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Hypercapnic acidosis minimizes endotoxin-induced gut mucosal injury in rabbits

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Abstract Objective: Recent evidence demonstrated that hypercapnic acidosis due to lung protective strategy was not only permissive but also even therapeutic for injured lung. Since the effects of hypercapnic acidosis on extra-pulmonary organs remain to be clarified, we tested the hypothesis that hypercapnic acidosis protects gut mucosal barrier function by modulating inflammation in a rabbit model of endotoxemia.

Design: Prospective randomized animal study. **Setting:** University research laboratory. **Subjects:** Male New Zealand white rabbits. **Interventions:** Thirty-two animals were randomly allocated into two groups: normocapnia ($n = 17$) and hypercapnia ($n = 15$). The latter group received $F_i\text{CO}_2$ 5% under mechanical ventilation to achieve hypercapnia throughout the study periods, whereas the former with $F_i\text{CO}_2$ 0%.

Measurements and results: Arterial blood gas, intramucosal pH (pHi) and portal blood flow were assessed at baseline, 2-h and 4-h infusion of lipopolysaccharide. At 4 h, ileal myeloperoxidase (MPO) activity and intestinal permeability were measured. The animals in the hypercapnia group showed apparent hypercapnic acidosis and progressive intramucosal acidosis at 4 h, accompanied by significantly lower intestinal permeability versus normocapnia group. Ileal MPO activity was comparable between the study groups. **Conclusions:** Hypercapnic acidosis attenuates endotoxin-induced gut barrier dysfunction possibly through neutrophil-independent mechanisms.

Keywords Hypercapnia · Bacterial translocation · Intramucosal pH · Myeloperoxidase

Introduction

Lung protective strategy has been recognized as a valuable approach to improve the outcome of critically ill patients with acute respiratory distress syndrome (ARDS) [1, 2]. With this strategy, low tidal volume ventilation to obviate alveolar overextension results in hypercapnic acidosis. Recent study demonstrated that hypercapnic acidosis had a protective or even therapeutic effect on injured lungs [3, 4], whereas the other group alarmed that hypercapnic acidosis retarded plasma membrane wound resealing in a model of ventilator-induced lung injury [5].

Although the key component to provide its protective property is primarily considered as being acidosis, hypercapnic acidosis is more effective to minimize lung injury rather than metabolic acidosis [6]. On the contrary, hypercapnia by itself has potent vasodilating property to provide sufficient oxygen delivery to tissues, particularly in the coronary and cerebral circulation [7, 8]. In addition, an increasing amount of evidence indicates that several components of inflammation, including production of reactive oxygen species and cytokines and activation of nuclear factor- κB , were diminished under hypercapnic acidosis [9, 10]. Collectively, protective effects of

hypercapnic acidosis on injured organs are mainly produced through its vasodilating and/or anti-inflammatory mechanisms.

While critically ill patients complicated by ARDS is believed to die not owing to respiratory failure but to the other organ failure, the effect of hypercapnic acidosis on extra-pulmonary organs remains to be fully determined. In *ex vivo* experimental condition, previous studies showed that reperfusion with hypercapnic perfusate augmented the recovery of myocardial function after prolonged ischemia and reduced the infarct size after coronary artery occlusion [11, 12]. On the other hand, the impact of hypercapnic acidosis on the gut, the most fragile organ against any type of insults [13], remains unclear and may differ depending on conditions of critically ill or study subjects. Besides, at the microcirculatory level of gut mucosa, hypercapnia by itself possesses the contra-lateral effects, i.e., direct local vasodilation and indirect local vasoconstriction by sympathetic simulation [10]. Since tissue accumulation of activated neutrophils plays consequential roles to regulate gut barrier function [14], we tested the hypothesis that hypercapnic acidosis preserved gut mucosal function through the modulation of neutrophil dynamics in splanchnic area in a rabbit model of endotoxemia.

Materials and methods

Detailed descriptions of all procedures are available in the online supplement.

Study protocol

After preparatory surgery as described previously [15, 16], 32 healthy rabbits were randomly assigned to two groups, normocapnia ($n = 17$) and hypercapnia ($n = 15$) groups: the former was mechanically ventilated with $F_{I}CO_2$ 0% and the latter with $F_{I}CO_2$ 5%. After baseline measurements (Fig. 1), lipopolysaccharide (LPS: 15 μ g/kg per minute) infusion was initiated with aggressive fluid

therapy throughout the study periods. At 4-h study period, we determined the changes of gut mucosal permeability using fluorescein isothiocyanate-conjugated dextran (FD4). Lung and ileal tissues were obtained for the measurement of wet to dry weight ratio. To examine the time-course effects of hypercapnic acidosis on normal rabbits, we also studied eight sham animals that received the same catheterization without LPS infusion under the same study protocol.

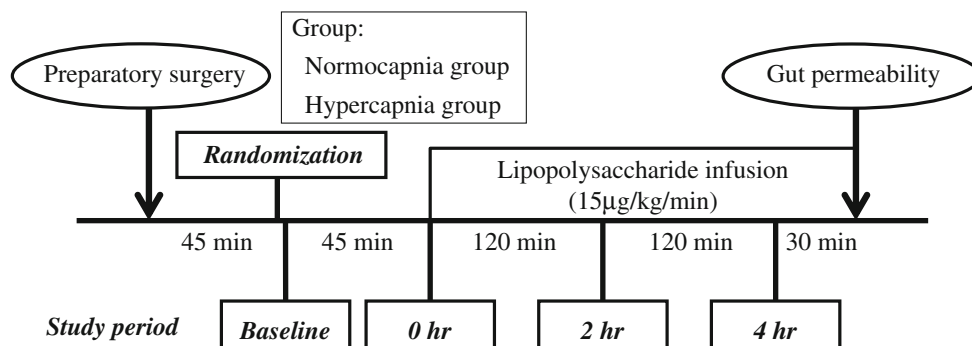
Measurement of intramucosal pH, gut mucosal permeability and biochemical analyses

Gut intramucosal pH (pHi) was monitored using an automated air tonometry (Tonocap, Datex Ohmeda, Helsinki, Finland) [16, 17] throughout the study period. To examine the effects of systemic acidosis on pHi over the hypercapnic and endotoxemic stages, we further examined the PCO_2 gap defined as the difference between measured $PrCO_2$ and $PaCO_2$. Gut mucosal permeability was measured using an *in situ* loop preparation as described previously [16, 18]. The results were corrected for the plasma protein contents measured by the Lowry method [19]. Arterial blood samples were used to determine white blood cell (WBC), blood gas and lactate. Residual arterial blood and portal blood samples were centrifuged and stored for measurements of lactate dehydrogenase (LDH) activity [16]. As an index of leukocyte sequestration, MPO activity of ileal wall was assayed [20]. At the completion of experiments, a randomly selected left or right lung was lavaged to measure protein concentration of bronchoalveolar lavage fluid (BALF) [21].

Statistical analysis

Data are expressed as mean \pm standard deviation unless otherwise specified. Analysis of variance with repeated measures and Friedman test was applied where appropriate. Differences were considered statistically significant if $P < 0.05$.

Fig. 1 Schematic drawing of experimental protocol



Results

Systemic effects of hypercapnic acidosis during LPS infusion

Table 1 shows the effects of hypercapnic acidosis and LPS infusion on systemic circulatory and biochemical parameters versus the sham animals. Hypercapnia group showed a marked hypercapnic acidosis to match the criteria of this group as described in “Materials and methods” versus the normocapnia group. By fluid resuscitation during LPS infusion, mean arterial pressure (MAP) and heart rate (HR) remained constant throughout the study period in both normocapnia and hypercapnia groups, while hypercapnia apparently increased MAP in sham group possibly through the activation of sympathetic system versus normocapnia. The number of circulating WBC in LPS-treated animals was significantly reduced to a similar extent in both study groups versus the baseline, whereas those in the sham groups remained constant throughout the study periods. Lactate dehydrogenase (LDH) activity to reflect the extent of LPS-induced tissue injury was increased in both the study groups, but those in normocapnia group were slightly but

significantly higher versus hypercapnia group at 4 h ($P < 0.05$), which was comparable to the sham. Simultaneously, arterial lactate in normocapnia group was significantly elevated versus hypercapnia group, indicating that tissue hypoxia caused by LPS infusion was reduced by hypercapnic acidosis.

Splanchnic effects of hypercapnic acidosis during LPS infusion

Figure 2 illustrates the changes of pHi, PCO_2 gap and portal blood flow at baseline and at 0–4 h of LPS infusion. The pHi values of both normocapnia and hypercapnia groups were progressively depressed, but the extent of the latter group was significantly greater versus the former. PCO_2 gap in hypercapnia group was significantly greater at 2 and 4-h study periods versus normocapnia group. On the other hand, portal blood flows in both normocapnia and hypercapnia groups increased at 2 and 4-h study periods compared to the baseline while the latter was increased significantly greater at 4 h versus the former.

Table 1 Effects of hypercapnic acidosis and lipopolysaccharide (LPS) infusion on systemic circulatory and biochemical parameters

		Group	Baseline	0 h	2 h	4 h
Mean arterial pressure (mmHg)	LPS	Normocapnia	55 ± 9	54 ± 4	53 ± 5	58 ± 11
		Hypercapnia	58 ± 5	58 ± 5	59 ± 12	60 ± 10
	Sham	Normocapnia	64 ± 1	60 ± 13	64 ± 12	67 ± 13
		Hypercapnia	67 ± 12	71 ± 10	70 ± 19	75 ± 25
Heart rate (beats/min)	LPS	Normocapnia	283 ± 24	273 ± 35	267 ± 21	268 ± 30
		Hypercapnia	275 ± 26	254 ± 27	259 ± 30	258 ± 25
	Sham	Normocapnia	272 ± 12	280 ± 24	262 ± 9	270 ± 24
		Hypercapnia	285 ± 12	287 ± 33	272 ± 22	272 ± 37
Arterial pH	LPS	Normocapnia	7.42 ± 0.06	7.43 ± 0.07	7.40 ± 0.05	7.38 ± 0.04
		Hypercapnia	7.25 ± 0.05**	7.27 ± 0.02**	7.25 ± 0.03**	7.24 ± 0.04**
	Sham	Normocapnia	7.40 ± 0.02	7.41 ± 0.01	7.46 ± 0.03	7.45 ± 0.02
		Hypercapnia	7.22 ± 0.07	7.26 ± 0.02	7.27 ± 0.03	7.27 ± 0.04
PaCO ₂ (mmHg)	LPS	Normocapnia	39 ± 4	37 ± 3	38 ± 3	40 ± 3
		Hypercapnia	68 ± 6**	70 ± 5**	68 ± 5**	71 ± 5**
	Sham	Normocapnia	42 ± 3	40 ± 2	39 ± 3	39 ± 3
		Hypercapnia	74 ± 5	73 ± 5	72 ± 7	70 ± 7
White blood cell count (/mm ³)	LPS	Normocapnia	5,440 ± 830	3,330 ± 1,420†	1,560 ± 430‡	1,870 ± 620‡
		Hypercapnia	5,530 ± 1,470	3,300 ± 1,560†	1,680 ± 930‡	2,020 ± 1,380‡
	Sham	Normocapnia	5,450 ± 500	5,460 ± 1,027	5,280 ± 1,800	5,360 ± 700
		Hypercapnia	5,330 ± 2590	5,470 ± 1,350	6,180 ± 1,500	5,660 ± 1,400
Lactate dehydrogenase (IU/L)	LPS	Normocapnia	120 ± 36	133 ± 41	174 ± 70	184 ± 94†
		Hypercapnia	135 ± 31	135 ± 31	145 ± 20	148 ± 22*
	Sham	Normocapnia	135 ± 21	145 ± 30	139 ± 12	141 ± 19
		Hypercapnia	124 ± 11	135 ± 7	127 ± 29	132 ± 38
Arterial lactate (mmol/L)	LPS	Normocapnia	2.9 ± 1.0	2.9 ± 1.6	3.4 ± 1.1	3.7 ± 1.4
		Hypercapnia	1.9 ± 0.8	1.8 ± 0.9	1.9 ± 0.6*	2.2 ± 0.7*
	Sham	Normocapnia	2.1 ± 0.5	2.1 ± 1.5	2.5 ± 0.8	2.2 ± 1.0
		Hypercapnia	1.9 ± 0.6	1.1 ± 0.3	1.2 ± 0.9	1.7 ± 1.2

Baseline = 45 min after preparatory surgery; 0, 2 and 4 h = study period during lipopolysaccharide infusion

* $P < 0.05$, ** $P < 0.01$ versus Normocapnia

† $P < 0.05$, ‡ $P < 0.01$ versus Baseline

Data are expressed as mean ± SD. No statistical analyses were performed between LPS and Sham

Fig. 2 Changes of intramucosal pH (pHi), PCO₂ gap and portal blood flow during 4-h study periods. Data are expressed as mean ± SD. Significance: * *P* < 0.05 versus normocapnia group. † *P* < 0.05, ‡ *P* < 0.01 versus baseline in each group

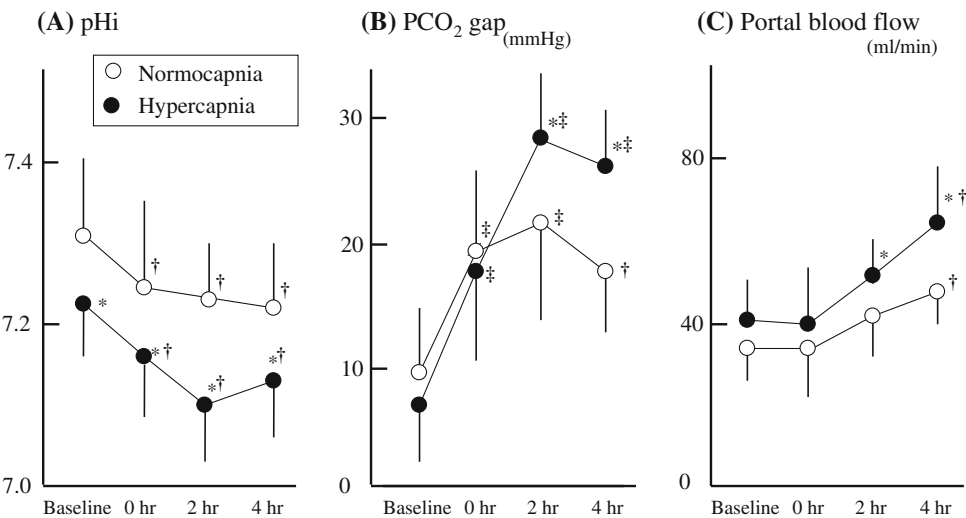


Figure 3 illustrates the changes of plasma FD4 concentration, wet/dry weight ratio of gut and ileal MPO activity. Plasma FD4 concentration in hypercapnia group, which was comparable with those in two sham groups was significantly less versus normocapnia group. Simultaneously, the wet/dry weight ratio of terminal ileum in hypercapnia group was significantly lower versus normocapnia group. However, ileal MPO activity to reflect neutrophil accumulation in the intestinal wall was similar

between the study groups, both of which appeared to be augmented versus the sham animals.

Pulmonary effects of hypercapnic acidosis during LPS infusion

To verify the effects of hypercapnic acidosis on injured lungs evoked by LPS infusion in this model, we measured

Fig. 3 Changes of plasma FD4 concentrations, wet/dry weight ratio of terminal ileum and ileal myeloperoxidase (MPO) activity. Data are expressed as mean ± SD. Significance: * *P* < 0.05, ** *P* < 0.01 versus normocapnia group

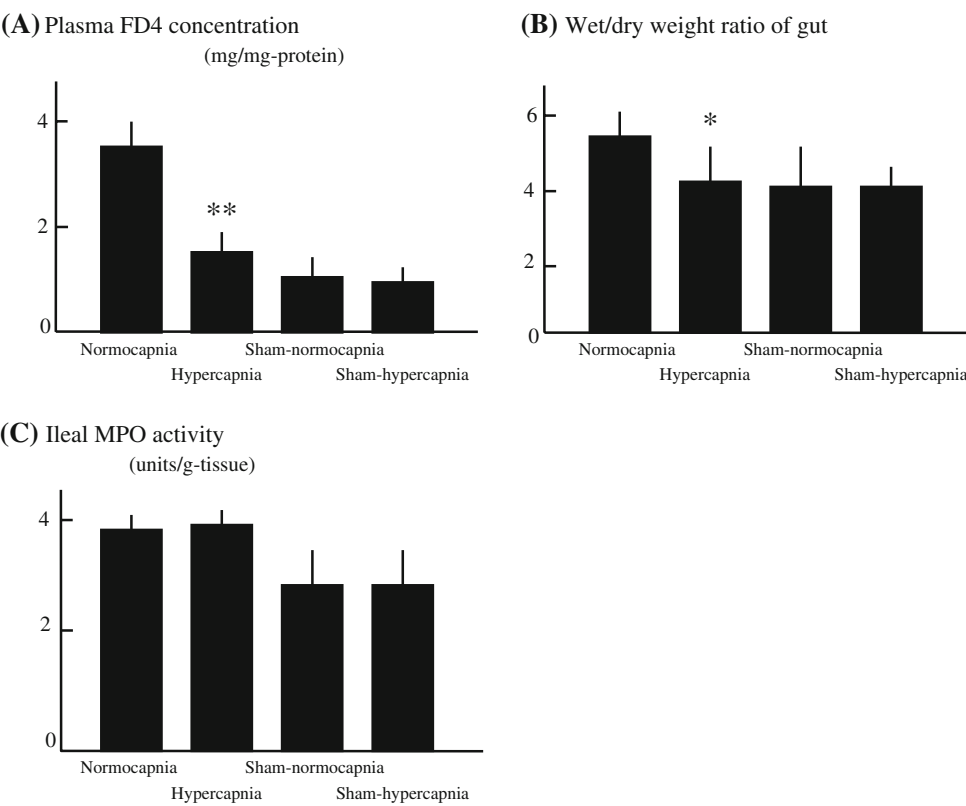
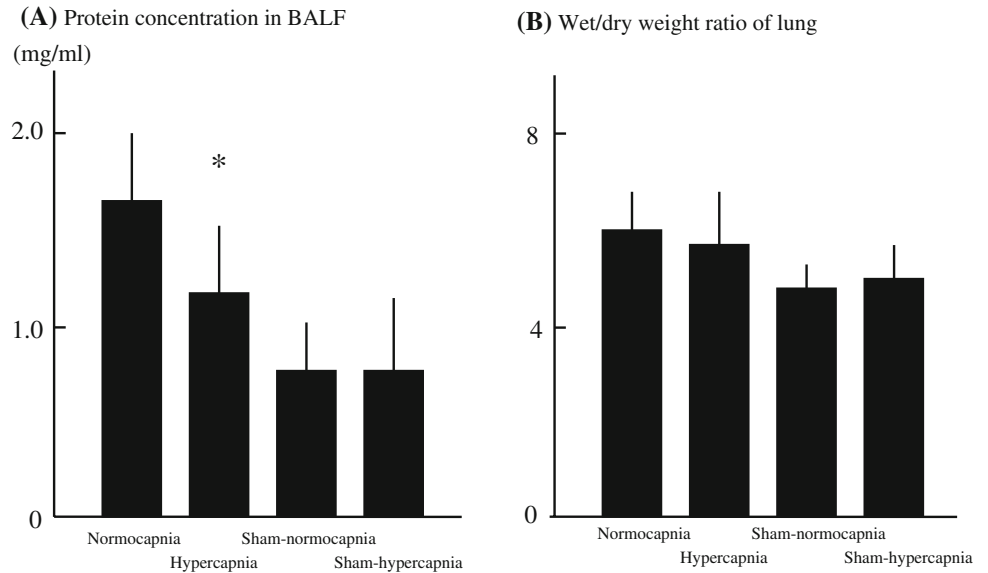


Fig. 4 Changes of protein concentration in bronchoalveolar lavage fluid (BALF) and wet/dry weight ratio of lung. Data are expressed as mean \pm SD. Significance: * $P < 0.05$ versus normocapnia group



the changes of protein concentration in BALF and wet/dry weight ratio of lung (Fig. 4). We then found that the protein concentration in BALF was significantly less in hypercapnia versus normocapnia group, whereas wet/dry weight ratio of lung was not significantly different between the groups.

Discussion

The present study indicates that hypercapnic acidosis, approximately at 70 mmHg of PaCO₂, ameliorates LPS-induced gut barrier dysfunction associated with the progression of intramucosal acidosis, and that intramucosal acidosis per se is not directly associated with deterioration of mucosal barrier function. In addition, overall tissue and cellular destruction, including the alterations of microvascular and alveolar permeability in endotoxemia, were minimized by short-term application of hypercapnic acidosis. These findings bring up a new hypothesis that lung protective strategy, subsequently inducing hypercapnic acidosis, could be another approach to prevent bacterial translocation from gut in critically ill state like endotoxemia.

To obviate confounding factors such as hypotension but concurrently to elicit gut mucosal injury, we applied low-dose endotoxin infusion model of rabbits with aggressive fluid resuscitation. These animals showed normotensive hyperdynamic circulatory responses as previously reported [16] although it remains unclear why MAP in the LPS-treated animals was kept slightly lower even at baseline period versus the sham (Table 1). Besides, progressive reduction of circulating WBC counts with mild elevation of arterial lactate fit well with the

finding in septic patients who were adequately resuscitated [22], suggesting that injected LPS was biologically active for 4-h study periods. Since hypercapnic acidosis ameliorated LPS-induced lung injury as shown in the protein leakage into BALF, our model could be comparable with previous studies, demonstrating protective property of hypercapnic acidosis on several types of injured lung [3, 4, 9]. Protective properties of hypercapnic acidosis on injured organs could be produced by its vasodilating and/or anti-inflammatory mechanisms. Tashkin et al. [23] demonstrated that hypercapnic acidosis caused a significant vasodilator effect, which was not related to arterial pH or β -adrenergic stimulation, on mesenteric arterial beds depending on the extent of hypercapnia. Indeed, we found that hypercapnic acidosis augmented the elevation of portal blood flow evoked by endotoxemia and fluid resuscitation. Such augmentation of blood flow to splanchnic area by hypercapnic acidosis might be able to reduce plasma FD4 concentration and show restoration of gut mucosal permeability by a simple dilution effect. However, portal blood flow was augmented to approximately 20% extent in hypercapnia group, which was unable to account for a marked increase of FD4 concentration, twice as much as higher versus normocapnia. Among inflammatory mediators and cells, tissue accumulation of activated neutrophils is a major contributor to develop gut mucosal injury [14]. Although tissue MPO activity alone may be insufficient to determine the precise involvement of activated neutrophils, the data in the LPS-treated animals showed apparently higher level compared to the sham group as previously reported in lung injury model [24]. However, it should be noted that the effects of hypercapnia on neutrophil function in situ such as phagocytosis and/or bactericidal capacity are not necessarily assessed using MPO activity.

Another important finding of this study was to show that PCO₂ gap progressively increased despite an augmentation of portal blood flow in hypercapnia versus normocapnia group. Paradoxically, arterial lactate in hypercapnia group was slightly but significantly reduced at 4-h study period versus the normocapnia group. As previously documented [25], arterial lactate per se, particularly in sepsis, does not always mirror the severity of tissue hypoxia. Although we did not directly assess the alterations of microvascular blood flow in gut mucosa, this finding suggests that the amelioration of LPS-induced gut barrier dysfunction under hypercapnic acidosis was not caused by the increase of intramucosal blood flow. High PCO₂ produces a rightward shift of the oxygen dissociation curve by increasing P50 value of hemoglobin, resulting in the augmentation of oxygen delivery to tissues as flow-independent mechanism [26]. While the exact mechanisms to enlarge PCO₂ gap remain unclear, hypercapnic acidosis might be able to redistribute blood flow to the others from mucosal layer of intestinal wall, augment intramucosal CO₂ production and/or produce progression of intramucosal hypoxia resulting in anaerobic glycolysis. In addition, the changes of intracellular pH exert a variety of actions on ionic conductance of cellular membranes, thus disturbing electric properties of excitable cells [27]. In gut mucosa, these alterations of membrane potential may be able to make intestinal wall rigid against macromolecules, i.e., hypercapnic acidosis preserves gut mucosal homeostasis possibly through the

modulation of membrane potential rather than the amelioration of intramucosal blood flow.

There are several limitations to interpret the data herein. Since the results of this study were obtained during 4 h of acute hypercapnia, prolonged effects remain to be fully determined. The present study clearly showed that gut mucosal barrier dysfunction caused by endotoxin infusion was ameliorated at least within 4 h of moderate hypercapnia. Second, this endotoxemic model may not be clinically relevant to mirror septic patients because hemodynamic parameters such as MAP and heart rate remained constant throughout the study periods. Although we did not measure cardiac output directly, increased portal blood flow up to 30% in normocapnia group suggests that cardiac output in this model was augmented by infusion of endotoxin and aggressive fluid therapy. Finally, since enzyme-linked immunoassay kits for many kinds of inflammatory cytokines are not commercially available for rabbits, we are unable to examine these markers, which might be more convincing to elucidate the mechanisms regarding the modulation of inflammation.

In conclusion, hypercapnic acidosis minimized endotoxin-induced gut barrier dysfunction possibly through neutrophil-independent mechanisms. Lung protective strategy inducing hypercapnic acidosis may serve to protect gut barrier function in critically ill patients.

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