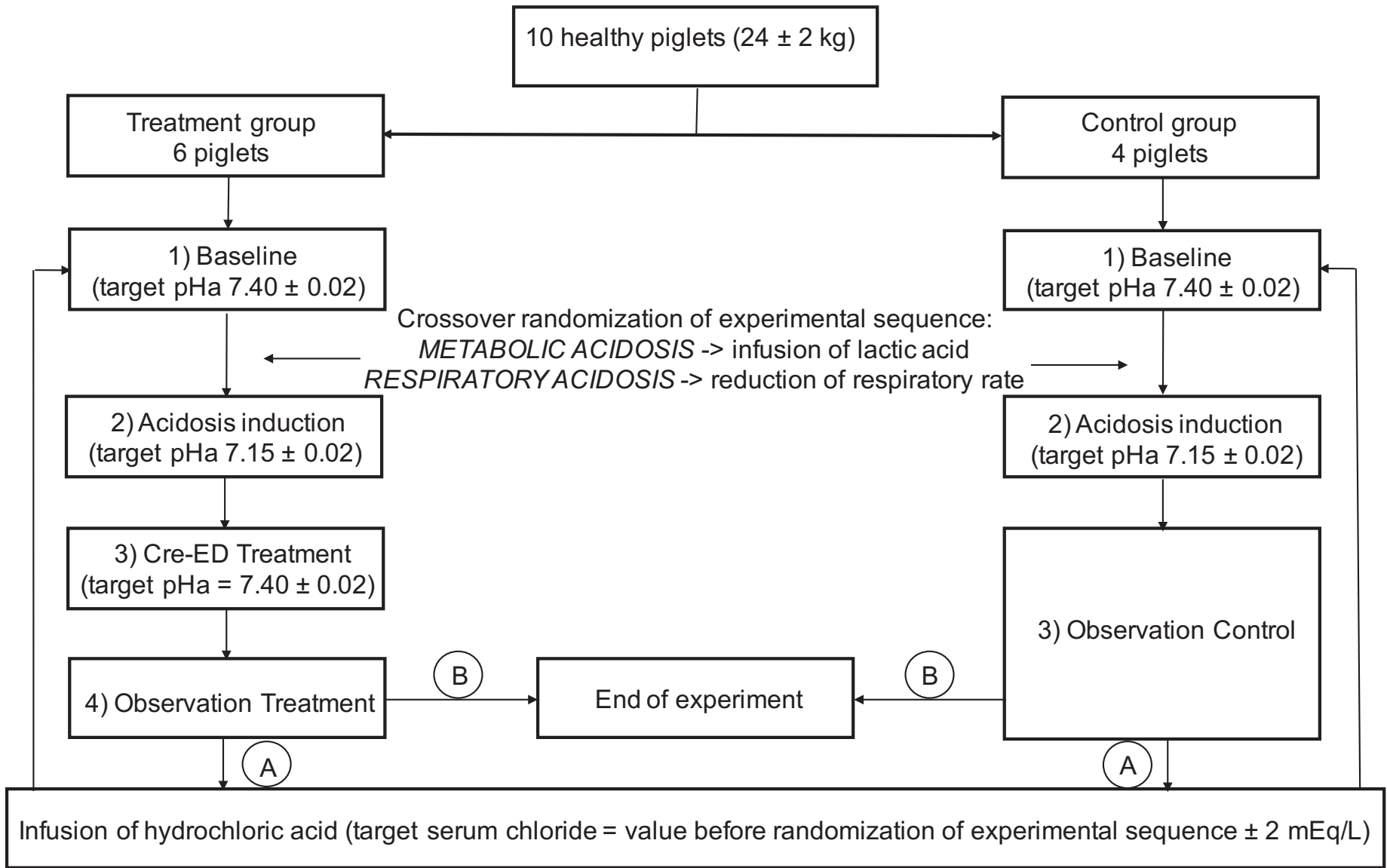


Figure 2

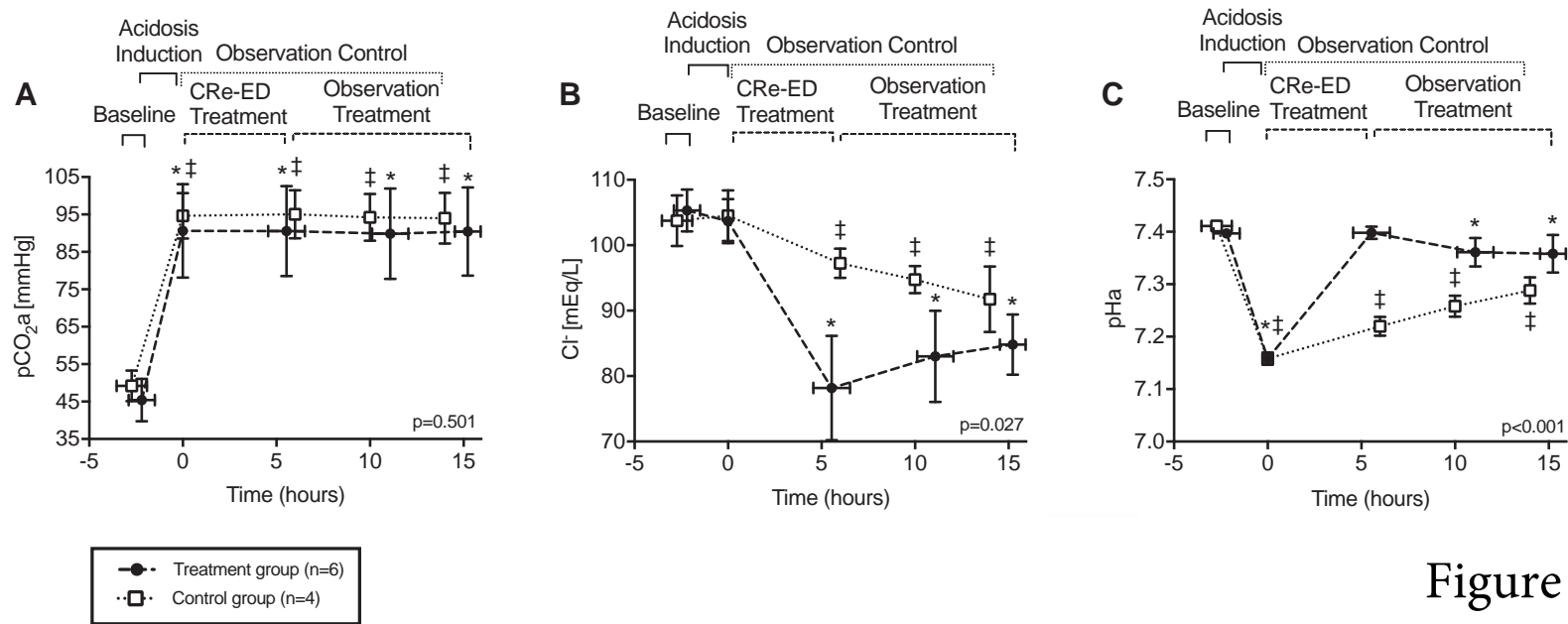


Figure 3

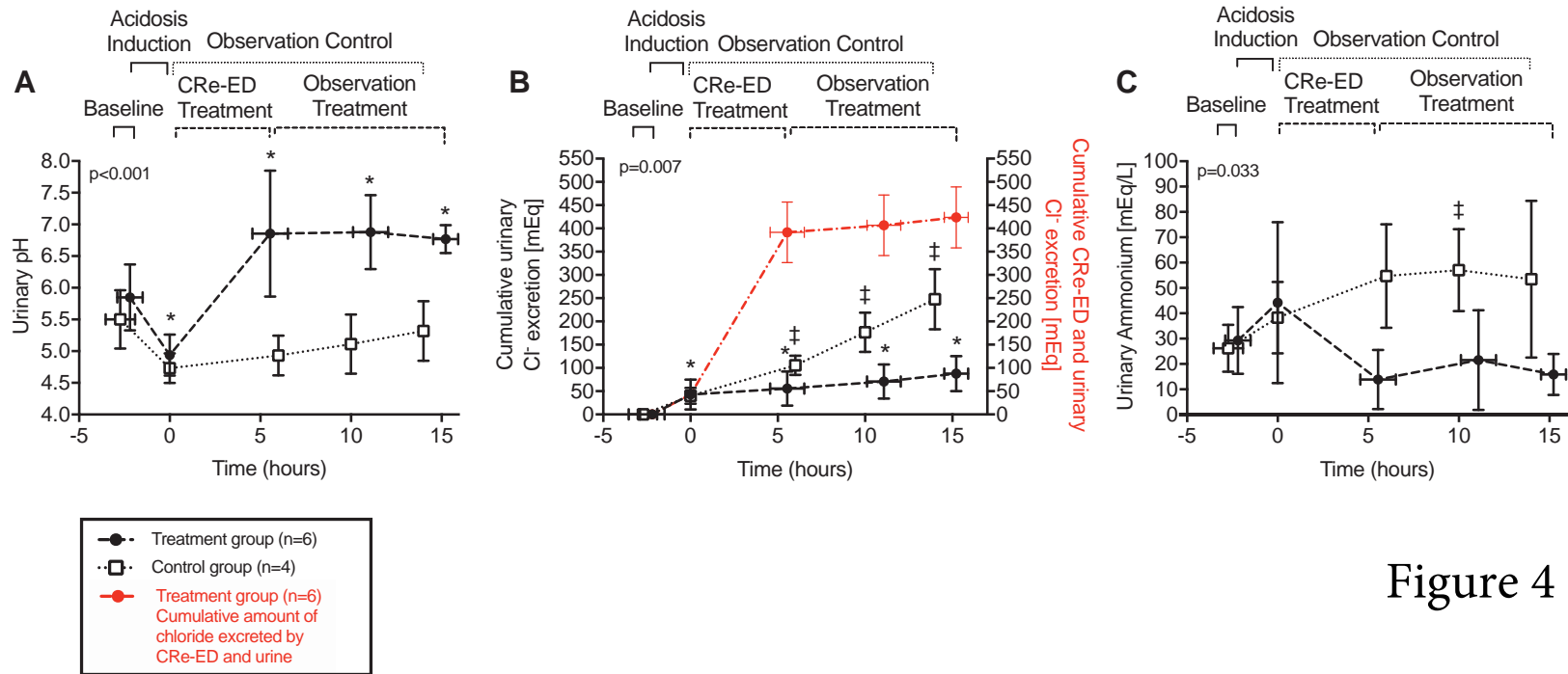


Figure 4

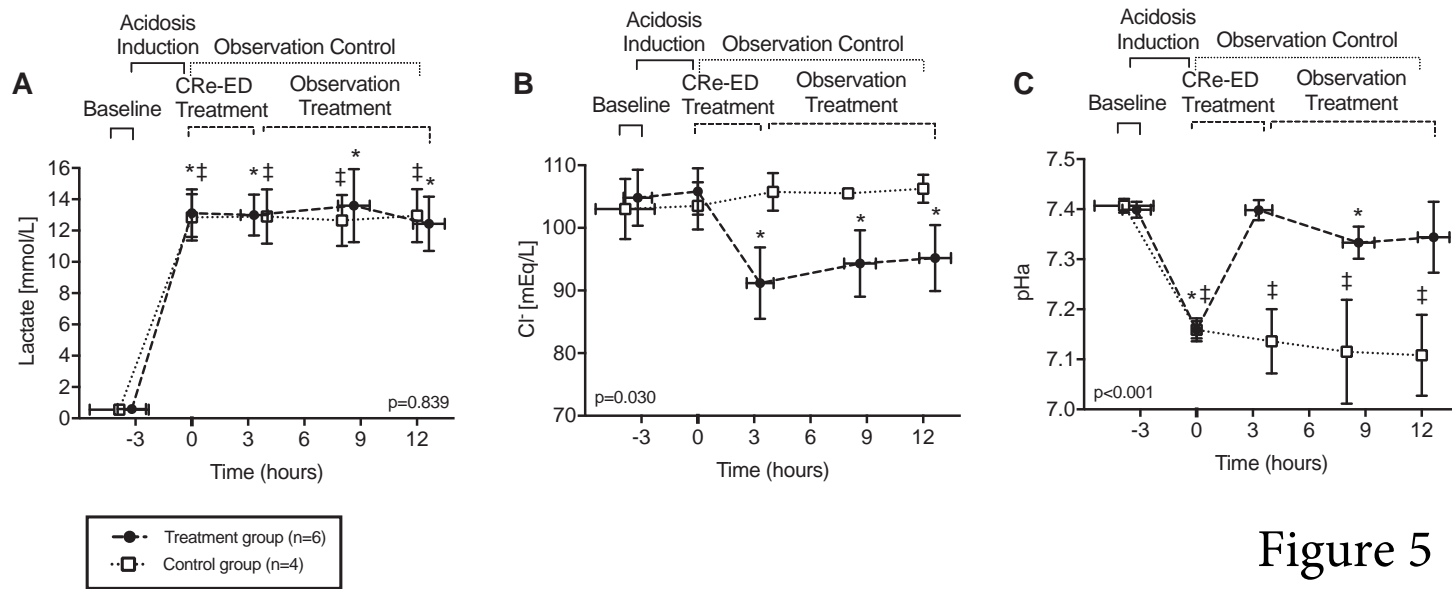


Figure 5

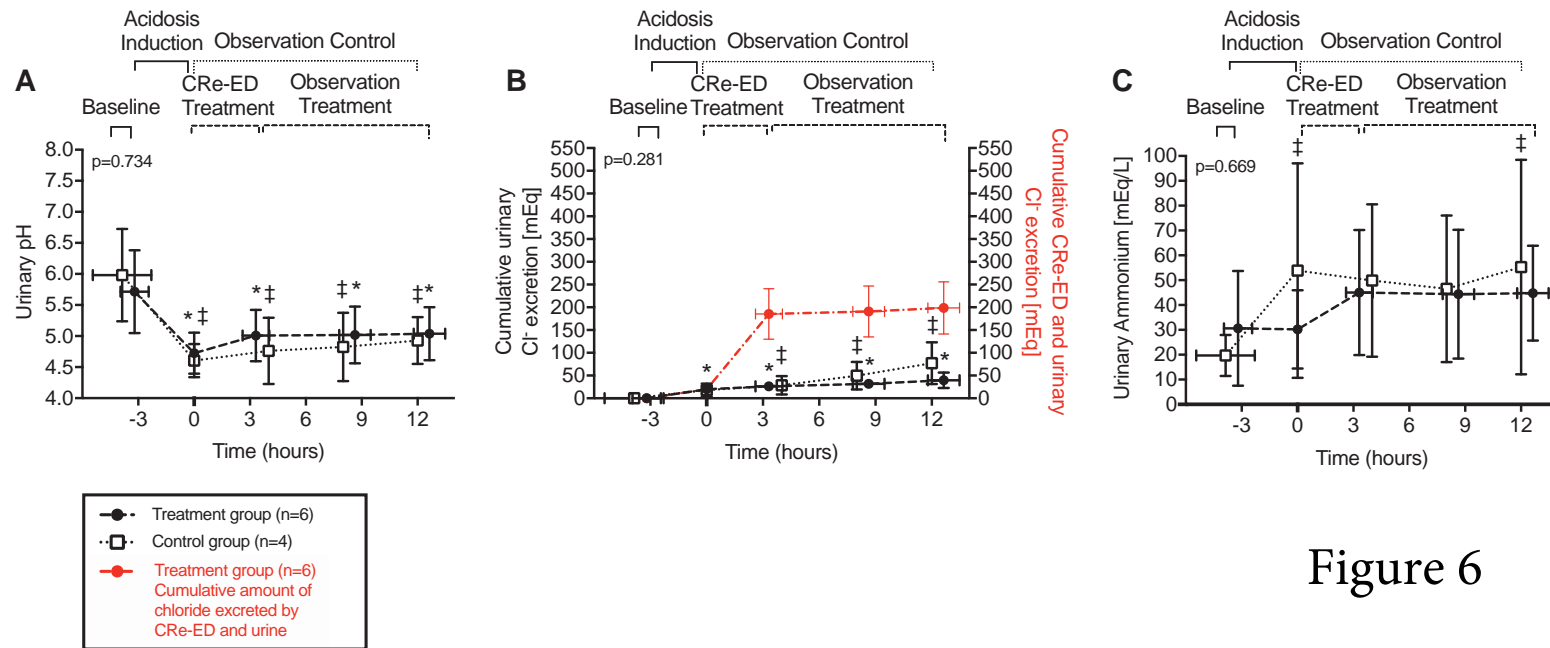


Figure 6

Online Data Supplement

Extracorporeal Chloride Removal by Electrodialysis (CRe-ED): A Novel Approach to Correct Acidemia.

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Methods

Animal Preparation and Management

The study was conducted within the animal research facility at the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico of Milan. The study protocol was approved by the Institutional Animal Care Committee, and the experiments conducted according to Italian National Regulations. After a 12-hour fasting period, ten healthy female piglets (weight 24 ± 2 kg) were sedated with an intramuscular injection of medetomidine (0.03 mg/kg) (Domitor, Orion corporation, Espoo, Finland) and tiletamine-zolazepam (4 mg/kg) (Zoletil, Virbac Srl, Milan, Italy). After catheterization of an ear vein, anesthesia was provided by continuous intravenous infusion of propofol (Diprivan, AstraZeneca, Basiglio, Milan, Italy) 10-15 mg/kg/h and medetomidine 3-6 mcg/kg/h. Analgesia was assured by intravenous administration of tramadol 50 mg every 12 hours. Thereafter, a surgical tracheostomy was performed and an endotracheal tube (diameter 7 mm) was secured in place. Once muscle paralysis was accomplished by intravenous administration of pancuronium-bromide (priming dose of 0.2 mg/Kg followed by a continuous infusion at a rate of 0.25 mg/kg/h), mechanical ventilation was started. The ventilator (Datex-Ohmeda Inc., General Electrics, Madison, WI, US) was set in volume controlled mode, with tidal volume of 10 mL/Kg, inspiratory to expiratory time ratio of 1:2, positive end expiratory pressure (PEEP) of 4 cmH₂O, inspiratory fraction of oxygen (FiO₂) of 40% and respiratory rate to obtain an arterial pH (pHa) of 7.40 ± 0.02 .

To monitor arterial and venous hemodynamics and to collect blood samples for laboratory analyses, left carotid artery and left internal jugular vein were surgically isolated and cannulated with an arterial catheter (5 Fr Seldicath, Prodimed,

Plastimed Division, Le Plessis Bouchard, France) and a central venous catheter (7 Fr, 3 lumen, Arrow, Reading, PA, US), respectively. To monitor pulmonary hemodynamics and to collect blood samples for laboratory analyses, a pulmonary arterial catheter (5 Fr, 4 lumen, 75cm, Edwards Lifesciences, Irvine, Canada) was inserted in the left internal jugular. To secure vascular access for the veno-venous extracorporeal circuit, a double-lumen hemodialysis catheter was surgically placed in the right internal jugular vein (12 Fr, 15 cm, Arrow, Reading, PA, US). Finally, cistostomy was performed and a Foley catheter (12 Ch Willy Rush AG, Kernen; Germany) was positioned into the bladder, to monitor urinary output and to collect urinary samples for electrolytes analyses.

Peripheral oxygen saturation (SpO_2), systemic and pulmonary arterial pressures, central venous pressure, urinary output, EKG, and core temperature were continuously monitored (SC 8000 MDLC, Siemens, Munich, Germany). Cardiac output measure was obtained by 5 mL of 5% dextrose bolus at room temperature into the central venous port of the pulmonary artery catheter (Vigilance, Edwards Lifesciences) within 3 seconds. Cardiac output was averaged after 3 measurements that were within 15% of each other. End-tidal carbon dioxide partial pressure ($ETCO_2$) was measured by a mainstream capnograph with an integrated airflow probe (CO_2SMO , Novametrix - Philips Respironics, Murrysville, PA, US). Expired minute volume and airways pressure were measured by the mechanical ventilator (Datex-Ohmeda Inc., General Electrics). During the entire experiment, Ringer's Lactate solution was infused at the rate of 4 mL/kg/h, unless otherwise specified.

Antibiotic prophylaxis was accomplished administering 1 g of ceftriaxone (Fidato, Fidia Spa, Padova, Italy) every 12 hour.

Following a parenteral injection of 150 IU/kg of unfractionated heparin (UFH), the animals were connected to a custom-made veno-venous extracorporeal circuit via the double lumen hemodialysis catheter. A continuous infusion of UFH was then titrated, with a target of activated clotting time (ACT) (Hemocron, ITC, Edison, NJ, US) between 200-250 seconds. During the whole experiment, an average UFH infusion of 52866 ± 22514 IU/day was required to obtain an ACT of 235 ± 56 seconds.

At the end of the experiment, all the animals were euthanized, while still under deep anesthesia, through an injection of 40 mEq of potassium chloride.

Extracorporeal Circuit

The extracorporeal circuit was composed by a *blood circuit*, an *electrodialysis (ED) circuit* and an *acid circuit* (see **Figure 1**). The *blood circuit* included a drainage limb, a roller pump (RS-7800 Minipump, Renal Systems, Minneapolis, MN, US), two serially connected hemofilters (FX60, surface area 1.4 m^2 , Fresenius Medical Care, Bad Homburg, Germany), and a re-infusion limb. Blood flow was kept constant at 150 and 200 mL/min in the experimental phase of respiratory acidosis and metabolic acidosis, respectively.

In the *ED circuit*, 30 mL/min of ultrafiltration fluid was generated from the first hemofilter by means of a peristaltic pump (PD 5206, Heidolph Instruments, Schwabach, Germany). This ultrafiltration fluid was diverted firstly towards a filter (calcium filter, FX50, Fresenius Medical Care), secondly into the ED base chamber, then through a pediatric membrane lung (ML) (Quadrox-iD Pediatric, Maquet Cardiopulmonary, Rastatt, Germany) and finally re-infused in the *blood circuit* among the two hemofilters. The gas inlet of the ML was connected to an air/CO₂ gas blender. To avoid potential embolization, we positioned an additional filter (safety

filter, FX50, Fresenius Medical Care) prior to the reinfusion of the ultrafiltrate fluid into the blood stream. An additional pump (PD 5206, Heidolph) was utilized to generate a re-circulating 30 mL/min of ultrafiltrate fluid from the outlet of the ED base chamber prior to the calcium filter. Finally, 30 mL/min of normal saline solution was driven by a roller pump (PD 5206, Heidolph) towards the ED acid chamber and then wasted.

In the *acid circuit*, outlet and inlet hemodiafiltrate ports of the second hemofilter were connected to create a closed loop circuit, so that the dialysate fluid flowed countercurrent to the blood flow at the rate of 600 mL/min by means of a peristaltic pump (PD 5206). Lactic acid 1.5 mol/L (Sigma-Aldrich, St. Louis, MO, US) or chloride acid 1.5 mol/L (Honeywell-Fluka, Morris Plains, NJ, US) was infused into the *acid circuit* during the experimental phases of the metabolic acidosis and re-chloration, respectively. All circuit tubing was standard $\frac{1}{4}$ or $\frac{3}{16}$ inches (inner diameter). Nine different withdrawal ports were arranged in the circuit for strict monitoring: 1 blood pre-filter, 2 blood post-filter, 3 ultrafiltrated fluid, 4 ED base inlet pre-filter, 5 ED base inlet post-filter, 6 ED base outlet pre-ML, 7 ED base outlet post-ML, 8 ED acid inlet and 9 ED acid outlet (see **Figure 1**). The ED unit was connected to a laboratory direct current (DC) power supply unit (EA-PS 3032-20B, EA Elektro Automatik, Munich, Germany) set to deliver a constant current output of 4 Ampere, maximum 20 Volt.

Electrodialysis

ED is an electrochemical process that utilizes the driving force of an electrical field to move ions from one solution to another one by ion-exchange membranes (E1). The ED unit (PCCell ED 200-OEM, PCCell GmbH, Heusweiler, Germany) (see Figure 1)

is composed of a customized four-chamber cell. Three membranes were aligned from the anode to the cathode: 1. a bipolar membrane (BPM); 2. an anionic membrane (AM), and 3. another BPM. By the separation of the three membranes within the ED cell, four compartments were created : anode, acid chamber, base chamber, and cathode. The anode compartment was immersed in a solution of sodium sulfate (300 mOsm/L), named the *anolyte*, and the cathode compartment was in a standard saline solution (0.9 %) called *catholyte*. Flow was continuous into the *anolyte* and *catholyte* compartments and was driven by two peristaltic pumps, respectively, into two closed loop circuits. An exit port was placed at the top of the 2 compartments to allow the removal of the gases produced into the cell (i.e. O₂ and H₂). An ion current was generated through the ED cell by featuring an electric field transversally to the fluid flow. Among the acid and base cell, the AM rectified the charge carriers to only negative ions. Furthermore, the two BPMs prevented electrolyte exchanges between the catholyte and anolyte. Contemporarily, the process of spontaneous water dissociation offered the necessary ion production to maintain the current flux inside the membranes, By peristaltic pumps, fluids flowed continuously in the “acid” and “base” chambers. Anions transitioned from the solution in the "base" chamber to the solution in the "acid" chamber through the anionic membrane, by means of electricity. The anion flux was kept constant by the external power supply, as operated at a constant current. Electrical current and ion transfer were related by the specific ion valence (v), $I=v \cdot 1/(e \cdot nA)$: “e” is the numerical value of the electron charge and nA is Avogadro’s number (1 Ampère corresponds to 0.622 mEq/min, in case of monovalent ions). A single ion species transfer depends on its concentration times its electrophoretic mobility. As Cl⁻ is the most represented anion in plasmatic water, the solution in the acid chamber mostly incorporates Cl⁻

ions. The combination of Cl^- ion with H^+ ions supplied by the BPM results in an acidic output solution. On the contrary, in the "base" chamber, OH^- ions are formed by hydrolysis in order to replace the loss of negative charges (i.e. Cl^-); therefore, the exit solution is alkaline.

At the time of the development of this technique, we reported the presence calcium crystals precipitation as the alkaline pH of the base chamber, which blocked the upcoming flow. To counteract this, we conceived a technique to prevent the precipitation of calcium inside the ED unit. Before the base chamber, we recirculated a portion of the alkaline solution exiting the base chamber itself. This led to controlled calcium precipitation before the ED unit and to the entrapment of the calcium crystals into the *calcium filter* placed before the base chamber. As a direct consequence, the concentration of calcium in the solution entering the base chamber was remarkably reduced. This prevented the damage of the ED unit by the calcium precipitation into the base chamber. As last, a *safety filter* was positioned downstream of the base chamber. This prevented that any residual of calcium crystals could enter the bloodstream.

Experimental design

Ten healthy female piglets (weight 24 ± 2 kg) were studied. Six subjects were assigned to treatment (i.e. chloride removal by ED, CRe-ED) and four to control (i.e. no treatment) (see **Figure 2**). Regardless of treatment assignment, the subjects underwent two randomly ordered experiments: called metabolic acidosis and respiratory acidosis. Briefly, each of these experiments was composed of:

1. a baseline step, to collect baseline data;

2. induction of acidosis, either metabolic by lactic acid infusion, or respiratory by reduction of minute ventilation, and achieving target acidosis (i.e. pH 7.15 ± 0.02);
3. correction of acidosis by CRe-ED, and thereby measuring the time necessary to pH correction; and finally, one hour after switching off CRe-ED system,
4. 8 hours of observation.

At the end of each step (i.e., baseline, start and end of CRe-ED treatment, 4h and end (8h) of observation period), every hour during CRe-ED treatment and every two hours during the control observation period, we collected the following data:

- 1) hemodynamic and ventilator parameters;
- 2) gas blood analyses, electrolytes plasma concentrations and laboratory analyses from blood samples obtained from the carotid artery, pulmonary artery and from the extracorporeal circuit;
- 3) urinary output, pH and urinary electrolytes (24);
- 4) glomerular filtration rate, which was estimated according to the following formula: $(\text{urinary creatinine} * \text{urine output}) / (\text{plasma creatinine}) / (\text{minutes}) = \text{mL/min}$.

In control subjects, ED was not applied, and thus the physiologic correction of acidosis was observed for 12 and 14 hours during metabolic and respiratory acidosis, respectively.

During the metabolic acidosis experiment, lactic acid was continuously infused in the *acid circuit* in both groups. Starting from the *baseline step* with an pHa of 7.40 ± 0.02 and maintaining arterial $p\text{CO}_2$ ($p\text{CO}_{2a}$) constant (value of baseline step ± 3 mmHg), the infusion of lactic acid was titrated to achieve a pH value of 7.15 ± 0.02 (*acidosis target step*). After achieving the *acidosis target step* until the end of the experiment, lactic acid infusion was set to maintain the arterial value of lactic acid as the same value reached at the *acidosis target step* (± 1.0 mEq/L). Then, in the treatment group during CRe-ED treatment, all peristaltic pumps of the *ED circuit* and power supply unit of the ED were on, and the gas mixture of CO_2 was delivered to the gas inlet port of ML to target the pH of ultrafiltration fluid at the ED base outlet post-ML port between 7.50-8.50. The CRe-ED system worked continuously until the restoration of an pHa 7.40 ± 0.02 and, after that, it was turned off. Finally, one hour after the end of the *CRe-ED treatment step*, 8-hour observation period (*CRe-ED observation step*) started. $p\text{CO}_{2a}$ was maintained equal to (± 3 mmHg) the $p\text{CO}_{2a}$ at *baseline step* and lactic acid was infused to maintain an arterial lactate levels equal to (± 1 mEq/L) the arterial lactate levels during the *acidosis target step*. In the control group, after the *acidosis target step*, 12-hour observation period (*control observation step*) started. $p\text{CO}_{2a}$ was maintained equal to (± 3 mmHg) the $p\text{CO}_{2a}$ at *baseline* and lactic acid was infused to maintain an arterial lactate levels equal to (± 1 mEq/L) the arterial lactate levels during the *acidosis target step*. During the experimental phase of metabolic acidosis, ringer lactate infusion was reduced of the same volume of the infused volume of lactic acid.

In both groups, during the respiratory acidosis experiment, starting with a baseline pHa of 7.40 ± 0.02 , the tidal volume was maintained constant at 10 mL/Kg and the respiratory rate was reduced to achieve an pHa of 7.15 ± 0.02 (*acidosis target step*).

After achieving the *acidosis target step* until the end of the experiment, the PEEP was set to 7 cmH₂O and FiO₂ was increased to keep SpO₂ above 98%, in order to avoid hypoxia determined by hypoventilation.

During the respiratory acidosis experiment, after achieving *acidosis target* pHa, the arterial pCO₂ was maintained equal to (\pm 3 mmHg) the pCO_{2a} reached during the *acidosis target step*, by changing the respiratory rate. Then, in the *treatment group* during *CRe-ED treatment step*, all the peristaltic pumps of the *ED circuit* and power supply unit of the ED were maintained on, and the gas mixture of CO₂ was inflated into the gas inlet port of ML to target the pH of ultrafiltration fluid at the ED base outlet post-ML port between 7.50-8.50. The CRe-ED system worked continuously until the restoration of an pHa of 7.40 ± 0.02 and after that it was interrupted. Finally, one hour after the end of the *CRe-ED treatment*, 8-hour observation period (*CRe-ED observation step*) started. PCO_{2a} was maintained equal to (\pm 3 mmHg) the pCO_{2a} during the *acidosis target step*. In the control group after the *acidosis target step*, a 12-hour observation period (*control observation step*) started. PCO_{2a} was maintained equal to (\pm 3 mmHg) the pCO_{2a} during the *acidosis target step*.

Since each animal was subjected to both experiments, after the end of the first randomized experiment, the arterial chloride level was restored as the same value (\pm 2 mEq/L) as during the *baseline step* of the first randomized experiment, by chloridric acid infusion into the *acid circuit*.

In both experiment, at the end of *baseline step* and *acidosis target step*, every hour during *CRe-ED treatment step* and every two hour during the *CRe-ED observation step* and *control observation step*, hemodynamics (i.e. heart rate, mean arterial pressure, mean pulmonary arterial pressure, pulmonary capillary wedge pressure,

central venous pressure, cardiac output, core temperature and urinary output), ventilatory parameters (i.e. minute ventilation, respiratory rate, tidal volume, peak pressure, plateau pressure, PEEP and inspiratory oxygen fraction), peripheral oxygen saturation (SpO₂) and end tidal CO₂ (EtCO₂) were recorded. During the same timepoints, blood was withdrawn for blood gas analyses (ABL 800 gas analyzer; Radiometer, Copenhagen, Denmark) from the carotid and the pulmonary artery.

Of note, bicarbonate concentration [HCO₃⁻] was calculated for each sample by applying known values for the solubility of CO₂ in plasma (S = 0.0307 mmol/(L·mmHg)) and the negative logarithm of the first apparent equilibrium dissociation constant of carbonic acid (pK'₁ = 6.120), according to the following formula:

$$[\text{HCO}_3^-] = S \cdot \text{PCO}_2 \cdot 10^{(\text{pH} - \text{pK}'_1)}$$

where PCO₂ = partial pressure of carbon dioxide (expressed in mmHg) and measured with the point-of-care blood gas analyzer and pH = pH measured with the point-of-care blood gas analyzer.

Furthermore, the serum strong ion difference (SID) and the urinary anion gap (AG) have been calculated as follows:

$$\text{Serum SID (mEq/L)} = (\text{Na}^+ \text{ mEq/L}) + (\text{K}^+ \text{ mEq/L}) + (\text{Ca}^{++*2} \text{ mEq/L}) + (\text{Mg mg/dL} * 10/24.3^*2) - (\text{Cl}^- \text{ mEq/L} + \text{Lactate}^- \text{ mEq/L})$$

$$\text{Urinary AG (mEq/L)} = (\text{Na}^+ \text{ mEq/L}) + (\text{K}^+ \text{ mEq/L}) - (\text{Cl}^- \text{ mEq/L})$$

Every hour during and at the end of the *CRe-ED treatment step*, blood was collected along the *blood circuit* from the blood pre-filter port and blood post-filter port.

Similarly, the ultrafiltrated fluid was withdrawn along the *ED circuit* from the ultrafiltration fluid port and the ED base outlet post-ML port for gas analyses (ABL 800 gas analyzer; Radiometer). Furthermore, the pH of every port of the ED circuit was measured simultaneously (HI9026, Hanna Instruments, San Benedetto del Tronto, Italy). Electrolytes (sodium, potassium, chloride, calcium, and magnesium) were measured in all blood or ultrafiltrate samples. Specifically, ionized calcium of blood samples was measured by a gas analyzer (ABL 800 gas analyzer; Radiometer). Sodium, potassium, chloride, totalcalcium and magnesium of all samples were measured by laboratory testing (ADVIA Chemistry, Siemens, Healthcare Diagnostics Inc., Tarrytown, NY, US). To prevent damage of the pH meter instruments, all ultrafiltrate samples with a pH greater than 8.0 were tonometered to obtain a pH between 7.0-8.0 by gaseous CO₂.

Urinary output, urinary pH and urinary electrolytes (sodium, potassium and chloride) and urinary ammonium were measured by the urinary analyzer KING (collaboration of Orvim and Kardia, Milan, Italy) and synchronized with blood data collection during both experiments.

Before starting and at the end of each step, blood was sampled to measure complete laboratory chemistry (hemoglobin, platelet count, serum electrolytes, urea, creatinine, urea, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, gamma-glutamyl transferase, total bilirubin, albumin (ADVIA Chemistry).

During the experimental phases with the ED on, calcium gluconate (10%) was continuously infused (average infusion rate=23±13 mL/h) to maintain an arterial ionized calcium concentration greater than 1.0 mmol/L.

Statistical Analysis

Data are presented as mean \pm standard deviation unless otherwise specified. To test the hypothesis that CRe-ED treatment (exposure variable) may correct blood pH after induction of respiratory or metabolic acidosis (outcome variable), variation in continuous physiological variables and electrolyte concentrations over time was compared using a 2-way analysis of variance (ANOVA) for repeated measurements (2-way ANOVA-RM over time) testing the difference among groups (treatment versus control group). Multiple comparisons analyses were tested among different time points in each group versus baseline, start and end of CRe-ED treatment using Dunnet's test. A p-value < 0.05 (two-tailed) was deemed statistically significant. Statistical analyses were performed using STATA-14/MP (StataCorp LP, College Station, TX, USA), GraphPad Prism 7a (GraphPad Software, San Diego, CA, USA) and Microsoft Excel for Mac 2017, Version 15.32.

References

E1. Zanella A, Castagna L, Salerno D, Scaravilli V, Abd El Aziz El Sayed Deab S, Magni F, Giani M, Mazzola S, Albertini M, Patroniti N, Mantegazza F, Pesenti A. Respiratory Electrodialysis. A Novel, Highly Efficient Extracorporeal CO₂ Removal Technique. *Am J Respir Crit Care Med*. 2015;192:719-26.

Tables

Table E1. Plasma laboratory biochemistry before and after induction of respiratory acidosis.

Variable	Baseline		CRe-ED treatment				Observation		p-value of difference between groups
	Treatment (n=6)	Control (n=4)	Start		End		8 hours		
			Treatment (n=6)	Control (n=4)	Treatment (n=6)	Control (n=4)	Treatment (n=6)	Control (n=4)	
Glycemia [mg/dL]	122±33	125±37	129±12	130±58	91±32*	103±35	98±17	123±41	0.563
AST [IU/L]	28±7	77±99	24±6	61±79	27±6	52±47	38±14	46±27	0.263
ALT [IU/L]	38±9†	56±12	39±9	54±11	37±9	52±12	38±11	52±8	0.037
Gamma-GT [IU/L]	27±9	17±2	27±8	20±7	25±8	17±3	33±12*	14±2	0.052
Total Bilirubin [mg/dL]	0.05±0.01	0.17±0.21	0.04±0.01	0.10±0.07	0.07±0.03	0.10±0.06	0.05±0.02	0.11±0.08	0.093
Albumin [g/dL]	2.6±0.7	2.8±0.5	2.6±0.7	2.7±0.6	2.5±0.6	2.5±0.4	2.3±0.4*	2.4±0.5	0.706
Phosphate [mg/dL]	8.4±1.6	10.2±0.7	9.8±1.7	11.9±1.6	9.5±1.9	10.9±1.1	10.3±0.8	8.7±1.5	0.450
Total Calcium [mg/dL]	9.7±1.5	9.8±1.8	9.4±1.1	9.2±1.3	9.2±1.1	8.9±1.3	8.7±1.4	9.3±1.2	0.447
Magnesium [mg/dL]	1.9±0.3	1.9±0.2	1.9±0.4	2.1±0.4	1.5±0.3*	1.8±0.2	1.7±0.2	1.8±0.2	0.387
LDH [IU/L]	518±132	643±112	538±146	618±100	498±162	573±59‡	505±126	536±29‡	0.408
Hb [g/dL]	7.2±1.7	7.8±1.7	8.2±1.5*	7.7±2.0	7.4±1.8	7.4±2.2	7.1±1.9	6.0±1.9‡	0.850
Platelets [10 ⁹ /L]	210±117	260±115	223±133	276±154	173±82	288±178	173±99	201±151	0.461

* p<0.05 vs baseline step in treatment group; ‡ p<0.05 vs baseline step in control group. p-value of difference between groups with a 2-way ANOVA for repeated measurements. AST=Aspartate-Aminotransferase; IU=international unit; ALT=alanine amino-transferase; Gamma-GT=Gamma-Glutamyl-Transferase; LDH=Lactate Dehydrogenase; Hb=hemoglobin.

Table E2. Respiratory variables before and after induction of respiratory acidosis

Variable	Baseline		CRe-ED treatment				Observation		p-value of difference between groups
	Treatment (n=6)	Control (n=4)	Start		End		8 hours		
			Treatment (n=6)	Control (n=4)	Treatment (n=6)	Control (n=4)	Treatment (n=6)	Control (n=4)	
T°C	37.5±1.0	37.8±1.5	38.0±1.0	38.3±0.1	37.9±1.0	38.2±0.4	38.2±1.2	38.2±0.6	0.730
PEEP, cmH ₂ O	5.0±1.4	4.8±1.0	6.0±1.3*	5.5±1.3	6.3±0.8*	5.5±1.3	6.7±1.0*	5.5±1.3	0.352
FiO ₂ , %	46±5	48±5	47±5	55±10	47±5	58±15	47±5	61±17‡	0.044
PaO ₂ , mmHg	194±34	187±41	116±19*	167±54	147±26*	164±43	144±40*	168±62	0.274
MAP, cmH ₂ O	9±1	9±1	9±1	9±1	9±1	9±1	10±1	9±2	0.713
Peak pressure, cmH ₂ O	24±2	24±7	23±2	24±3	24±2	23±3	23±2	27±7	0.575
Plateau pressure, cmH ₂ O	18±3	18±5	19±3	20±3	20±2	19±2	19±2	22±6‡	0.630
Alveolar ventilation, L/min	2.4±0.7	2.1±0.5	1.0±0.2*	0.9±0.0‡	1.0±0.2*	1.2±0.2‡	1.0±0.2*	1.0±0.3‡	0.984

* p<0.05 vs baseline step in treatment group; ‡ p<0.05 vs baseline step in control group. p-value of difference between groups with a 2-way ANOVA for repeated measurements. FiO₂=inspired fraction of oxygen; MAP=mean airway pressure; PEEP=positive end expiratory pressure.

Table E3. Change of pH, pCO₂ and electrolytes at the end of CRe-ED treatment in the blood pre-filter and blood-post-filter withdrawal port along the extracorporeal circuit after induction of respiratory acidosis.

Variable	1-blood pre-filter		2-blood post-filter	
	1 hour CRe-ED treatment	End of CRe-ED treatment	1 hour CRe-ED treatment	End of CRe-ED treatment
pH	7.19±0.02	7.34±0.01*	7.27±0.03	7.37±0.02*
pCO ₂ [mmHg]	102.4±16.8	105.3±13.6	106.8±11.4	112.9±11.8
pO ₂ [mmHg]	52.0±2.8	43.9±2.2*	45.5±9.8	40.0±7.3*
FiO ₂ , %	42±2	43±3	42±2	43±3
HCO ₃ ⁻ [mEq/L]	38.9±3.8	55.3±7.2*	48.8±6.8	63.0±7.0*
Lactate [mEq/L]	0.6±0.1	0.8±0.2*	0.5±0.1	0.8±0.2*
Na ⁺ [mEq/L]	136±3	136±4	136.2±3.4	136.4±4.0
K ⁺ [mEq/L]	4.0±0.4	3.3±0.1*	4.0±0.4	3.4±0.1*
Cl ⁻ [mEq/L]	97±5	77±8*	88±7	71±8*
Ca ⁺⁺ (mmol/L)	1.3±0.0	1.2±0.1*	0.9±0.1	0.9±0.0
Total Calcium [mg/dL]	9.7±0.3	10.4±0.9	7.2±0.9	7.9±0.8*
Phosphate [mg/dL]	9.9±1.3	9.6±1.7	8.2±1.3	8.0±1.7
Magnesium [mg/dL]	1.7±0.3	1.5±0.4	1.4±0.3	1.4±0.3

* p < 0.05 vs 1 hour ED treatment same withdrawal port by paired t-test.

Electrolytes were all measured by gas analysis (ABL 800 gas analyzer; Radiometer), except for total Calcium, phosphate and magnesium that were measured by laboratory testing (ADVIA Chemistry, Siemens).

Table E4. Change of pH and electrolytes at the end of CRe-ED treatment in the different withdrawal ports along the extracorporeal circuit after induction of respiratory acidosis.

Variables	3 Ultrafiltrated fluid		4 ED base inlet pre-filter		5 ED base inlet post-filter		6 ED base outlet pre-ML		7 ED base outlet post-ML		8 ED acid inlet		9 ED acid outlet	
	1 hour CRe-ED treatment	End of CRe-ED treatment	1 hour CRe-ED treatment	End of CRe-ED treatment	1 hour CRe-ED treatment	End of CRe-ED treatment	1 hour CRe-ED treatment	End of CRe-ED treatment	1 hour CRe-ED treatment	End of CRe-ED treatment	1 hour CRe-ED treatment	End of CRe-ED treatment	1 hour CRe-ED treatment	End of CRe-ED treatment
pH	7.49±0.13	7.63±0.11*	10.14±0.48	9.92±0.52*	10.14±0.39	9.91±0.56*	11.93±0.83	11.47±1.03*	7.98±0.53	8.01±0.14	6.34±2.14	6.08±1.99	2.37±1.72	2.36±1.47
Na ⁺ [mEq/L]	145±1	142±4	-	-	143±5	141±7	146±3	142.3±5	-	-	-	-	-	-
K ⁺ [mEq/L]	4.2±0.3	3.6±0.2*	-	-	4.3±0.3	3.9±0.4	4.2±0.2	3.9±0.6	-	-	-	-	-	-
Cl ⁻ [mEq/L]	106±6	90±8*	-	-	94±5	81±4*	82±3	72±4*	-	-	-	-	-	-
Phosphate [mg/dL]	9.2±1.6	8.5±1.4*	-	-	6.0±1.6	5.8±1.3	5.2±1.4	4.9±1.4	-	-	-	-	-	-
Total Calcium [mg/dL]	7.0±0.5	7.3±1.0	-	-	1.1±0.6	1.6±0.7*	0.9±0.4	1.3±0.9	-	-	-	-	-	-
Magnesium [mg/dL]	1.2±0.1	1.1±0.1*	-	-	0.7±0.1	0.7±0.1	0.6±0.1	0.6±0.2	-	-	-	-	-	-

* p < 0.05 vs 1 hour ED treatment same withdrawal port by paired t-test.

PH of all fluids were measured with a dedicated pH-meter (HI9026, Hanna Instruments), while electrolytes were all measured by laboratory testing (ADVIA Chemistry, Siemens).

Table E5. Plasma laboratory biochemistry exams before and after induction of metabolic lactic acidosis.

Variables	Baseline		CRe-ED treatment				Observation		p-value of difference between groups
	Treatment (n=6)	Control (n=4)	Start		End		8 hours		
			Treatment (n=6)	Control (n=4)	Treatment (n=6)	Control (n=4)	Treatment (n=6)	Control (n=4)	
Glycemia [mg/dL]	96±16	96±37	158±53*	152±26	158±18*	200±39‡	148±17	190 ± 92‡	0.279
AST [IU/L]	37±11	46±25	29±8	39±11	27±9	38±6	28±10	29 ± 10‡	0.240
ALT [IU/L]	36±7	45±7	36±6	45±6	36±8	45±6	36±5	46 ± 5	0.047
Gamma-GT [IU/L]	36±23	19±5	23±6*	16±3	24±8*	15±4	22±4*	17 ± 4	0.091
Total Bilirubin [mg/dL]	0.06±0.02	0.11±0.09	0.04±0.02	0.05±0.01	0.07±0.07	0.11±0.11	0.07±0.08	0.06 ± 0.03	0.257
Albumin [g/dL]	2.5±0.5	2.6±0.5	2.4±0.4	2.5±0.5	2.4±0.5	2.4±0.5	2.3±0.4	2.4 ± 0.5	0.828
Phosphate [mg/dL]	8.5±1.4	10.4±1.5	7.3±1.0	9.6±2.4	6.6±0.7	7.9±1.3	7.7±1.2	8.9 ± 1.9	0.453
Total Calcium [mg/dL]	10.0±0.4	10.0±0.6	10.9±0.7	11.0±0.8	10.1±0.6	10.4±0.2	9.9±0.7	9.9 ± 0.3	0.447
Magnesium [mg/dL]	1.8±0.1	2.1±0.4	1.8±0.1	2.1±0.4	1.4±0.2*	1.9±0.2	1.7±0.3	2.0 ± 0.3	0.024
LDH [IU/L]	507±86	699±248	485±85	640±188	494±89	626±153	480±75	611 ± 123‡	0.108
Hb [g/dL]	7.1±0.9	7.4±1.0	7.1±0.9	7.2±1.2	7.1±0.8	7.2±1.7	6.3±1.0*	6.6 ± 1.6	0.761
Platelets [10⁹/L]	221±147	262±46	209±136	271±55	190±110	231±54	122±94*	178 ± 53‡	0.510

* p<0.05 vs baseline step in treatment group; ‡ p<0.05 vs baseline step in control group. p-value of difference between groups with a 2-way ANOVA for repeated measurements. AST=Aspartate-Aminotransferase; IU=international unit; ALT=alanine aminotransferase; Gamma-GT=Gamma-Glutamyl-Transferase; LDH=Lactate Dehydrogenase; Hb=hemoglobin.

Table E6. Respiratory variables before and after induction of metabolic lactic acidosis/

Variables	Baseline		CRe-ED treatment				Observation		p-value of difference between groups
	Treatment (n=6)	Control (n=4)	Start		End		8 hours		
			Treatment (n=6)	Control (n=4)	Treatment (n=6)	Control (n=4)	Treatment (n=6)	Control (n=4)	
T°C	36.8±1.4	37.2±0.6	37.4±1.3	37.3±0.2	37.5±1.0	37.3±0.2	37.6±1.2*	37.5±0.5	0.972
PEEP, cmH₂O	5±1	6±3	4±1	6±3	4±1	6±1	5±1	7±3	0.181
FiO₂, %	47±5	48±5	47±5	48±5	47±5	48±5	47±5	48±5	0.807
PaO₂, mmHg	191±39	188±43	165±20	181±49	203±21	177±55	189±34	164±42	0.632
MAP, cmH₂O	9±1	10±3	10±1	12±3	10±1	13±2‡	11±3*	14±3‡	0.099
Peak pressure, cmH₂O	23±1	26±1	30±4*	29±3	31±5*	30±5	33±7*	34±6‡	0.821
Plateau pressure, cmH₂O	17±1	21±3	21±3*	20±3	21±3*	23±5	21±3*	25±3	0.187
Alveolar ventilation, L/min	2.1±0.4	2.3±0.2	3.2±0.7*	3.4±0.6‡	3.1±0.6*	3.7±0.6‡	3.3±0.9*	3.9±0.7‡	0.298

* p<0.05 vs baseline step in treatment group; ‡ p<0.05 vs baseline step in control group. p-value of difference between groups with a 2-way ANOVA for repeated measurements. p-value of group-by-time interaction with a 2-way ANOVA for repeated measurements. FiO₂=inspiratory fraction of oxygen; MAP=mean airway pressure; PEEP=positive end expiratory pressure.

Table E7. Change of pH, pCO₂ and electrolytes at the end of CRe-ED treatment in the blood pre-filter and blood-post-filter withdrawal port along the extracorporeal circuit after induction of metabolic acidosis.

Variables	1-blood pre-filter		2-blood post-filter	
	1 hour CRe-ED treatment	End of CRe-ED treatment	1 hour CRe-ED treatment	End of CRe-ED treatment
pH	7.18±0.05	7.27±0.09*	7.23±0.07	7.29±0.09*
pCO ₂ [mmHg]	62.2±8.6	68.4±13.2	70.2±11.6	78.2±15.8*
pO ₂ [mmHg]	42.7±5.1	37.4±3.5	41.9±5.2	36.8±3.1
FiO ₂	40±3	41±3	40±3	41±3
HCO ₃ ⁻ [mEq/L]	22.6±3.4	30.2±4.4*	28.0±4.1	36.6±6.7*
Lactate [mEq/L]	13.6±1.6	14.0±1.4	13.1±1.8	13.4±1.7
Na ⁺ [mEq/L]	137±3	136±2	137±3	136±3
K ⁺ [mEq/L]	3.3±0.4	3.1±0.5*	3.3±0.4	3.1±0.5
Cl ⁻ [mEq/L]	99±4	89±5*	95±4	85±7*
Ca ⁺⁺ (mmol/L)	1.5±0.1	1.3±0.1*	1.2±0.2	1.1±0.2
Total Calcium [mg/dL]	10.9±0.5	10.4±0.6*	9.8±1.9	9.1±1.6
Phosphate [mg/dL]	6.9±0.8	6.7±0.7	6.2±0.9	6.0±0.9
Magnesium [mg/dL]	1.7±0.1	1.4±0.2*	1.5±0.2	1.3±0.2*

* p < 0.05 vs 1 hour ED treatment same withdrawal port by paired t-test.

Electrolytes were all measured by gas analyses (ABL 800 gas analyzer; Radiometer), except for total Calcium, phosphate and magnesium that were measured by laboratory testing (ADVIA Chemistry, Siemens).

Table E8. Change of pH and electrolytes at the end of CRe-ED treatment in the different withdrawal ports along the extracorporeal circuit after induction of metabolic acidosis.

Variable	3 ultrafiltrated fluid		4 ED base inlet pre-filter		5 ED base inlet post-filter		6 ED base outlet pre-ML		7 ED base outlet post-ML		8 ED acid inlet		9 ED acid outlet	
	1 hour CRe-ED treatment	End of CRe-ED treatment	1 hour CRe-ED treatment	End of CRe-ED treatment	1 hour CRe-ED treatment	End of CRe-ED treatment	1 hour CRe-ED treatment	1 hour CRe-ED treatment	1 hour CRe-ED treatment	End of CRe-ED treatment	1 hour CRe-ED treatment	End of CRe-ED treatment	1 hour CRe-ED treatment	End of CRe-ED treatment
pH	7.39±0.21	7.48±0.25	10.90±0.29	10.46±0.18*	10.81±0.14	10.47±0.24*	12.43±0.22	12.25±0.30	7.96±0.20	7.85±0.22	5.18±1.84	6.36 ± 1.94	1.39 ± 0.97	1.82 ± 1.46
Na⁺ [mEq/L]	144±3	147±6	-	-	144±3	144±2	145±4	143±3	-	-	-	-	-	-
K⁺ [mEq/L]	3.8±0.8	3.3±0.7	-	-	3.8±0.6	3.4±0.8*	3.4±0.3	3.3±0.6	-	-	-	-	-	-
Cl⁻ [mEq/L]	103±4	91±10*	-	-	91±3	84±4*	78±3	72±4*	-	-	-	-	-	-
Phosphate [mg/dL]	7.2±1.0	6.6±1.7	-	-	3.1±0.9	3.6±1.0*	2.6±0.9	2.9±0.7*	-	-	-	-	-	-
Total Calcium [mg/dL]	8.4±0.5	6.9±3.2	-	-	0.7±0.2	1.0±0.2*	0.7±0.1	1.3±0.8	-	-	-	-	-	-
Magnesium [mg/dL]	1.3±0.1	1.1±0.3	-	-	0.6±0.1	0.6±0.1	0.6±0.1	0.7±0.1	-	-	-	-	-	-

* p < 0.05 vs 1 hour ED treatment same withdrawal port by paired t-test.

PH of all fluids were measured with a dedicated pH-meter (HI9026, Hanna Instruments), while electrolytes were all measured by laboratory testing (ADVIA Chemistry, Siemens).