

Hypercapnic acidosis attenuates shock and lung injury in early and prolonged systemic sepsis

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Objective: To investigate whether acute hypercapnic acidosis—induced by adding CO₂ to inspired gas—would protect against severe systemic sepsis-induced lung and systemic organ injury resulting from cecal ligation and puncture. Acute hypercapnic acidosis protects against lung injury after both nonseptic and early pneumonia-induced lung injury. In contrast, prolonged hypercapnia worsens pneumonia-induced lung injury. The effects of hypercapnia and acidosis in the setting of systemic sepsis remain to be determined.

Design: Prospective randomized animal study.

Setting: University research laboratory.

Subjects: Adult male Sprague-Dawley rats.

Interventions: In the early systemic sepsis series, post induction of anesthesia and tracheostomy placement, animals were randomized to normocapnia (Fico₂ = 0.00, n = 12) or hypercapnic acidosis (Fico₂ = 0.05, n = 12). Cecal ligation and puncture were performed and the animals were ventilated for 3 hrs. In the prolonged systemic sepsis series, rats were anesthetized, cecal ligation and puncture were performed, and the animals were allowed to recover. The animals were then randomized to housing

under conditions of environmental normocapnia (Fico₂ = 0.00, n = 20) or hypercapnia (Fico₂ = 0.08, n = 20). After 96 hrs, the animals were reanesthetized, and the severity of lung and hemodynamic injury was assessed.

Results: In early systemic sepsis, hypercapnic acidosis attenuated the development and severity of hypotension, and reduced lactate accumulation and the decrement in central venous oxyhemoglobin levels, compared with normocapnia. Hypercapnic acidosis reduced bronchoalveolar lavage neutrophil infiltration, and lung wet/dry weight ratios. In prolonged systemic sepsis, hypercapnic acidosis reduced histologic indices of lung injury. There was no evidence that hypercapnia worsened prolonged systemic sepsis-induced lung injury. Hypercapnic acidosis did not alter lung or systemic bacterial loads in early or prolonged systemic sepsis.

Conclusion: Hypercapnic acidosis exerts beneficial effects in early and prolonged cecal ligation and puncture-induced polymicrobial systemic sepsis. (Crit Care Med 2009; 37:2412–2420)

KEY WORDS: acute lung injury; hypercapnia acidosis; acute respiratory distress syndrome; shock; sepsis

Mechanical ventilation is an essential life-supporting therapy in patients suffering from acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). Large-scale clinical trials have shown that outcome is improved when a “protective” ventilatory strategy is used in patients with ALI/ARDS (1, 2). These ventilation strategies result generally in increased levels of Paco₂ termed “permissive hypercapnia,” which is toler-

ated to minimize pulmonary overdistension. Conventionally, the protective effect “permissive hypercapnia” is considered to be solely due to reductions in lung stretch. However, hypercapnic acidosis (HCA) has been demonstrated to attenuate acute lung injury induced by free radicals (3), pulmonary (4) and systemic ischemia-reperfusion (5), pulmonary endotoxin instillation (6), and excessive lung stretch (7, 8) in preclinical models. The protective action of HCA seems due,

in part, to its anti-inflammatory effects, which include: attenuation of cellular immune function (9–11), reduction of free radical generation (3) and oxidant-induced tissue damage (6), and reduction in the levels of key cytokines, such as tumor necrosis factor (TNF)- α , interleukin (IL)-1 β (12), and IL-8 (11). Thus, HCA may have therapeutic potential in the critically ill (13–15).

The commonest cause of severe ALI is sepsis, whether primary pulmonary or secondary to systemic sepsis (16–21). Of concern, HCA may exert deleterious effects in the context of bacterial infection (14, 22). The anti-inflammatory actions of HCA could impair the immune response to an invading pathogen. We have demonstrated that acute HCA does not worsen (23), and may protect against (24), early pneumonia-induced lung injury. In contrast, sustained hypercapnia worsened the lung injury induced by prolonged (i.e., 48 hrs) bacterial sepsis in a rodent pneumonia model (25). The potential for HCA to attenuate the hemodynamic effects of systemic sepsis has

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Drs. Costello and Higgins contributed equally to this work.

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recently been demonstrated (26). However, the potential for hypercapnia to reduce lung injury, and its effects in the setting of early vs. prolonged systemic sepsis are not known.

Based on the foregoing issues, we hypothesized that acute HCA would attenuate early (Series 1) systemic sepsis-induced lung and systemic organ injury resulting from cecal ligation and puncture. In the setting of prolonged systemic sepsis (Series 2), we hypothesized that HCA would worsen lung injury.

MATERIALS AND METHODS

Specific-pathogen-free adult male Sprague Dawley rats (Harlan, Bicester, United Kingdom), weighing between 350 g and 450 g, were used in all experiments. All work was approved by the Animal Ethics Committee of the National University of Ireland, Galway, and conducted under license from the Department of Health, Ireland.

Anesthesia and Dissection. Anesthesia was induced with intraperitoneal ketamine 80 mg·kg⁻¹ (Ketalar, Pfizer, Cork, Ireland) and xylazine 8 mg·kg⁻¹ (Xylapan, Vétoquinol, Dublin, Ireland). After confirming depth of anesthesia by absence of response to paw compression, intravenous access was gained via the dorsal penile vein and further anesthesia was maintained with an intravenous infusion (Alfaxadone 0.9% and alfadadolone acetate 0.3%; Saffan, Schering-Plough, Welwyn Garden City, United Kingdom) at 5 to 20 mg·kg⁻¹·hr⁻¹. A tracheostomy catheter (2-mm internal diameter) was inserted and secured and intra-arterial access (22-gauge cannulae, Becton Dickinson, Cowley, United Kingdom) was sited in the right external carotid artery. Cisatracurium besilate (0.5 mg; Glaxo-SmithKline, Dublin, Ireland) was administered intravenously to produce muscle relaxation. Intravenous access was also sited at the internal jugular vein for fluid infusion and central venous pressure and oxygen saturation (ScvO₂) measurement. The animals were ventilated, using a small animal ventilator (683, Harvard Apparatus, Kent, United Kingdom), with an inspired gas mixture of F_{IO₂} of 0.3, respiratory rate of 90 breaths/min, tidal volume of 6 mL·kg⁻¹, and positive end-expiratory pressure of 2 cm H₂O. To minimize lung derecruitment, a recruitment maneuver consisting of a positive end-expiratory pressure of 10 cm H₂O for 25 breaths was applied every 15 mins throughout the protocol.

Depth of anesthesia was assessed every 15 mins by monitoring the cardiovascular response to paw clamp. Body temperature was maintained at 36°C to 37.5°C, using a thermostatically controlled blanket system (Harvard Apparatus, MA). Systemic arterial pressure, peak airway pressures, and temperature were measured continuously throughout the experimental protocol. After 20 mins, an arterial blood gas sample was drawn for blood gas measurement (ABL 705, Radiometer, Copenhagen,

Table 1. Early systemic sepsis

| Variable | Normocapnia | HCA |
|-------------------------------------------------------|-------------------------|----------------------------------|
| Number of animals | 12 | 12 |
| Animal weight, g | 375 ± 7 | 388 ± 10 |
| Animal survival, n/N (%) | 12/12 (100) | 12/12 (100) |
| Time to development of shock, min | | |
| Time to 25% MAP decrease | 21 ± 5 | 103 ± 19 ^a |
| Time to 50% MAP decrease | 104 ± 16 | 171 ± 9 ^a |
| Central venous pressure (mm Hg) | | |
| Baseline | 4.5 ± 0.5 | 5.0 ± 0.4 |
| Final | 4.6 ± 0.6 | 4.8 ± 0.3 |
| Delta (final–baseline; CI) | 0.1 (–1.0 to 1.2) | –0.2 (–0.9 to 0.5) |
| Central venous oxygen saturation (ScvO ₂) | | |
| Baseline | 75 ± 2 | 73 ± 3 |
| Final | 57 ± 3 | 65 ± 3 |
| Delta (final–baseline; CI) | –18 (–23 to –13) | –9 (–17 to –1) ^b |
| Serum bicarbonate (mMol/L) | | |
| Baseline | 23.0 ± 0.2 | 23.4 ± 0.3 |
| Final | 14.2 ± 0.9 ^c | 16.2 ± 0.7 ^c |
| Delta (final–baseline; CI) | –8.8 (–10.6 to –7) | –7.1 (–8.5 to –5.7) |
| Base excess | | |
| Baseline | –1.7 ± 0.2 | –1.8 ± 0.3 |
| Final | –13.3 ± 1.4 | –8.2 ± 0.8 |
| Delta (final–baseline; CI) | –11.7 (–14.4 to –9) | –6.4 (–8.2 to –4.6) ^c |
| Arterial O ₂ tension (torr) | | |
| Baseline (F _{IO₂} 0.3) | 141 ± 2 | 141 ± 2 |
| 1-hr post CLP (F _{IO₂} 0.3) | 137 ± 2 | 156 ± 2 ^b |
| Final (F _{IO₂} 0.3) | 153 ± 2 | 152 ± 3 |
| (F _{IO₂} 1.0) | 492 ± 8 | 510 ± 8 |
| Alveolar-arterial O ₂ gradient (torr) | 181 ± 24 | 155 ± 23 ^b |
| Peak airway pressure (mm Hg) | | |
| Baseline | 4.8 ± 0.2 | 4.8 ± 0.1 |
| Final | 5.3 ± 0.2 | 5.0 ± 0.2 |
| Delta (final–baseline; CI) | 0.5 (0.1 to 0.9) | 0.2 (–0.1 to 0.5) |
| Static lung compliance, mL·mm Hg ⁻¹ | | |
| Baseline | 0.75 ± 0.04 | 0.72 ± 0.03 |
| Final | 0.57 ± 0.04 | 0.60 ± 0.04 |
| Delta (final–baseline; CI) | –0.18 (–0.24 to –0.12) | –0.12 (–0.16 to –0.08) |
| Wet/dry weight ratio | 4.6 ± 0.2 | 4.0 ± 0.2 ^b |
| BAL protein concentration, μg·mL ⁻¹ | 867 ± 62 | 479 ± 62 ^b |
| BAL TNF-α concentration (pg·mL ⁻¹) | 154 ± 65 | 92 ± 23 |
| BAL IL-6 concentration, pg·mL ⁻¹ | 1794 ± 180 | 1664 ± 217 |
| Bacterial counts, cfu | | |
| Blood-T90, ×10 ¹² ·mL ⁻¹ | 4.3 ± 0.8 | 2.9 ± 0.5 |
| T180, ×10 ¹² ·mL ⁻¹ | 7.0 ± 1.3 | 4.9 ± 1.1 |
| BAL, ×10 ⁶ ·mL ⁻¹ | 4.3 ± 0.9 | 3.3 ± 0.6 |
| Peritoneal fluid, ×10 ¹² ·mL ⁻¹ | 8.4 ± 1.7 | 8.8 ± 2.0 |

HCA, hypercapnic acidosis; MAP, mean arterial pressure; CI, confidence interval; CLP, cecal ligation and puncture; BAL, bronchoalveolar lavage; TNF, tumor necrosis factor; IL, interleukin; T90, 90 mins post-CLP; T180, 180 mins post-CLP.

^aSignificantly different from normocapnia ($p < .01$); ^bsignificantly different from normocapnia ($p < .05$); ^csignificantly different from baseline ($p < .05$). Data are expressed as mean ± standard deviation or median (interquartile range). Final data indicates data collected upon completion of the experimental protocol.

Denmark), and lung compliance was measured to confirm baseline stability. These measurements were repeated at hourly intervals over the course of the experimental protocol.

Cecal Ligation and Puncture Protocol. The lower half of the abdomen was shaved and disinfected with 100% alcohol and the cecum was mobilized through an approximately 2-cm long, median abdominal incision. The cecum was filled by gently “milking back” colon contents and then ligated with a 3-0 silk suture distal to the ileo-cecal valve, without causing bowel obstruction. In the early sepsis experiments, 50% of the cecum was ligated, as this process pro-

duced a severe early injury in pilot studies. In the prolonged sepsis experiments, 25% of the cecum was ligated, as this increased the duration of animal survival, facilitating a study of the effects of HCA in a prolonged sepsis model. In both series, the ligated cecum was then subjected to a single “through and through” perforation with a sterile 18-gauge needle and gently compressed until its contents began to exude, to ensure patency of the perforation sites. The bowel was then repositioned in the abdomen and the incision was closed in layers with 4-0 silk sutures. All rats were given 10 mL·kg⁻¹ of Gelofusine (B. Braun, Dublin, Ireland) intra-

venously for fluid resuscitation over a 15-min period and then a continuous infusion of $10 \text{ mL}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ over the course of the experimental protocol.

Early Sepsis Experimental Protocol

The purpose of this series was to investigate the effect of HCA in the setting of severe early systemic sepsis. Post anesthesia and dissection, confirmation of the absence of baseline exclusion criteria, the cecum was ligated and punctured, and animals were randomized to receive either normocapnia or HCA. Control animals were ventilated with an inspired gas mixture of $\text{FICO}_2 = 0.0$, $\text{FIO}_2 = 0.3$, and $\text{FIN}_2 = 0.7$. HCA animals were ventilated with an inspired gas mixture of $\text{FICO}_2 = 0.05$, $\text{FIO}_2 = 0.3$, and $\text{FIN}_2 = 0.65$. The animals were then ventilated for 6 hrs, and the severity of lung and systemic organ injury was assessed.

Exclusion and Termination Criteria. Before entry into the experimental protocol, the following baseline values were required for continuation with the protocol: Paco_2 in the range of 30 and 40 torr (4–5.3 kPa), Pao_2 of >120 torr (16 kPa), HCO_3^- of $>20 \text{ mmol}\cdot\text{L}^{-1}$, and temperature of 36.0°C and 37.5°C . Where the criteria were not fulfilled, variables were reassessed after an additional 15 mins, during which no specific interventions were performed apart from adjusting the respiratory rate if Paco_2 was not in the target range. Failure to meet the criteria at this point mandated exclusion from the protocol. Thereafter, if at any stage during the protocol, the mean arterial pressure decreased $<30 \text{ mm Hg}$ for >15 mins, the experiment was terminated.

Prolonged Sepsis Experimental Protocol

The purpose of this series was to investigate the effect of prolonged hypercapnia in the setting of ongoing systemic sepsis. The animals were anesthetized and the cecum was ligated and punctured according to protocol, and the animals were allowed to recover. After recovery from anesthesia, the animals were then randomized to hypercapnia or normocapnia. Animals randomized to environmental hypercapnia were housed in an environmental chamber in which ambient oxygen was maintained at 21% and CO_2 at 8%, using automated controllers (ProOx 110 and ProCO2 120, Biospherix, NY). Rats randomized to environmental normocapnia were maintained in 21% oxygen without added CO_2 during this time. After 96 hrs, surviving animals in each group were reanesthetized with intraperitoneal ketamine $80 \text{ mg}\cdot\text{kg}^{-1}$ and xylazine $8 \text{ mg}\cdot\text{kg}^{-1}$, and underwent insertion of intravascular cannulas and tracheostomy, and mechanical ventilation.

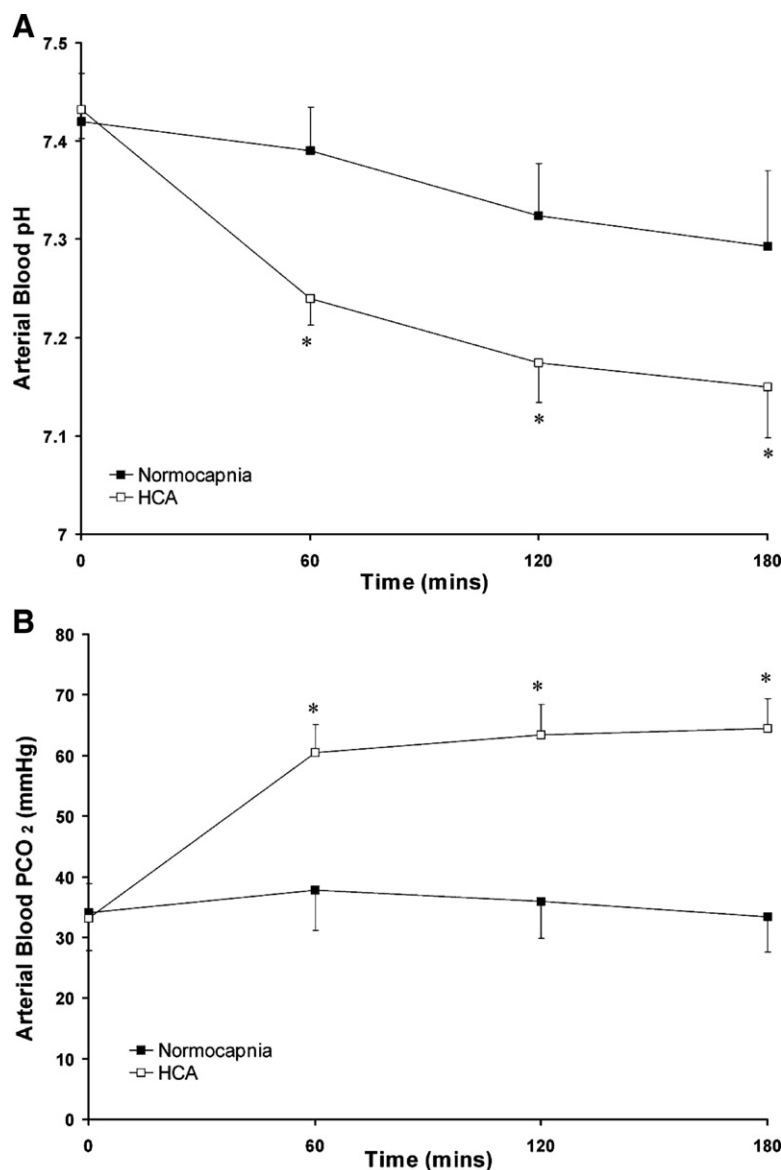


Figure 1. A, Graph representing mean (standard deviation) arterial pH at baseline and over the course the protocol in early cecal ligation and puncture-induced systemic sepsis. B, Graph representing mean (standard deviation) Paco_2 at baseline and over the course the protocol in early cecal ligation and puncture-induced systemic sepsis. Normocapnia ($\text{FICO}_2 = 0.00$); HCA, hypercapnic acidosis ($\text{FICO}_2 = 0.05$). *Significantly different normocapnia ($p < 0.05$, analysis of variance).

Control animals were ventilated with an inspired gas mixture of $\text{FICO}_2 = 0.0$, $\text{FIO}_2 = 0.3$, and $\text{FIN}_2 = 0.7$. HCA animals were ventilated with an inspired gas mixture of $\text{FICO}_2 = 0.05$, $\text{FIO}_2 = 0.3$, and $\text{FIN}_2 = 0.65$. The animals were then ventilated for 20 mins, and the severity of lung and systemic organ injury was assessed.

Measurement of Physiologic Variables

In the early sepsis protocol, intra-arterial blood pressure, peak airway pressures, and rectal temperature were recorded continuously over the 3-hr duration of the protocol.

Arterial blood gas analysis and static inflation lung compliance measurements were performed at baseline and hourly throughout the protocol. Incremental 1-mL volumes of room air were injected via the tracheostomy tube, and the pressure was attained 3 secs after each injection was measured, until a total volume of 5 mL was injected.

In the prolonged systemic sepsis series, these measurements were taken in the first eight surviving animals from each group at the end of the protocol. In both experimental series, at the end of the protocol, the inspired gas was altered to a FIO_2 of 1.0 for 15 mins, and an arterial blood sample was then taken for calculation of alveolar-arterial oxygen gradi-

ent. Heparin (400 IU·kg⁻¹, CP Pharmaceuticals, Wrexham, United Kingdom) was then administered intravenously, and the animals were then killed by exsanguination.

Tissue Sampling and Assays

Immediately postmortem, the heart-lung block was dissected from the thorax and bronchoalveolar lavage (BAL) collection was performed (23, 25). Total cell numbers per milliliter in the BAL fluid were counted, and differential cell counts were performed. The concentrations of IL-6 and TNF- α in BAL fluid were determined, using commercially available rat quantitative sandwich enzyme-linked immunosorbent assays (R&D Systems Europe, Abingdon, United Kingdom). The concentration of total protein in BAL fluid was determined, using a Micro BCA Protein assay kit (Pierce, Rockford, IL) (27).

The concentration of bacteria in BAL, blood, and abdominal fluid was determined by plating serial dilutions on blood agar plates and carrying out a colony count 24 hrs later. Wet/dry lung weights were determined by tying off and removing the lowest lobe of the right lung, before BAL collection, and drying the lung at 37°C for 72 hrs before reweighing.

Histologic and Stereologic Analysis

The left lung was isolated and fixed for morphometric examination (6, 23, 28). The pulmonary circulation was first perfused with normal saline at a constant hydrostatic pressure of 25 cm H₂O until the left atrial effluent was clear of blood. The left lung was then inflated through the tracheal catheter, using paraformaldehyde (4% wt.vol⁻¹) in phosphate-buffered saline (300 mOsmol) at a pressure of 25 cm H₂O. Paraformaldehyde was then instilled through the pulmonary artery catheter at a pressure of 62.5 cm H₂O. After 30 mins, the pulmonary artery and trachea were ligated and the lung was stored in paraformaldehyde (28). The extent of histologic lung damage was determined, using quantitative stereological techniques (29, 30).

Data Presentation and Analysis

The distribution of all data were tested for normality, using the Kolmogorov-Smirnov test. Results are expressed as mean \pm standard deviation for normally distributed data, and as median (interquartile range, IQR) if nonnormally distributed. Data that were obtained at multiple time points throughout the experiment, such as Pao₂ and Pco₂ and pH and airway pressures, were analyzed using a two-way repeated-measures analysis of variance, with group allocation (HCA vs. Control) as the group factor and time as the repeated mea-

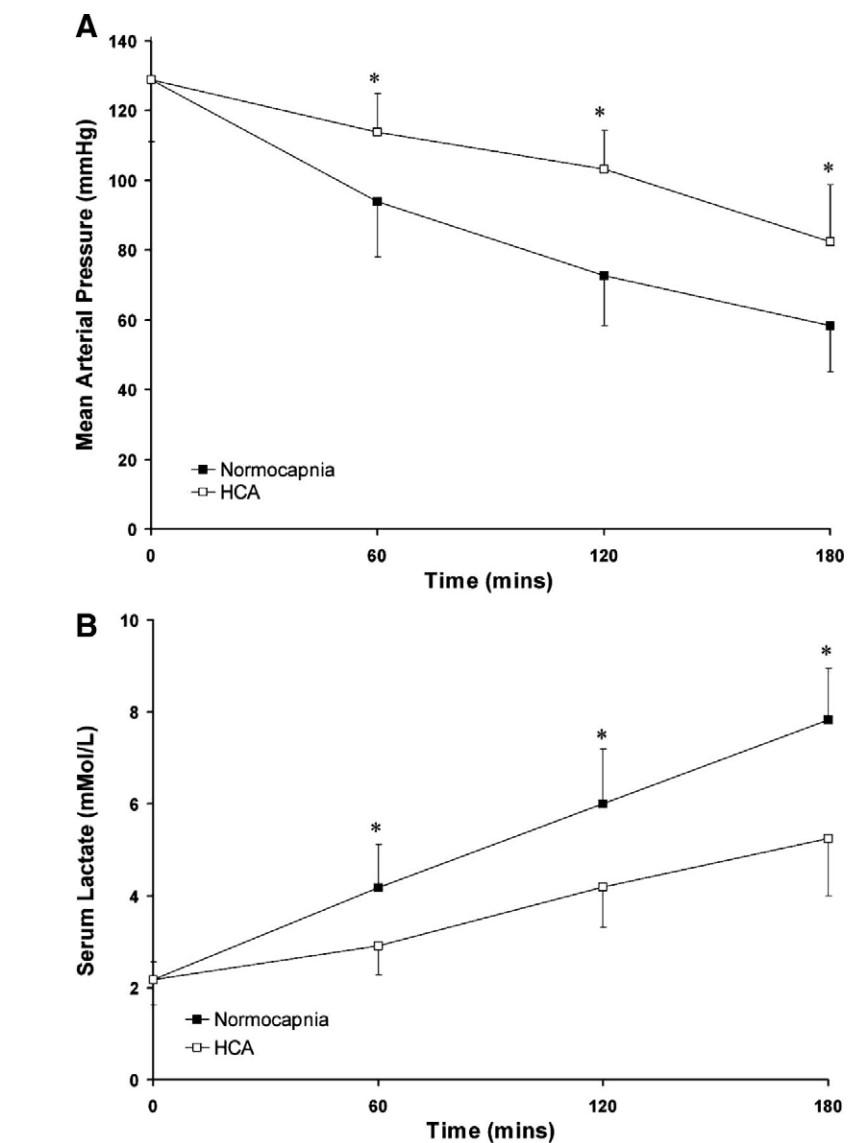


Figure 2. A, Graph representing mean (standard deviation) arterial blood pressure at baseline and over the course the protocol in early cecal ligation and puncture-induced systemic sepsis. B, Graph representing mean (standard deviation) arterial lactate concentrations at baseline and over the course the protocol in early cecal ligation and puncture-induced systemic sepsis. Normocapnia (Fico₂ = 0.00); HCA, hypercapnic acidosis (Fico₂ = 0.05). *Significantly different normocapnia ($p < .05$, analysis of variance).

sure. Lung histology was analyzed by two-way analysis of variance, with group as the first factor and histologic classification (airspace, intra-alveolar tissue, extra-acinar tissue) as the second factor. Underlying model assumptions were deemed appropriate on the basis of suitable residual plots.

Data obtained at baseline and again at the end of the experiment were analyzed by comparing the differences between baseline and final values, using a Student's t test, and reported with confidence intervals. Data obtained at a single time point were analyzed, using a Student's t test or Mann-Whitney U test, with the Bonferroni correction as appropriate. Mortality data were analyzed, using a Fisher's exact test. A two-tailed $p < .05$ was considered significant.

RESULTS

Series 1: Early Systemic Sepsis

Twenty-four animals were entered into this study. No animals were excluded before randomization, and all 24 animals were randomized to receive normocapnia ($n = 12$) or HCA ($n = 12$). There were no differences between the groups at baseline with regard to animal weight, mean arterial blood pressure (MAP), central venous pressure (CVP), Scvo₂, Pao₂, Paco₂, arterial pH, serum lactate and bicarbonate, peak airway pressure (Paw), and static compliance (Table 1; Figs. 1–3). All

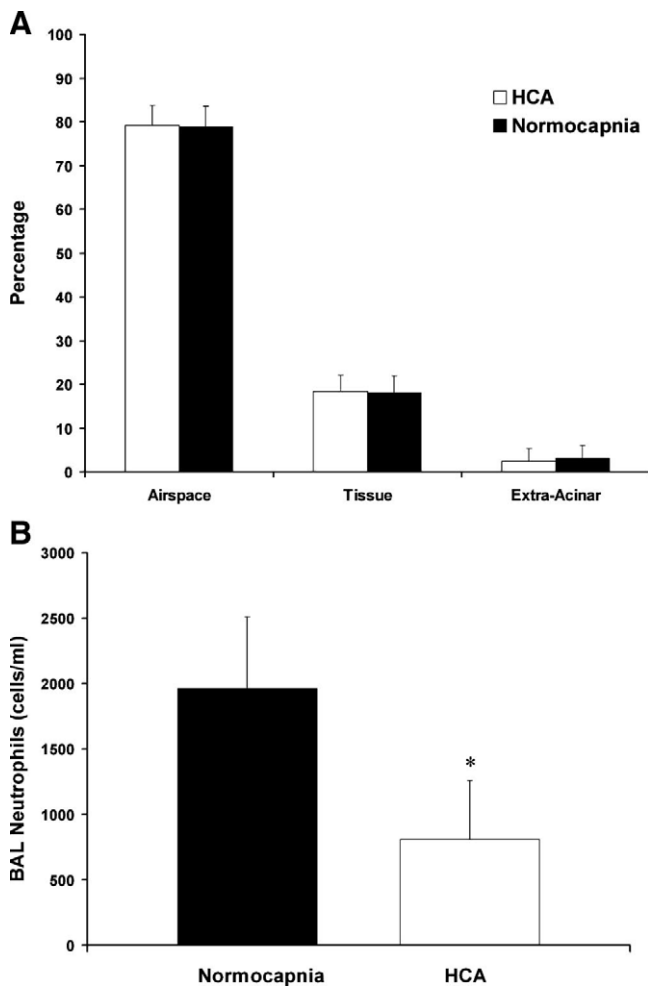


Figure 3. A, Histogram representing stereologic assessment of the extent of histologic injury in early cecal ligation and puncture-induced systemic sepsis. B, Histogram representing the mean (standard deviation) bronchoalveolar lavage (BAL) neutrophil counts at the end of the protocol in early cecal ligation and puncture-induced systemic sepsis. Normocapnia ($F_{iCO_2} = 0.00$); HCA, hypercapnic acidosis ($F_{iCO_2} = 0.05$). *Significantly different from normocapnia ($p < .05$, Student's t test).

animals in both groups survived the injury protocol (Table 1).

Arterial CO_2 Tension and Acid Base. Arterial pH and P_{aCO_2} were similar in the normocapnia and HCA groups at baseline (Fig. 1A, B). There was an initial rapid increase in P_{aCO_2} and decrease in pH in the HCA group post induction of hypercapnia. At each hourly time point during the experiment, P_{aCO_2} was higher and pH was lower in the HCA than in the normocapnia group (Fig. 1A, B). Serum bicarbonate decreased significantly in both groups over the course of the protocol, but there was no difference between the groups (Table 1). Serum base excess decreased significantly in both groups over the course of the protocol, and decreased to a significantly greater extent with normocapnia vs. HCA (Table 1).

Effect on Hemodynamic Profiles. HCA attenuated the decreased in MAP over the

course of the protocol. The MAP was significantly lower with normocapnia compared with HCA at each hourly time point after cecal ligation and puncture (Fig. 2A). The time required for the MAP to decrease by 25% and 50% from baseline values was significantly shorter in the normocapnia group compared with HCA (Table 1). There were no changes in CVP in either group over the course of the protocol (Table 1). $ScvO_2$ decreased significantly in both groups over the course of the protocol, but the decrease in the normocapnia group was significantly greater than with HCA (Table 1). HCA attenuated the increase in serum lactate over the course of the protocol, and serum lactate was significantly lower at each time point, compared with normocapnia (Fig. 2B).

Effect on Lung Injury. P_{aO_2} changed significantly in both groups over the

course of the protocol. There was a significant effect of HCA on P_{aO_2} , with P_{aO_2} significantly greater with HCA at 60 mins compared with normocapnia (Table 1). HCA reduced the alveolar-arterial oxygen gradient compared with normocapnia (Table 1). Peak airway pressure (P_{aw}) increased significantly in the normocapnia group, but not with HCA over the course of the experiment (Table 1). Static lung compliance decreased significantly in both groups over the course of the protocol, but there was no difference between the groups (Table 1). HCA significantly reduced lung wet/dry weight ratios and BAL protein concentrations (Table 1) compared with normocapnia. Quantitative stereological analysis demonstrated that there was no significant difference between normocapnia and HCA in regard to acinar tissue volume fraction or acinar air-space volume fraction (Fig. 3A). These data are consistent with a similar degree of structural lung damage in normocapnia and HCA.

Effect on Inflammation. HCA significantly reduced BAL neutrophil counts compared with normocapnia (Fig. 3B). There were no differences between the groups in BAL IL-6 or TNF- α levels (Table 1).

Pulmonary and Systemic Bacterial Load. There were no significant differences between the groups in the bacterial loads of the lungs, as assessed by BAL colony counts (Table 1). Similarly, there were no significant differences between the groups in the bacterial loads in the blood at 90 mins post cecal ligation and puncture, or at the end of the protocol (Table 1). Peritoneal bacterial loads were similar in both groups (Table 1).

Series 2: Prolonged Systemic Sepsis

Forty animals were entered into this study. No animals were excluded before randomization, and all 40 animals were randomized to receive normocapnia ($n = 20$) or HCA ($n = 20$).

Duration of Survival. Thirteen animals randomized to environmental hypercapnia survived the protocol, compared with 12 animals in the normocapnia group (Table 2). Median survival time was identical in both groups (Table 2). Animals in both groups lost weight to a similar extent over the course of the protocol (Table 2).

Arterial CO_2 Tension and Acid Base. Arterial pH was significantly lower and arterial bicarbonate and base excess were significantly higher in the prolonged hy-

Table 2. Prolonged systemic sepsis

| Variable | Normocapnia | HCA |
|-------------------------------------------------------|------------------|--------------------------|
| Number of animals | 20 | 20 |
| Animal survival, n/N (%) | 12/20 (60) | 13/20 (65) |
| Animal weight, g | | |
| Baseline | 416 ± 38 | 395 ± 29 |
| Final | 388 ± 42 | 365 ± 31 |
| Delta (final–baseline; CI) | –28 (–36 to –20) | –30 (–37 to –23) |
| Median survival time, hr | 96 (48, 96) | 96 (48, 96) |
| Arterial pH | 7.46 ± 0.04 | 7.29 ± 0.04 ^a |
| Arterial CO ₂ tension, torr, kPa | 30 ± 5.4 | 57 ± 3.3 ^a |
| Mean arterial pressure, mm Hg | 132 ± 6 | 134 ± 6 |
| Serum bicarbonate, mMol/L | 22.8 ± 0.9 | 25.4 ± 1.7 ^a |
| Base excess | –3.0 ± 0.3 | +2.1 ± 0.7 ^a |
| Lactate, mMol/L | 3.5 ± 1.0 | 2.5 ± 0.9 ^b |
| Arterial O ₂ tension, torr, kPa | | |
| F _{IO₂} 0.3 | 149 ± 10 | 160 ± 6 ^b |
| F _{IO₂} 1.0 | 566 ± 34 | 570 ± 34 |
| Alveolar-arterial oxygen gradient, torr | 108 ± 28 | 95 ± 40 |
| Peak airway pressure, mm Hg | 4.6 ± 0.3 | 4.6 ± 0.3 |
| Static lung compliance, mL/mm Hg ^{–1} | 0.68 ± 0.13 | 0.67 ± 0.04 |
| Wet/dry weight ratio | 4.4 (4.2, 4.6) | 4.3 (4.2, 4.6) |
| BAL protein concentration, μg·ml ^{–1} | 159 ± 59 | 163 ± 54 |
| BAL TNF-α concentration, pg·ml ^{–1} | 0 ± 0 | 0 ± 0 |
| BAL IL-6 concentration, pg·ml ^{–1} | 0 (0, 33) | 0 (0, 8) |
| Bacterial counts, cfu | | |
| Blood, ×10 ¹¹ ·ml ^{–1} | 2.6 ± 1.4 | 2.2 ± 1.6 |
| BAL, ×10 ⁶ ·ml ^{–1} | 8.6 ± 3.0 | 7.6 ± 2.7 |
| Peritoneal fluid, ×10 ¹³ ·ml ^{–1} | 4.1 ± 1.2 | 3.7 ± 1.1 |

HCA, hypercapnic acidosis; CI, confidence interval; BAL, bronchoalveolar lavage; TNF, tumor necrosis factor; IL, interleukin.

^aSignificantly different from normocapnia ($p < .01$); ^bsignificantly different from normocapnia ($p < .05$). Data are expressed as mean ± standard deviation or median (interquartile range). Final data indicates data collected upon completion of the experimental protocol.

percapnia group compared with the normocapnia group (Table 2), a finding compatible with persistent HCA. The degree of acidosis with chronic hypercapnia was less than that seen with acute HCA (Series 1), and serum bicarbonate was higher, indicating that partial buffering had taken place over the course of the exposure.

Effect on Hemodynamic Profile. There was no difference in MAP between the groups at the end of the protocol (Table 2). MAP was similar to that seen at baseline in anesthetized animals before exposure to cecal ligation and puncture (CLP) (Series 1). In contrast, serum lactate was significantly lower in animals exposed to HCA compared with normocapnia (Table 2).

Effect on Lung Injury. Pao₂ was higher in animals exposed to HCA (Table 2). However, this seems to be a physiologic effect of inhaled CO₂ because Pao₂ was not different between the groups when the animals were exposed to 100% oxygen, and there was no difference in alveolar-arterial oxygen gradient at the end of the protocol (Table 2). Furthermore, there was no dif-

ference in peak Paw or static lung compliance between the groups at the end of the protocol (Table 2). Lung wet/dry weight ratios were not different between the groups (Table 2). Quantitative stereological analysis demonstrated HCA modestly but significantly reduced acinar tissue volume fraction and increased acinar air-space volume fraction (Fig. 4A). These data are consistent with a small reduction in structural lung damage in animals exposed to hypercapnia.

Effect on Inflammation. HCA did not alter BAL neutrophil counts compared with normocapnia (Fig. 4B). There were no differences between the groups in BAL IL-6 or TNF-α levels, or in BAL protein concentrations compared with normocapnia (Table 2). Of interest, TNF-α was not detected in the BAL in either group, and BAL IL-6 levels were much lower than that seen in the early sepsis series (Table 2).

Pulmonary and Systemic Bacterial Load. There were no significant differences between the groups in the bacterial loads of the lungs, as assessed by BAL colony counts (Table 2). Similarly, there

were no significant differences between the groups in the bacterial loads in the blood at the end of the protocol (Table 2). These data suggest that prolonged hypercapnia did not alter bacterial proliferation or killing in this model.

DISCUSSION

Hypercapnia is a common component of protective ventilation strategies in critically ill patients with ALI/ARDS. Sepsis, whether due to pneumonia or systemic infection, is a major cause of ALI/ARDS (17–21), and is associated with the poorest outcome (20, 31). In addition, sepsis may complicate critical illness due to other causes, as demonstrated by the frequency of ventilator-associated pneumonia in the critically ill (32). The safety of HCA and its potential to cause adverse effects in the context of live bacterial sepsis remains a concern. Specifically, HCA may retard the immune response to bacterial infection, reducing bacterial killing, and ultimately worsening lung damage and injury caused by live bacterial infection (14, 22).

The effect of HCA in the setting of sepsis may differ depending on the phase of the infective process into the it is introduced. Early in the infective process, the contribution of bacterial toxins and the host response to the infection may predominate over direct bacterial injury and damage that occur later. The anti-inflammatory effects of HCA (13), and its potential to reduce endotoxin-induced ALI (6), may limit early tissue injury. This contention is supported by our previous finding that acute HCA reduces the severity of early acute lung injury induced by intratracheal *Escherichia coli* instillation (24). In prolonged systemic sepsis, HCA may impair the host response, reducing neutrophil recruitment and microbicidal (33, 34) and chemotactic activity (35), impairing bacterial killing, ultimately leading to increased bacterial load and more tissue damage (14, 22). In this regard, our group has reported recently that sustained hypercapnia worsened the lung injury induced by prolonged bacterial sepsis, by a mechanism involving inhibition of neutrophil phagocytosis (25). We therefore wished to determine the effects of HCA in both early and late systemic sepsis induced by cecal ligation and puncture, a well-characterized animal model of polymicrobial sepsis-induced septic shock and lung injury (36).

Acute HCA reduced the severity of early septic shock and lung injury pro-

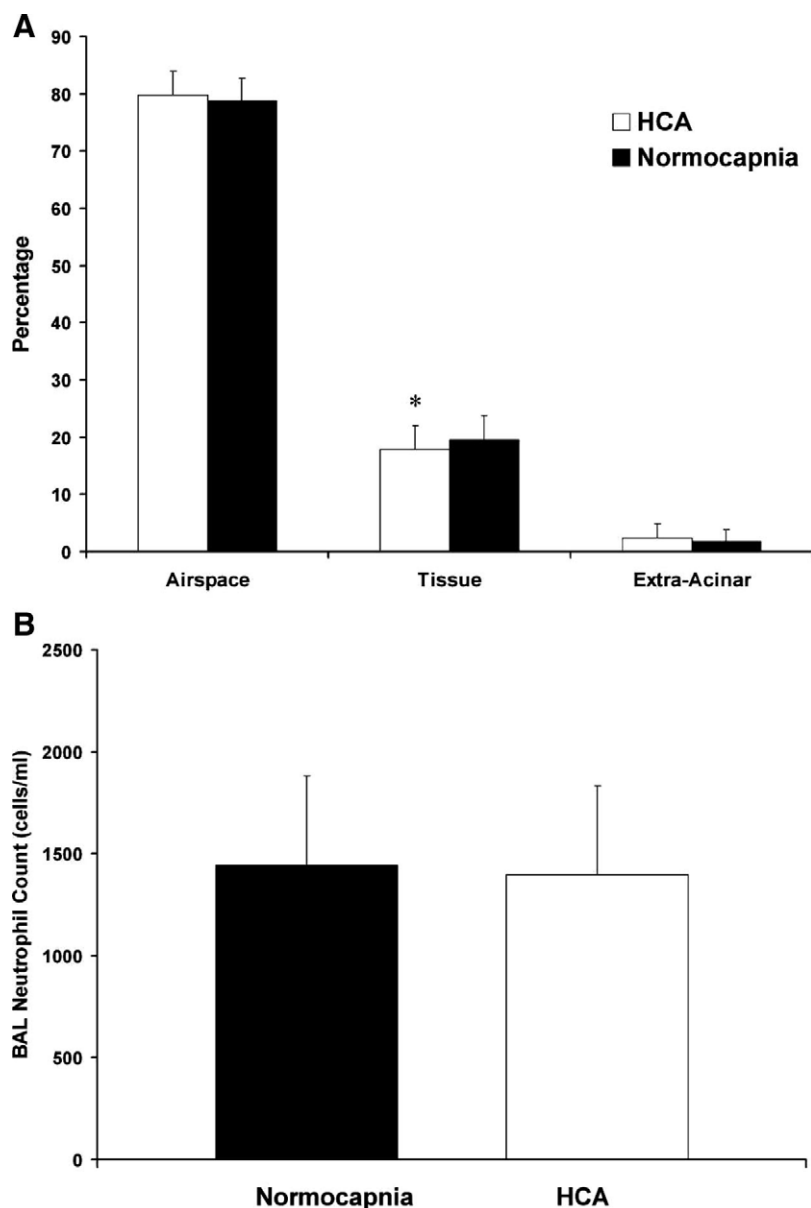


Figure 4. A, Histogram representing stereologic assessment of the extent of histologic injury in prolonged cecal ligation and puncture-induced systemic sepsis. B, Histogram representing the mean (standard deviation) bronchoalveolar lavage (BAL) neutrophil counts at the end of the protocol in prolonged cecal ligation and puncture-induced systemic sepsis. Normocapnia ($F_{iCO_2} = 0.00$); HCA, hypercapnic acidosis ($F_{iCO_2} = 0.05$). *Significantly different from normocapnia ($p < .05$, Student's t test).

duced by cecal ligation and puncture in these studies. HCA slowed the development of hypotension, preserved central venous oxygen saturations, and attenuated the increase in serum lactate, compared with normocapnia. CVPs did not change throughout the protocol, reducing the likelihood that differences in fluid volume status contributed to the hypotension in either group. HCA reduced the severity of lung injury, as demonstrated by a reduction in the alveolar-arterial oxygen gradient, and reduced lung permeability, compared with normocapnia.

HCA reduced BAL neutrophil counts but did not alter BAL IL-6 or TNF- α levels compared with normocapnia. There was no effect of HCA on indices of histologic injury. Of importance, there was no effect of HCA on the bacterial load in the bronchoalveolar lavage, or in the blood at 90 mins post CLP, or the end of the protocol. Peritoneal fluid bacterial loads were also similar in both groups. These findings confirm and extend the reported findings of Wang et al (26), who demonstrated that acute HCA improved indices of tissue oxygenation in septic shock produced by

CLP in an ovine model (26). HCA also reduced certain indices of lung injury in this setting (26).

HCA did not worsen the severity of prolonged systemic sepsis, in contrast to our hypothesis. HCA did not alter survival from prolonged systemic sepsis. The exposure of the animals to prolonged environmental hypercapnia had induced partial tissue buffering, as shown by a higher pH and serum bicarbonate than that seen post acute exposure to hypercapnia. However, these animals had a persistent HCA, as had been previously reported during sustained exposure to environmental hypercapnia (57–59). The hemodynamic profile in surviving animals in both groups was comparable and not dissimilar from that seen in anesthetized animals not exposed to CLP. Prolonged HCA did not alter physiologic indices of lung injury in comparison with normocapnia. There was no effect of HCA on BAL neutrophil counts or BAL IL-6 or TNF- α levels compared with normocapnia. Of importance, HCA did reduce acinar tissue volume fraction and increased acinar air-space volume fraction, a finding consistent with modest reduction in structural lung damage in animals exposed to hypercapnia. Furthermore, there was no effect of prolonged HCA on the bacterial load in the BAL, or in the blood at the end of the protocol. Peritoneal fluid bacterial loads were also similar in both groups.

Our finding that HCA exerted beneficial effects in the setting of prolonged systemic sepsis provides reassurance regarding the effects of sustained exposure to hypercapnia in systemic sepsis. Of importance, there was no evidence to suggest that HCA worsened lung injury induced by prolonged CLP. The finding that HCA did not alter pulmonary, blood, or peritoneal bacterial loads in the setting of either early or prolonged systemic sepsis provides reassurance regarding the effects of HCA on bacteria killing. These findings contrast with our earlier reported findings of deleterious effects of sustained HCA in the setting of pneumonia-induced HCA (25). In this study, sustained hypercapnia worsened pneumonia-induced lung injury and increased bacterial load in the lungs (25). The increased bacterial numbers in this study seem to have been due to reduced bacterial killing, as shown by reduced neutrophil phagocytic activity rather than from hypercapnia-enhanced growth of *E. coli* (25). In fact, the growth rate of *E. coli* is

unaltered by CO₂ values of >20%, concentrations of CO₂ that markedly exceed those used in either study (37). Taken together, these findings suggest that the effects of sustained HCA may depend on the location of primary infection and bacterial burden.

The literature attesting to beneficial effects of direct intra-abdominal administration of CO₂—by means of a CO₂ pneumoperitoneum—further supports the safety and efficacy of HCA in abdominal sepsis. CO₂ pneumoperitoneum improved survival compared with helium pneumoperitoneum in animals subjected to combined laparotomy and endotoxemia injury (38). Most recently, CO₂ pneumoperitoneum has been demonstrated to increase survival in mice and rabbits with polymicrobial peritonitis induced by CLP (39, 40). These effects seem to be due to the immunomodulatory effects of HCA (41), which include an IL-10 mediated down-regulation of TNF- α (42). Importantly, these effects seem to be mediated by the localized peritoneal acidosis produced (43), rather than by any systemic effect (44).

There are a number of aspects of this study that indicate the need for caution before extrapolation to the clinical scenario. First, this study utilized a concentration of 5% to 8% CO₂, which was based on our previous demonstration that this concentration range was both safe and effective (5). This produced a degree of HCA similar to that commonly observed when using protective ventilatory strategies. The effects of higher concentrations of CO₂ in systemic polymicrobial sepsis are not known, but potentially important given that higher doses are deleterious in other ALI models (5). However, the beneficial effects of a CO₂ pneumoperitoneum, which likely results in high intraperitoneal CO₂ tensions, may allay these concerns in regard to abdominal sepsis. Furthermore, in our experiments, HCA was introduced around the time of commencement of the CLP injury. It is not clear what effect induced HCA might have if introduced well after the establishment of infection. Finally, the finding that HCA reduced the decrement in Pao₂ in early systemic sepsis may be explained, in part, by its potential to improve V/Q matching (45). However, HCA did reduce the severity of other physiologic and histologic indices of lung injury in these studies.

CONCLUSION

We report that acute HCA reduced the severity of early septic shock and lung injury induced by CLP. Furthermore, we have demonstrated that the severity of lung injury induced by prolonged polymicrobial sepsis was modestly reduced—not worsened—by sustained HCA. These findings provide reassurance regarding the safety of HCA in the setting of systemic sepsis.

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