Injurious Effects of Hypocapnic Alkalosis in the Isolated Lung

JOHN G. LAFFEY, DOREEN ENGELBERTS, and BRIAN P. KAVANAGH

Department of Critical Care Medicine and The Lung Biology Program, The Research Institute, The Hospital for Sick Children; Department of Anaesthesia and the MSICU, The Toronto General Hospital, University Health Network; and Departments of Anaesthesia and Medicine, University of Toronto, Toronto, Ontario, Canada

Mechanical ventilation can worsen morbidity and mortality by causing ventilator-associated lung injury, especially where adverse ventilatory strategies are employed. Adverse strategies commonly involve hyperventilation, which frequently results in hypocapnia. Although hypocapnia is associated with significant lung alterations (e.g., bronchospasm, airway edema), the effects on alveolar-capillary permeability are unknown. We investigated whether hypocapnia could cause lung injury independent of altering ventilatory strategy. We hypothesized that hypocapnia would cause lung injury during prolonged ventilation, and would worsen injury following ischemia–reperfusion. We utilized the isolated buffer-perfused rabbit lung model. Pilot studies assessed a range of levels of hypocapnic alkalosis. Experimental preparations were randomized to control groups ($F_{ICO_2} = 0.06$) or groups with hypocapnia ($F_{ICO_2} = 0.01$). Following prolonged ventilation, pulmonary artery pressure, airway pressure, and lung weight were unchanged in the control group but were elevated in the group with hypocapnia; elevation in microvascular permeability was greater in the hypocapnia versus control groups. Injury following ischemia–reperfusion was significantly worse in the hypocapnia versus control groups. In a preliminary series, degree of lung injury was proportional to the degree of hypocapnic alkalosis. We conclude that in the current model (1) hypocapnic alkalosis is directly injurious to the lung and (2) hypocapnic alkalosis potentiates ischemia–reperfusion-induced acute lung injury.

Hypocapnia has been a standard therapeutic goal in critically ill patients, especially in situations in which pulmonary vascular resistance or intracranial pressure is increased. However, it is becoming clear that uncritically targeting hypocapnic blood gas tensions—specifically in patients following head injury (1) or in neonates with persistent fetal circulation (2)—is associated with worsened clinical outcome. Furthermore, in the context of respiratory failure, development of lower versus higher Pa_{CO_2} may be associated with worse outcome in adult acute respiratory distress syndrome (ARDS) (3), and a greater incidence of chronic bronchopulmonary dysplasia in children (4).

Several lines of evidence complement these clinical concerns relating to hypocapnia. *First*, a significant body of laboratory data confirm that experimentally induced hypocapnia results in significant adverse pulmonary effects, including bronchospasm (5), increased airway permeability (6), dysfunctional surfactant (7), and reduced lung compliance (8). *Second*, hypocapnia is associated with significant adverse systemic consequences. Hypocapnia results in accentuation of ischemic injury in the brain (9) and myocardium (10), and worsens systemic oxygenation (11, 12). *Third*, management of

ventilatory strategy and control of Pa_{CO_2} are inextricably linked. Because choice of ventilatory strategy may have an impact on patient outcome* (3), investigation of the potential independent role of Pa_{CO_2} on outcome is important. Preliminary reports that randomization to low- versus high-stretch ventilatory strategies is associated with lower mortality, despite comparable Pa_{CO_2}, controls for, but does not address, any potential roles that hypocapnia or hypercapnia may play in determining outcome. *Finally*, hypocapnia is a central component of several critical disease states, including systemic inflammatory response syndrome (13), asthma, and high-altitude pulmonary edema (14). The general assumption that low CO_2 tension simply reflects hyperventilation, but does not exacerbate or cause the underlying illness, has not been proven.

These concerns, together with reports demonstrating protective effects of hypercapnia in experimental lung injury from our group (15, 16) and protective effects against systemic organ injury from other groups (9, 10, 17), have led us to propose that hypocapnia might represent a pathogenic parameter in critically ill patients, that should perhaps be independently avoided or corrected (18).

We therefore investigated the effects of hypocapnia in an *ex vivo* perfused and ventilated lung model. Based on pilot data indicating a dose–response relationship between degree of hypocapnic alkalosis and degree of lung injury, we specifically hypothesized (1) that hypocapnic alkalosis might be directly injurious and have adverse effects on microvascular permeability and (2) that hypocapnic alkalosis might potentiate ischemia–reperfusion-induced acute lung injury.

METHODS

Male New Zealand white rabbits (3.0–4.0 kg) were used in all experiments. All experimental work conformed to the guidelines of the Canadian Council for Animal Care, and was approved by the Animal Care Committee of The University Health Network.

Experimental Outline

Following pilot studies examining the effects of graded hypocapnic alkalosis on injury, the experiments were organized into two series of *ex vivo* isolated lung experiments. Series I examined the effects of control conditions ($F_{ICO_2} = 0.06$) versus hypocapnic alkalosis ($F_{ICO_2} = 0.01$) on preparations subjected to a standard 3-h period of ventilation and perfusion. Series II examined the effects of control conditions versus hypocapnic alkalosis on acute lung injury following warm ischemia–reperfusion.

Surgical Dissection

After premedication with intramuscular ketamine (85 mg kg^{-1}), anesthesia was induced with pentobarbital sodium (15–25 mg kg^{-1}) given intravenously, and heparin (1,000 IU) was administered. The surgical preparation used in this study was similar to that previously reported

*Data from NIH-sponsored trial comparing high-stretch versus low–stretch ventilation; preliminary report available at website: http://hedwig.mgh.harvard.edu/ardsnet/nih.html

(15, 19), with several modifications. Incremental boluses of pentobarbital (5 mg kg^{-1}) were administered as required. Briefly, a tracheotomy was performed and pancuronium bromide (1 mg given intravenously) was administered after depth of anesthesia was confirmed by absence of response to paw clamp. The lungs were ventilated using a small animal ventilator (Model No. 683; Harvard Apparatus, Holliston, MA) with $F_{I_{O_2}}$ = 1.0, rate 20 min^{-1}, tidal volume 4 ml kg^{-1}, and 2 cm H_2O positive end-expiratory pressure (PEEP). The carotid artery was cannulated for arterial pressure measurement, with additional pentobarbital administered for any elevation in baseline mean arterial pressure $\geqslant$ 10%. A sternotomy was performed, and the pulmonary artery and left atrium were cannulated. The lungs were then ventilated with 6% CO_2–75% O_2–19% N_2 and flushed with blood-free Krebs–Henseleit solution containing 3% bovine serum albumin, using a peristaltic pump (Model No. M312; Gilson, Villier, France). The heart and lungs were excised from the chest and suspended by the tracheotomy tube from a counterbalance force-displacement transducer (Model No. 60-2995; Harvard Apparatus), connected to a chart recorder (Recordall Series 5000; Fisher Scientific, Nepean, ON, Canada) for continuous measurement of lung weight. The lungs were then perfused in a recirculating manner with Krebs–Henseleit solution containing 3% bovine serum albumin at a flow rate of 150 ml min^{-1}. The perfusate temperature was maintained at 37° C, and the total volume in the circuit was 500 ml. Pulmonary arterial (Ppa) and left atrial pressures (Pla) were referenced to the height of the left atrium and measured continuously via T-piece stopcock connections in the tubing leading to each cannula. The pulmonary cannula ended in a single end-lumen, whereas the left atrial cannula had side holes in addition to the end lumen to prevent occlusion in the event of atrial collapse. The venous reservoir height was adjusted to maintain Pla at +2 mm Hg. The left atrium was wrapped loosely with string and glued to maintain constant left atrial volume to prevent it from acting as a pressure "capacitor" during vascular occlusion, and thus causing distortion (underestimation) of pulmonary capillary hydrostatic pressure measurement. All gas mixtures were supplied premixed in cylinders (Praxair, Mississauga, ON, Canada).

Measurements

Perfusate gases. Samples of perfusate were taken from the reservoir and were measured for pH, P_{CO_2}, and P_{O_2}, using an ABL-300 blood gas analyzer (Radiometer, Copenhagen, Denmark).

Vascular and airway pressures. Ppa, Pla, and airway pressures were recorded from a standard monitor (Spacelabs Monitor Model No. 90303B; Spacelabs Medical Products Ltd., Mississauga, ON, Canada), with hard copy tracings for determination of microvascular pressures recorded using a five-channel recorder (Gould 8 channel Recorder 2800; Gould Inc., Instruments Division, Cleveland, OH).

Pulmonary capillary hydrostatic pressure (Pcap). To determine Pcap, mechanical ventilation was stopped, and the lungs inflated with continuous positive airway pressure (CPAP) of 2 cm H_2O. The pulmonary arterial and left atrial cannulas were simultaneously occluded using the double occlusion technique (19, 20) and Pcap was calculated as the mean of the arterial and venous pressures at 3 s after occlusion.

Isogravimetric pressure. Isogravimetric pressure (Piso) is the Pcap at which the lung neither gains nor loses weight, indicating that the Starling forces are balanced (19, 20). Piso was determined by discontinuing flow through the lung and opening a shunt between arterial and venous tubing so that Ppa and Pla were identical and thus equal to Pcap. Pla was then altered in 1 mm Hg increments, by alteration of the height of the venous reservoir, and the effect on lung weight was examined. Piso was defined as the highest Pcap at which the lung did not gain weight over a 4-min period.

Pulmonary capillary filtration coefficient (Kf,c). Kf,c was then calculated by a modification of the methods of Kavanagh and coworkers (19) and Drake and coworkers (20). When Pcap equals Piso, the Starling forces are balanced so that there is no net transfer of fluid across the pulmonary capillary membrane. When the Pcap is suddenly increased from Piso to Piso +7 mm Hg, the other Starling forces initially remain unchanged so that pulmonary edema formation occurs at rate of 7 × Kf,c. However, lung weight gain after the increase in Pcap is due to both intravascular volume expansion and edema formation. The expansion is rapid and essentially complete within 3 min.

Therefore, the rate of weight gain was recorded on a semilog plot every minute from 3 to 10 min and extrapolated back to time = 0, by linear regression.

Exclusion Criteria

Prior to randomization, baseline perfusion was commenced for 10 min with 75% O_2–19% N_2–6% CO_2. For all preparations, based on both previous work (15, 19) and preexperimental pilot data, the following exclusion criteria were applied: air leak from preparation, appearance of gas bubbles or emboli in perfusate cannulas, weight gain > 2 g during baseline perfusion, weight gain > 3 g during estimation of Kf,c, perfusate leakage > 0.3 ml/min from preparation during baseline perfusion, Ppa > 10 mm Hg, weight gain > 1 g during the third 5-min period of perfusion following measurement of baseline Kf,c and weight gain during 5 min immediately postaddition of purine to the preparation of > 1 g. Preparations were discarded if any of these parameters were exceeded.

Pilot Studies: Dose–Response with Graded Hypocapnic Alkalosis

A model of warm ischemia–reperfusion was developed, modified from a previous report (21). The duration of ischemia necessary to produce a "threshold" injury postreperfusion in the control group was determined during preliminary pilot experiments (n = 10). After baseline perfusion, and provided none of the exclusion criteria was met, four additional preparations were assigned to ventilation with the following gas mixtures: (*1*) 0% CO_2, 75% O_2, 25% N_2, (*2*) 1% CO_2, 75% O_2, 24% N_2, (*3*) 3% CO_2, 75% O_2, 21% N_2, or (*4*) 6% CO_2, 75% O_2, 19% N_2. The target pH and P_{CO_2} were achieved over a 20-min period. After stabilization at target pH and P_{CO_2}, purine (0.006 g) was added to replace physiological sources, and perfusion continued for an additional 10 min. Ventilation and then perfusion were then stopped, and lung inflation was maintained using CPAP 2 cm H_2O with the same gas mixture, and the temperature maintained at 37° C in a humidified chamber. After 20 min, mechanical ventilation was recommenced. Perfusion was then commenced at 15 ml min^{-1}, and increased in a standardized fashion, increasing by 15 ml min^{-1}, each minute, for the first 10 min. The reperfusion continued for an additional 30 min, at which stage final lung weight and elevation in airway pressure were measured.

Series I: Prolonged Mechanical Ventilation

After baseline values were determined during ventilation with 6% CO_2, 75% O_2, 19% N_2 and exclusion criteria had not been met, preparations were randomly allocated to one of two groups (n = 8 in each) as follows: control ventilated with 6% CO_2, 75% O_2, 19% N_2 or hypocapnic alkalosis ventilated with 1% CO_2, 75% O_2, 24% N_2. Final values were determined after 3 h of ventilation.

Series II: Injury Induced by Warm Ischemia and Reperfusion

After baseline perfusion, and provided none of the exclusion criteria was met, the preparations were then randomized (n = 8 per group) to ventilation with one of the following gas mixtures: 6% CO_2, 75% O_2, 19% N_2 (producing control conditions) or 1% CO_2, 75% O_2, 24% N_2 (producing hypocapnic alkalosis). The target pH and P_{CO_2} were achieved over a 20-min period. After stabilization at target pH and P_{CO_2}, purine (0.006 g) was added to replace physiological sources, and reperfusion continued for an additional 10 min. Ventilation and then perfusion were then stopped, and lung inflation was maintained using CPAP 2 cm H_2O with the same gas mixture, and the temperature maintained at 37° C in a humidified chamber. After 20 min, mechanical ventilation was recommenced. Perfusion was then commenced at 15 ml min^{-1}, and increased in a standardized fashion, increasing by 15 ml min^{-1}, each minute, for the first 10 min. The perfusion continued for an additional 30 min, at which stage final values for Ppa, Pcap, Piso, and Kf,c were measured.

Data Analysis and Statistics

All data were entered into a standard spread sheet (Excel 7.0, Microsoft Corp.) and exported for analysis using Sigmastat (Jandel #2,

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TABLE 1

SERIES I: PROLONGED VENTILATION

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Variable	Control ($F_{I_{CO_2}}$, 0.06)	Hypocapnia ($F_{I_{CO_2}}$, 0.01)
Rabbit weight, kg	3.4 ± 0.1	3.3 ± 0.1
pH baseline	7.38 ± 0.01	7.40 ± 0.01
pH final	7.36 ± 0.01	$7.84 \pm 0.02^{*,\dagger}$
P_{CO_2} baseline, mm Hg	33.1 ± 1.1	32.3 ± 0.6
P_{CO_2} final, mm Hg	35.0 ± 1.0	$13.5 \pm 0.3^{*,\dagger}$
Pcap baseline, mm Hg	4.1 ± 0.3	4.9 ± 0.4
Pcap final, mm Hg	4.4 ± 0.4	5.9 ± 1.2
Piso baseline, mm Hg	5.6 ± 0.2	5.5 ± 0.3
Piso final, mm Hg	$4.8 \pm 0.2^{\dagger}$	$4.9 \pm 0.4^{\dagger}$
Ppa baseline, mm Hg	6.3 ± 0.4	7.6 ± 0.3
Ppa final, mm Hg	8.1 ± 0.8	$14.3 \pm 3.6^{\dagger}$

Definition of abbreviations: Pcap = pulmonary capillary hydrostatic pressure; Piso = isogravimetric pressure; Ppa = pulmonary arterial pressure. Results are means ± SEM; n = 8 per group.
* $p < 0.05$ between groups.
† $p < 0.05$ versus baseline (within group).

San Rafael, CA). The data are summarized as means ± standard error of the mean (SEM). Statistical analysis utilized Kruskal–Wallis and Student–Newman–Keuls tests for nonparametric data, and ANOVA with Student–Newman–Keuls or paired *t*-tests for parametric data. We considered differences significant where $p < 0.05$.

RESULTS

Seventy isolated rabbit lung preparations were utilized over the three experimental series. Twenty-four preparations (34%) were discarded prior to randomization because they fulfilled one or more of the exclusion criteria.

Series I: Prolonged Mechanical Ventilation

Baseline. The values for animal weight, Pcap, Piso, Kf,c, and Paw, at baseline, in the animals that were randomized, were comparable (Table 1). The values for pH and P_{CO_2} before and after randomization are presented (Table 1).

Microvascular pressure measurements. There were no significant differences in Pcap among or within the groups at any time during the experiment (Table 1).

Pulmonary capillary permeability measurements. Kf,c at baseline was similar in both groups (Table 1). Final Kf,c was significantly greater than baseline Kf,c in both groups (Figure 1). Final Kf,c was significantly greater in the group with hypocapnic alkalosis compared with the control group (Figure 1). Furthermore, the increase in Kf,c (i.e., delta Kf,c: final Kf,c − baseline Kf,c) was significantly greater in the group with hypocapnic alkalosis compared with the control group (Figure 1).
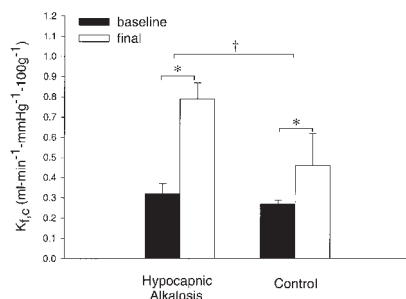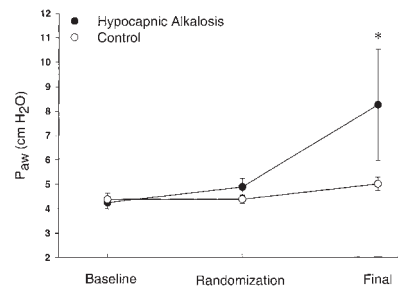

Figure 2. Peak airway pressure [Paw] at baseline, before and after prolonged ventilation. Paw was significantly elevated in the group with hypocapnia (*$p < 0.05$) but not in the control group.

Isogravimetric pressure measurements. There was no difference in Piso between the groups at baseline (Table 1). Piso was significantly decreased in both groups by the end of the series, and the magnitude of the decrease was similar in each group (Table 1).

Pulmonary artery pressure measurements. There was a significant increase in Ppa in the group with hypocapnic alkalosis following 3 h of ventilation and perfusion, but Ppa did not increase in the control group (Table 1).

Airway pressure measurements. There was no difference in Paw between the groups at baseline. There was a significant increase in Paw in the group with hypocapnic alkalosis over time. Furthermore, the increase in Paw (ΔPaw: final Paw − baseline Paw) was significantly greater in the group with hypocapnic alkalosis compared with the control group (Figure 2).

Experimental weight gain. There was no significant weight gain in the control group, but a significant weight gain developed in the group with hypocapnia over the experimental time course (Figure 3).

Series II: Injury Induced by Ischemia–Reperfusion (IR)

Baseline. The values for animal weight, Pcap, Piso, Kf,c, Ppa, and Paw, were comparable at baseline (Table 2). The values for pH and P_{CO_2} before and after randomization are presented (Table 2).

Microvascular pressure measurements. There were no significant within- or between-group differences in Pcap during the experiment (Table 2).

Pulmonary capillary permeability measurements. There was no difference between the groups in baseline Kf,c. Final versus baseline Kf,c was significantly greater in both groups (Figure 4). The magnitude of ΔKf,c, was significantly greater in the group with hypocapnic alkalosis compared with the control group (Figure 4).

Figure 1. Kf,c before and after prolonged ventilation. Final Kf,c was significantly greater than baseline in both groups (*$p < 0.05$); the magnitude of the increase was significantly greater in the group with hypocapnia versus the control group (†$p < 0.05$).
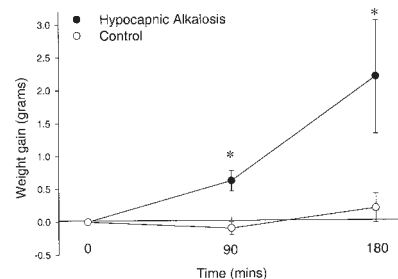

Figure 3. Increase in lung weight after prolonged ventilation. Lung weight was significantly increased in the group with hypocapnia (*$p < 0.05$) but not in the control group.

TABLE 2
SERIES II: ISCHEMIA–REPERFUSION

Variable	Control IR (F_{ICO_2}, 0.06)	Hypocapnic IR (F_{ICO_2}, 0.01)
Rabbit weight, kg	3.7 ± 0.1	3.7 ± 0.1
pH baseline	7.44 ± 0.01	7.43 ± 0.01
pH final	7.43 ± 0.01	$7.93 \pm 0.02^{*,\dagger}$
P_{CO_2} baseline, mm Hg	30.3 ± 0.6	31.2 ± 1.0
P_{CO_2} final, mm Hg	31.1 ± 0.6	$12.4 \pm 0.4^{*,\dagger}$
Pcap baseline, mm Hg	3.8 ± 0.2	4.5 ± 0.6
Pcap final, mm Hg	3.7 ± 0.3	4.3 ± 0.4
Ppa baseline, mm Hg	5.5 ± 0.5	5.9 ± 0.5
Ppa final, mm Hg	5.8 ± 0.3	$6.8 \pm 0.5^{\dagger}$

Definition of abbreviations: Pcap = pulmonary capillary hydrostatic pressure; Ppa = pulmonary arterial pressure. Results are means ± SEM; n = 8 per group.
* $p < 0.05$ between groups.
† $p < 0.05$ versus baseline (within group).

Figure 5. Piso measured before and after IR injury. Piso was significantly depressed after IR in the group with hypocapnia (*$p < 0.05$) but not in the control group.

Isogravimetric pressure measurements. There was no difference in Piso between the groups at baseline (Figure 5). Following IR, there was a significant decrease in Piso in the group with hypocapnic alkalosis, but not in the control group (Figure 5).

Pulmonary artery pressure measurements. There was no difference in Ppa between groups at baseline. There was a significant increase in Ppa in the group with hypocapnic alkalosis, but not in the control group, following IR (Table 2).

Airway pressure measurements. There was no difference in Paw between groups at baseline. There was a significant increase in Paw in the group with hypocapnic alkalosis, but not in the control group, following IR (Figure 6).

Pilot Studies: Dose–Response of Hypocapnic Alkalosis

The degree of hypocapnic alkalosis was correlated with the degree of lung injury as measured by corrected lung weight ($r^2 = 0.97$, $p < 0.02$) and elevation in airway pressure ($r^2 = 0.96$, $p < 0.02$) in a preliminary series of experiments (Figure 7).

DISCUSSION

The current study supports our hypothesis that hypocapnia causes lung injury during prolonged mechanical ventilation, and potentiates lung injury following ischemia–reperfusion.

Rationale for Investigating the Role of CO_2 in ALI

The importance of CO_2 in critically ill patients with respiratory failure is becoming increasingly recognized (18, 22–25). There are two complementary lines of reasoning to account for this. *First*, in the context of current practice, it is now clearly recognized that ventilatory techniques involving hyper-
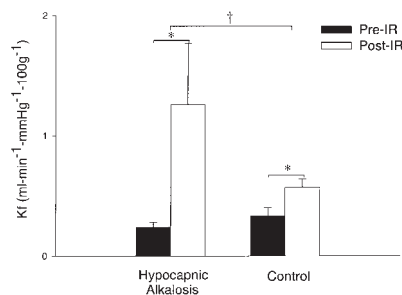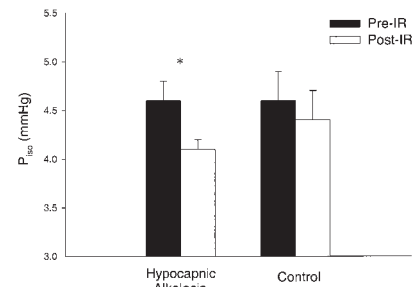
ventilation might cause or potentiate acute lung injury. Hypocapnia may accompany the development of such hyperventilation-associated ventilator-induced lung injury (VALI). In this paradigm, worsened outcome is thought to be due to nonprotective ventilatory strategies involving increased pulmonary production of cytokines (26) and/or pulmonary to systemic translocation of bacteria (27) or endotoxin (28) with hypocapnia fulfilling a passive role. However, hypocapnia has been *associated* with adverse outcome in patients with lung injury receiving mechanical ventilation (3, 4). Furthermore, an early report suggests that small concentrations (3.8% F_{ICO_2}), utilized to restore normocapnia, appeared to delay, but not prevent, the development of ventilator-induced lung injury (29). *Second*, "protective" ventilator strategies that minimize lung stretch necessitate use of smaller tidal volumes, and often lead to an elevation in Pa_{CO_2} (3, 30). This "permissive hypercapnia" is *associated* with improved outcome (3, 30). Alternatively stated, use of smaller tidal volumes in the presence of elevated Pa_{CO_2} appears to be beneficial. Whereas current concepts generally presuppose that improved outcome is due to limitation of lung stretch, evidence exists to support an independent protective effect of elevated CO_2 tension in experimental models of pulmonary (15, 16) and systemic organ (9, 10, 17) injury.

These phenomena have raised logical questions about the role of CO_2 in the determination of outcome (15, 18). CO_2 tension and tidal volume may play distinct roles, and by manipulation of respiratory frequency (or F_{ICO_2}), they can be separately controlled. The need to separately evaluate the contribution of P_{CO_2} and lung stretch to outcome has been highlighted by the preliminary data from the recently completed NIH-sponsored trial that demonstrated that adoption of a low tidal volume ventilatory strategy, while maintaining comparably elevated Pa_{CO_2} levels, resulted in a 25% reduction
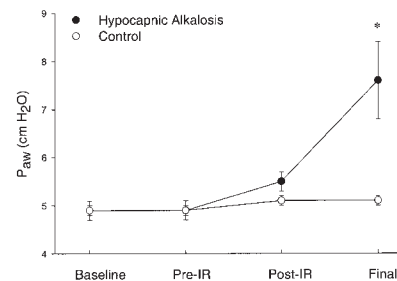

Figure 4. Kf,c before and after IR injury. Kf,c was significantly increased following IR in the control group and the group with hypocapnia (*$p < 0.05$); the magnitude of the increase was significantly greater in the group with hypocapnia versus the control group (†$p < 0.05$).

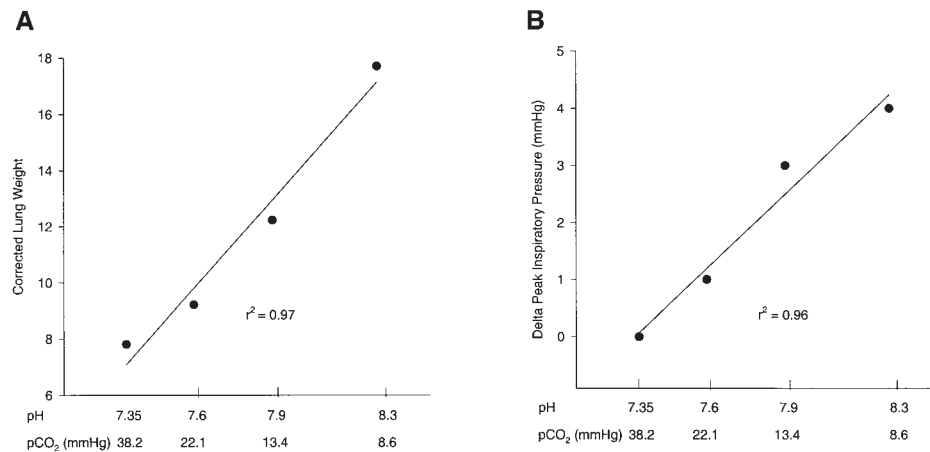*Figure 6.* Peak airway pressure (Paw) measured at baseline, before and immediately after IR injury, and at the end of the experiment. Paw was significantly increased following IR in the group with hypocapnia (*$p < 0.05$) but not in the control group.

A

B

Figure 7. (*A*) Dose–response data for hypocapnic alkalosis versus corrected wet lung weight ($r^2 = 0.97$; $p < 0.02$). (*B*) Dose–response data for hypocapnic alkalosis versus elevation in airway pressure ($r^2 = 0.96$; $p < 0.02$).

in mortality in patients with ARDS (*see above*; NIH preliminary communication). This finding clearly demonstrates that reduced lung stretch is highly beneficial independent of Pa_{CO_2} levels. In contrast, the clinical significance of CO_2 tension in the context of acute lung injury, independent of ventilation strategy, remains to be elucidated. Encouragingly, increasing laboratory evidence suggests that hypercapnic acidosis can exert direct protective effects on tissue injury in several different organ systems, including the lung (18, 24). However, the role of hypocapnia in the context of VALI is less clear. Because an evolving body of literature indicates advantages of hypercapnia versus normocapnia, and disadvantages of hypocapnia versus normocapnia, the potential adverse pulmonary effects of hypocapnia versus normocapnia merit investigation.

Deleterious Consequences of Hypocapnia

Significant concerns exist regarding potential adverse effects of lowered CO_2 tension on several aspects of both pulmonary (5–8, 12) and systemic organ (9, 10) dysfunction. Hypocapnia has been associated with worsened lung injury in the context of bronchopulmonary dysplasia (4) and ARDS (3). Although the pathogenic links between hypocapnia and adverse pulmonary outcome are incomplete, several potential contributory factors have been determined. Hypocapnia causes and exacerbates bronchospasm (5) and attenuates hypoxic pulmonary vasoconstriction, worsening intrapulmonary shunt and systemic oxygenation (12). In addition, laboratory data examining interactions of hypocapnia on ischemia-related injury in systemic organ systems are confined to the central nervous system (9) and the heart (10). The treatment of patients with acute head injury formerly employed the use of hyperventilation to produce hypocapnic alkalosis in order to decrease intracranial pressure, and was assumed to be beneficial. Prospective data now clearly show that hyperventilation is associated with *worsened* outcome (1), possibly as a result of hypocapnia-induced vasoconstriction and left shifting of the hemoglobin oxygen dissociation curve. In addition, hypocapnia prior to neonatal extracorporeal membrane oxygenation increases the risk of adverse neurological sequelae (31, 32). Finally, long-term neurological sequelae from exposure to extreme altitude are associated not with exposure to minimum Pa_{O_2}, but rather with the ability to generate extremely low levels of Pa_{CO_2} (33).

Mechanisms of Injury induced by Hypocapnia

Hypocapnia may exert adverse pulmonary effects via several distinct mechanisms. Airway hypocapnia increases tracheal microvascular permeability (6). Furthermore, hypocapnia decreases lung compliance in healthy volunteers and in patients with chronic obstructive lung disease (8), probably as a result of increased production of dysfunctional surfactant (7). Furthermore, we have demonstrated in this study that hypocapnia is both directly deleterious to the lung and potentiates acute lung injury following ischemia-reperfusion. The cellular basis for injury may relate to specific adverse processes associated with hypocapnia. The physiological basis for injury involves increases in microvascular permeability, which is consistent with the previously described effects of hypocapnia on tracheal microvascular permeability (6), possibly involving products of cyclooxygenase activation. Additional specific cellular effects associated with hypocapnia include increased inositol phosphate turnover (34) and activation of ATP-sensitive K^+ channels (35) and voltage-sensitive Ca^{2+} channels (36), all of which may play pathogenic roles in reperfusion injury.

Finally, mechanistic insights may be gained from knowledge of the possible mechanisms involved in the protective effects of hypercapnia. Hypercapnia can exert protective effects on mechanisms known to be involved in reperfusion injury. These include attenuation of leukocyte superoxide formation (37), cell adhesion molecule expression (38), and xanthine oxidase (15). Furthermore, hypercapnia increases production of endogenous NO (39) and cyclic nucleotides (40). It is possible that the adverse effects associated with hypocapnia may result from reversal of these protective mechanisms.

Hypocapnia and Hypercapnia: A Continuum of Effect?

It is not yet clear whether the range of hypocapnia–normocapnia–hypercapnia may represent a "therapeutic" (or pathogenic) continuum, with the deleterious effects of hypocapnia alkalosis forming the corollary of the protection seen with hypercapnic acidosis. However, we believe that this may be the case for several reasons. *First,* as discussed, a dose–response relationship appears to exist for hypocapnic alkalosis, with the degree of injury proportional to the severity of the hypocapnic alkalosis. This range includes normocapnia. *Second,* we have previously demonstrated that a dose–response relationship exists for the protective effects of hypercapnic acidosis (15). *Third,* supportive evidence from central nervous system ischemia exists. Vannucci and coworkers demonstrated histological indices of stroke severity were ranked highest with hypocapnia, lowest with hypercapnia, and intermediate with normocapnia, in an *in vivo* rabbit model (9). In summary, the data presented here provide further support for the contention that the same diseases or types of injuries that are protected by hypercapnia may be adversely affected by hypocapnia (and vice versa). In this regard, administration of CO_2

therapeutic hypercapnia (18), may be beneficial both through prevention of hypocapnia and/or generation of hypercapnia.

Limitations of Study

Extrapolation of our results to the clinical situation must be restricted by the inherent limitations of an isolated buffer-perfused model. The perfused lung is denervated, isolated from the systemic circulation, and perfused with a blood-free perfusate. The particular concerns relate to the lack of pulmonary–systemic interactions in the context of organ injury and the potential systemic effects of profound hypocapnia. Accurate assessment of Kf,c in the current model may be limited by the inability to quantify a contribution arising from epithelial leakage. This may be significant in the context of alveolar edema. Future research could investigate the specific mechanisms of the effects observed in the current study, and, in addition, examine possible synergy between adverse ventilatory strategies and varying degrees of hypocapnia.

Clinical Significance

Although the levels of hypocapnia examined in this study are severe, they are of human relevance in several contexts. Hypocapnia of comparable severity is *rarely* attained in several diverse clinical situations including cardiopulmonary bypass (41), neonatal respiratory failure (31), and under conditions of real (42, 43) and simulated (44, 45) extreme altitude. Severe alkalosis (pH > 7.7) has also been reported in humans, particularly in the context of extreme altitude (42, 43). Such levels of hypocapnia have been associated with adverse neurological outcome following cardiopulmonary bypass (41), and with treatment of neonatal respiratory failure (31). Finally, if the dose–response characteristics in the current study are confirmed and extended, it is possible that qualitatively, although not quantitatively, comparable effects of hypocapnia on lung injury may be clinically common. Therefore, although current concerns relating to Pa_{CO_2} may mirror our contemporary approach to Pa_{O_2} in terms of targets (46) and practice variations (47), future use of "therapeutic hypercapnia" may place CO_2 management in a new perspective (18).

The findings in the current study support the hypothesis that hypocapnia can cause and potentiate acute lung injury, and extend our knowledge regarding the adverse pulmonary and systemic effects of hypocapnia. Given the interplay between mechanical ventilation and arterial CO_2 tension, these and previous (15, 16) findings suggest that future investigations into adverse outcome associated with mechanical ventilation in critically ill patients should involve assessment of the role of CO_2. The current data support, in addition, the hypothesis (18) that where hypocapnia commonly coexists in medical conditions, it may exert directly harmful effects.

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