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THAM reduces CO_2-associated increase in pulmonary vascular resistance – an experimental study in lung-injured piglets

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Abstract

Introduction: Low tidal volume (V_T) ventilation is recommended in patients with acute respiratory distress syndrome (ARDS). This may increase arterial carbon dioxide tension ($PaCO_2$), decrease pH, and augment pulmonary vascular resistance (PVR). We hypothesized that Tris(hydroxymethyl)aminomethane (THAM), a pure proton acceptor, would dampen these effects, preventing the increase in PVR.

Methods: A one-hit injury ARDS model was established by repeated lung lavages in 18 piglets. After ventilation with V_T of 6 ml/kg to maintain normocapnia, V_T was reduced to 3 ml/kg to induce hypercapnia. Six animals received THAM for 1 h, six for 3 h, and six serving as controls received no THAM. In all, the experiment continued for 6 h. The THAM dosage was calculated to normalize pH and exhibit a lasting effect. Gas exchange, pulmonary, and systemic hemodynamics were tracked. Inflammatory markers were obtained at the end of the experiment.

Results: In the controls, the decrease in V_T from 6 to 3 ml/kg increased $PaCO_2$ from 6.0±0.5 to 13.8±1.5 kPa and lowered pH from 7.40±0.01 to 7.12±0.06, whereas base excess (BE) remained stable at 2.7±2.3 mEq/L to 3.4±3.2 mEq/L. In the THAM groups, $PaCO_2$ decreased and pH increased above 7.4 during the infusions. After discontinuing the infusions, $PaCO_2$ increased above the corresponding level of the controls (15.2±1.7 kPa and 22.6±3.3 kPa for 1-h and 3-h THAM infusions, respectively). Despite a marked increase in BE (13.8±3.5 and 31.2±2.2 for 1-h and 3-h THAM infusions, respectively), pH became similar to the corresponding levels of the controls. PVR was lower in the THAM groups (at 6 h, 329±77 dyn·s/m^5 and 255±43 dyn·s/m^5 in the 1-h and 3-h groups, respectively, compared with 450±141 dyn·s/m^5 in the controls), as were pulmonary arterial pressures.

Conclusions: The pH in the THAM groups was similar to pH in the controls at 6 h, despite a marked increase in BE. This was due to an increase in $PaCO_2$ after stopping the THAM infusion, possibly by intracellular release of CO_2. Pulmonary arterial pressure and PVR were lower in the THAM-treated animals, indicating that THAM may be an option to reduce PVR in acute hypercapnia.

Introduction

Low tidal volume (V_T) ventilation has been shown to reduce ventilator-induced lung injury (VILI) and to improve survival in patients with acute respiratory distress syndrome (ARDS) [1]. In addition, it seems to reduce the risk of lung complications after surgery in which patients are under general anesthesia [2]. Therefore, low V_T ventilation is recommended for ventilating patients with ARDS and has also gained support in anesthesia.

It has been suggested that ventilation with V_T even less than 6 ml/kg would further reduce the risk of VILI in ARDS [3]. However, very low V_T ventilation may increase arterial carbon dioxide tension ($PaCO_2$) substantially. Although hypercapnic acidosis has been shown to have both negative and positive effects on immune function, it has unequivocal and clinically relevant negative effects on the pulmonary circulation, increasing pulmonary vascular resistance (PVR) [4–7]. This, in combination with

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high positive end-expiratory pressure (PEEP) levels, may induce acute right heart failure [8, 9].

Different methods to reduce $PaCO_2$ during low V_T ventilation have been suggested, such as reduction of apparatus dead space by a tracheal double-lumen tube, tracheal gas insufflation, expiratory flushing of the dead space by a tracheal catheter, or prolonging the end-inspiratory pause [10–13]. One of the most common methods is to increase minute ventilation by increasing respiratory rate (RR). However, this sometimes results in an unwanted buildup of auto-PEEP. Furthermore, it might be that the increased RR in itself, owing to increased energy transfer to the lungs, induces VILI. Indeed, very high RR combined with very low V_T using high-frequency oscillation has not been shown to be beneficial and might even increase mortality in ARDS [14, 15].

Other methods are extracorporeal CO_2 removal using an arteriovenous or venovenous approach with a low blood flow through a small membrane oxygenator/CO_2 remover. However, this method has not yet been shown to improve clinical outcome [16]. Thus, in many situations with low V_T ventilation, hypercapnic acidosis is unavoidable.

Because the acidosis caused by the increased $PaCO_2$ may be the main reason for the side effects of "permissive hypercapnia," it has been speculated whether treatment with sodium bicarbonate ($NaHCO_3$) would be useful [1, 17, 18]. In fact, in order for NaHCO3 to work as a buffer, it has to generate CO_2, which in turn has to be removed via the lungs increasing ventilatory demands [19]. Because the problem with low V_T ventilation is that the CO_2 excretion via the lungs is impaired, $NaHCO_3$ is theoretically not a good choice for buffering in these conditions. However, Tris(hydroxymethyl)aminomethane (THAM), or trometamol, which is a pure proton acceptor that exerts its buffer effects without generating CO_2, could be a more logical choice in cases of excessive CO_2 accumulation [20]. In addition, there are experimental data indicating that THAM may attenuate VILI [21]. THAM has already been used with success in cases with severe asthma and as an adjunct in ARDS [5, 20, 22]. However, in all published studies so far, THAM has been used at a modest dose, for a short period of time, and not with the aim of full pH correction. Using a porcine model, we have previously shown that THAM could be used to correct pH for at least 3 h during total apnea and that PVR was not severely increased during this period [23]. The aims of the present study were to explore how THAM, administered during two limited periods of very low V_T ventilation, would affect, first, pH and PVR and, second, lung inflammatory parameters in a porcine lung lavage model. We hypothesized that, during hypercapnia, THAM infusion would keep pH normal by increasing the metabolic base component, would prevent increase of PVR, and would not increase lung injury.

Methods

The study was approved by the Animal Research Ethics Committee at Uppsala University, and the National Institutes of Health guidelines for animal research were followed. The study was performed at the Hedenstierna Laboratory, Uppsala University, Uppsala, Sweden.

Anesthesia, ventilation, instrumentation, and monitoring

Eighteen pigs (23.0–30.5 kg body weight) were premedicated with 6 mg kg^{-1} tiletamine and zolazepam (Zoletil Forte; Boehringer Ingelheim Vetmedica, Ingelheim, Germany) and 2.2 mg kg^{-1} intramuscular xylazine (Rompun; BayerDVM, Shawnee Mission, KS, USA). After 5–10 minutes, the animal was placed supine on a table and a tracheotomy was performed by inserting an 8-mm I.D. endotracheal tube (Mallinckrodt Medical, Dublin, Ireland). Ventilation was started using a volume-controlled mode on a SERVO-i ventilator (Maquet Critical Care, Solna, Sweden) with V_T 8 ml kg^{-1}, inspiratory/expiratory ratio (I:E) 1:1, fraction of inspired oxygen (FiO_2) 0.5, RR 25 cycles/min, and PEEP 5 cmH_2O. Just before the tracheostomy, a bolus of fentanyl 10–20 μg kg^{-1} was given intravenously. Anesthesia was then maintained with ketamine 30 mg kg^{-1} h^{-1}, midazolam 0.1 mg kg^{-1} h^{-1}, and fentanyl 4 μg kg^{-1} h^{-1}. After checking that anesthesia was sufficient to prevent responses to painful stimulation between the front toes, muscle relaxation was added by a continuous infusion of pancuronium 0.3 mg kg^{-1} h^{-1}.

During the first hour, 10 ml kg^{-1} h^{-1} Ringer's acetate was infused intravenously, after which the infusion rate was lowered to 5 ml kg^{-1} h^{-1} intravenously. After open dissection of the neck vessels, an arterial catheter was inserted into the right carotid artery for blood sampling and blood pressure monitoring, and a central venous catheter was inserted via the right external jugular vein. In addition, a pulmonary arterial catheter (Criti Cath No7; Ohmeda, Singapore) for measurement of cardiac output (CO) and pulmonary arterial pressure was introduced via the right external jugular vein, and its correct position was verified by pressure monitoring. CO was obtained as the mean of three values measured by thermodilution after injection of 10 ml of ice-cold saline into the central venous catheter (Siemens SC 9000XL; Dräger, Lübeck, Germany). A bladder catheter was inserted suprapubically to measure hourly urine production. Electrocardiographic monitoring was started, and peripheral capillary oxygen saturation was monitored at the base of the tail by pulse oximetry (Siemens SC 9000XL). Next, a lung recruitment maneuver was performed with I:E 1:1, RR 6 breaths/min, pressure control, inspiratory pressure 40 cmH_2O, and PEEP 20 cmH_2O for 60 seconds. If the animal was considered hemodynamically unstable (mean arterial blood pressure [MAP] <50 mmHg) at this point,

50-ml boluses of hydroxyethyl starch (Hesra, Baxter Medical, Kista, Sweden) were given until a MAP of at least 50 mmHg was reached. All recruitment maneuvers were performed in the manner detailed above.

Estimation of required amounts of buffer to equilibrate protons

Using data from a pilot study and previous experiments, we targeted a pH of 7.35 and a $PaCO_2$ of 15 kPa at the 6-h endpoint. The pH target of 7.35 was chosen because, as seen in the study by Weber et al. [5], already at pH of approximately 7.2 there was a rise in mean pulmonary arterial pressure (MPAP). Inserting these values into the Henderson-Hasselbalch equation and solving for HCO_3^- = $10^{(7.2-6.1)}$·0.23 × 15 = 43 mEq/L, we could find the mEq/L amount of buffer needed as the difference between this value and normal bicarbonate (HCO_3^-), which was assumed to be 20 mEq/L. The buffering capacity needed for 1 h of infusion was then estimated as weight in kilograms × 23 × 0.4 = 9.2 × weight. The recommended dosing for THAM (Addex-THAM; Fresenius Kabi, Uppsala, Sweden) is normally calculated with a coefficient of 0.3, not 0.4, but in a pilot study a 1h infusion with coefficient 0.3 was found to be an inadequate dose; therefore, the coefficient was raised.

Experimental protocol

Preparations

After the instrumentation, a lung recruitment maneuver was performed to homogenize lung volume history, FiO_2 was set to 1.0, and the animal was allowed to stabilize for at least 15 minutes before baseline blood gas values were recorded; that is, arterial and venous blood was sampled for measurement of oxygen tension (PaO_2), $PaCO_2$, mixed venous oxygen saturation, hemoglobin, lactate, sodium, chloride, potassium, calcium, glucose content, pH, and base excess (BE) (ABL800 FLEX; Radiometer, Copenhagen, Denmark), and oxygen hemoglobin saturation (OSM3; Radiometer). The following baseline hemodynamic parameters were measured: MAP, MPAP, central venous pressure (CVP), pulmonary capillary wedge pressure, and CO. Brody's formula [24] for body surface area of pigs was used for the cardiac index (CI) calculations. PEEP was set to 0 cmH_2O, and an airway pressure-volume (PV) loop was obtained by slow insufflation to 40 cmH_2O followed by slow exsufflation via an occluder [25], and functional residual capacity was obtained (FRC) using a sulfur hexafluoride washin/washout method [26, 27].

After baseline measurements, lung injury was induced by repeated lung lavages with 30 ml/kg of 37–39 °C normal saline (eight in total). Blood gases were sampled again, and MAP, MPAP, and CVP, as well as FRC and compliance of the respiratory system as obtained from the maximum slope of the expiratory PV loop, were recorded [25].

Next, the tracheal tube was replaced with a double lumen endotracheal tube with both distal openings in the trachea [10]. The inspiratory tubing was connected to one of the endotracheal tube lumens and the expiratory tubing was attached to the other lumen, separating inspiration from expiration. Thus, the apparatus dead space was eliminated. A second lung recruitment maneuver was performed, and the ventilator was set to PEEP 10 cmH_2O, V_T 6 ml/kg, and I:E 1:2, and the RR was adjusted to target a pH between 7.38 and 7.42 in two successive blood samples obtained within a 15-minute time window.

Main experiment

After confirming that pH was normal by arterial and venous blood gas sampling, hemodynamic measurements (time point 0) were registered. Hypoventilation was initiated by reducing V_T to 3 ml/kg, and THAM infusion was started at the same time through a central venous line, in the two buffering groups. Arterial and mixed venous blood gases were obtained at 5, 10, 15, 30, 60, 90, and 120 minutes and then every hour up to 6 h from time point 0. In addition, the hemodynamic variables were registered at similar time points, except at 5 and 10 minutes.

After 6 h of low V_T ventilation, further data were collected by drawing venous samples for cytokine analyses, measuring FRC and compliance and performing a lavage of the basal right lung with 30 ml of normal saline. Thereafter the left lung was removed and dissected to obtain tissue samples from the ventral, medial, and dorsal parts at the hilar level. The tissue samples were frozen in liquid nitrogen and stored at −80 °C. The animals were killed with an intravenous dose of potassium chloride under deep anesthesia.

Cytokine analysis

Pieces weighing between 80 and 320 mg were homogenized in lysis buffer (15 mM Tris, pH 7.4, 150 mM NaCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 0.5 % Triton X-100), with addition of protease inhibitor (Thermo Scientific, Waltham, MA, USA) using an IKA Ultra-Turrax T8 homogenizer (IKA-Werke, Staufen im Breisgau, Germany). Homogenates were centrifuged at 300×*g* at 4 °C for 10 minutes. Supernatants were kept at −80 °C until assay. Cytokine content was assessed using a DuoSet enzyme-linked immunosorbent assay (ELISA) system (R&D Systems, Minneapolis, MN, USA), for porcine tumor necrosis factor (TNF)-α, interleukin (IL)-1β/IL-1F2, and IL-6 according to the manufacturer's instructions. The detection limits of the assays were 125 pg/ml for TNF-α, 62.5 pg/ml for IL-1β/IL-1F2, and 125 pg/ml for IL-6. Total protein content of the supernatant was measured using a Coomassie Plus Assay (Thermo Scientific) according to the manufacturer's

instructions. Cytokine content of tissue lysates were normalized against total protein content of the homogenate.

Statistics

A power analysis (analysis of variance [ANOVA]) indicated that six animals in each group would be enough to detect a pH difference of 0.1 with a standard deviation of 0.05 with a p <0.05 and a power of 0.8, assuming a normal distribution.

If there was a clear resemblance with the exponential distribution, a log transformation was performed to improve normality. If the variables did not pass the Shapiro-Wilk normality test, Kruskal-Wallis one-way ANOVA was performed. Tukey's honestly significant difference was assessed post hoc. The data are presented as mean and standard deviation. The statistical calculations were performed using R 3.1.1 [28] and SigmaPlot 11.0 (Systat Software, San Jose, CA, USA).

Results

All values reported in groups of three are in the order controls (NT), 1-h infusion (1T), 3-h infusion (3T) of THAM. p-Values reported are with regard to NT unless otherwise specified.

Blood gas and acid–base status

Blood gas and acid–base data are reported in Table 1 and graphed in Fig. 1.

As evident in Fig. 1, arterial pH was largely different between the groups during THAM infusion. These differences diminished over time, showing no differences between groups at 360 minutes. $PaCO_2$ rose in all groups. At the end of infusions, both the 1T and 3T groups showed a second phase of $PaCO_2$ increase, whereas NT changed very little after 120 minutes. There was a large difference in $PaCO_2$ at 360 minutes (13.8±1.5 kPa vs 15.2±3.3 kPa, p =0.55; 22.6±1.7 kPa, p <0.001). The difference between mixed venous and arterial carbon dioxide tension was lower in both 1T and 3T groups starting from the 15-minute time point (1.8±0.5 kPa vs 0.8±0.4 kPa, p =0.001; 0.6±0.2 kPa, p <0.001). After the infusion, the 1T group rebounded to levels similar to that of NT. At 360 minutes, the values were 2.1±0.8 kPa vs 2.2±0.6 kPa (p =0.96) and 1.0±0.7 kPa (p =0.03). At 360 minutes, the BE values were 3.4±3.2 mEq/L vs 10.2±2.1 mEq/L (p =0.002) and 27.8±3.1 mEq/L (p <0.001). HCO_3^- showed an increase similar to that of BE.

Arterial oxygen tension (PaO_2) trended toward decrease and was lower at 360 minutes in the 3T group (p <0.001) compared with controls and the 1T group. The 3T group had a higher shunt fraction than the 1T group at 360 minutes (p =0.03).

Hemodynamics

Hemodynamic data are reported in Table 2 and graphed in Fig. 2.

The MPAP was lower in the 3T and 1T groups from the 30- and 60-minute time points, respectively, onward; at 360 minutes, however, only the 3T group was different from the control animals (25±5 mmHg vs 21±2 mmHg, p =0.17; 18±2 mmHg, p =0.008). PVR was similar to MPAP, and the 3T group was still different at 360 minutes (450±141 dyn·s/m^5 vs 329±77 dyn·s/m^5, p =0.11; 255±43 dyn·s/m^5, p =0.0081). CI exhibited a rising trend over time in all the groups, with 3T separating from the rest at 360 minutes (3.5±1.0 L/min/m^2 vs 3.8±0.75 L/min/m^2, p =0.42; 5.0±0.57 L/min/m^2, p <0.001). MAP and systemic vascular resistance (SVR) did not differ between the groups at the examined time points, but SVR showed a decreasing trend over time in all three groups.

Inflammatory markers

Inflammatory marker data are depicted in Fig. 3.

Because many values were at levels too low to be detected with the ELISA, they are not presented in a table. Most of the missing (i.e., below the detection limit) values were in the 1T and 3T groups, indicating that they might be generally lower than in the controls.

In the tissue samples, there was an effect on the IL-6 concentration (detected by two-way ANOVA) in the THAM strata, and post hoc analysis showed a p-value of 0.014 compared with controls, with the 3T group being higher (11.8±11.7 pg/$mg_{protein}$) than the 1T group (4.5±2.0 pg/$mg_{protein}$) and non-significant values found for the rest (p =0.086 for 3T vs NT and p =0.423 for 1T vs NT). Two-way ANOVA of the TNF-α and IL-1β concentrations was not done, owing to many missing values. Kruskal-Wallis one-way ANOVA on ranks was performed for every tissue strata, with the missing values set below the minimum measured values. No differences were detected.

Lung mechanics

Data regarding lung mechanics are provided in Table 3.

FRC and compliance of the respiratory system decreased with the lavages (p <0.001) but did not differ between the groups.

Electrolytes

Electrolyte data are reported in Table 4.

Arterial sodium concentration fell during the THAM infusion in both the 1T and 3T groups, and arterial potassium concentration increased.

Discussion

This study shows the following results in a porcine lung lavage model:

Table 1 Arterial acid–base values, arterial and venous oxygenation, oxygen consumption, and venous admixture

Parameter	Group	Baseline	Start	60 min	180 min	360 min
pH	NT	7.40 (0.04)	7.40 (0.01)	7.14 (0.03)	7.11 (0.05)	7.12 (0.06)
	1T	7.40 (0.05)	7.41 (0.02)	7.34 (0.05)[a]	7.18 (0.05)[a]	7.16 (0.07)
	3T	7.43 (0.02)	7.40 (0.01)	7.35 (0.02)[a]	7.39 (0.01)[a,b]	7.16 (0.03)
$PaCO_2$ (kPa)	NT	6.6 (1.2)	6.0 (0.5)	12.3 (1.2)	13.5 (1.4)	13.8 (1.5)
	1T	6.0 (0.6)	5.8 (0.3)	10.2 (0.9)[a]	14.6 (2.4)	15.2 (3.3)
	3T	6.3 (0.7)	5.9 (0.7)	10.6 (0.7)[a]	13.1 (0.5)	22.6 (1.7)[a,b]
PaO_2 (kPa)	NT	62.6 (12.7)	63.7 (7.4)	48.1 (12.0)	45.2 (12.3)	46.4 (11.8)
	1T	65.2 (4.8)	65.0 (8.6)	59.8 (6.3)	49.4 (9.0)	53.3 (5.0)
	3T	55.5 (15.5)	56.0 (4.6)	49.7 (4.1)	32.6 (7.6)§	30.0 (7.7)[a,b]
Base excess (mEq/ml)	NT	4.8 (2.2)	2.7 (2.3)	1.3 (2.2)	1.8 (2.8)	3.4 (3.2)
	1T	3.0 (3.2)	2.4 (1.5)	13.8 (3.5)[a]	10.9 (3.1)[a]	10.2 (2.1)[a]
	3T	6.2 (2.1)	2.4 (3.0)	16.0 (0.6)[a]	31.2 (2.2)[a,b]	27.8 (3.1)[a,b]
HCO_3^- (mmol/L)	NT	28.5 (1.8)	27.2 (2.5)	29.7 (2.2)	30.8 (2.5)	32.3 (2.8)
	1T	27.0 (2.8)	26.7 (1.5)	40.1 (3.2)[a]	39.3 (3.2)[a]	38.8 (2.5)[a]
	3T	29.8 (1.9)	26.8 (3.1)	42.3 (0.9)[a]	58.6 (2.2)[a,b]	57.2 (2.8)[a,b]
SaO_2 (%)	NT	98 (0.3)	98 (0.2)	97 (0.4)	97 (0.6)	97 (0.6)
	1T	98 (0.2)	98 (0.3)	98 (0.3)[a]	98 (0.2)	97 (0.2)
	3T	98 (0.8)	98 (0.1)[c]	98 (0.2)[a]	98 (0.3)	97 (0.8)
SvO_2 (%)	NT	71 (11)	53 (11)	63 (6.1)	67 (5.8)	67 (11)
	1T	68 (4.6)	43 (5.2)	54 (10)	65 (6.5)	66 (10)
	3T	70 (7.6)	47 (11)[c]	61 (7.9)	69 (4.6)	74 (2.1)
VO_2 (ml/min)	NT	133 (32)	124 (22)	118 (23)	120 (9)	121 (21)
	1T	128 (28)	142 (18)	131 (20)	139 (14)[c]	140 (26)
	3T	133 (30)[c]	123 (18)[c]	117 (7)	127 (7)	134 (13)
Q_s/Q_t (fraction)	NT	0.15 (0.03)	0.11 (0.04)	0.17 (0.04)	0.20 (0.07)	0.20 (0.08)
	1T	0.13 (0.03)	0.09 (0.02)	0.11 (0.03)[a]	0.17 (0.05)[c]	0.15 (0.04)
	3T	0.16 (0.02)[c]	0.12 (0.02)[c]	0.15 (0.03)[b]	0.24 (0.04)	0.26 (0.06)[b]
Lactate (mmol/L)	NT	1.1 (0.26)	1.3 (0.23)	0.7 (0.08)	0.6 (0.14)	0.6 (0.14)
	1T	1.6 (0.81)	1.4 (0.14)	1.1 (0.19)[a]	0.6 (0.14)	0.7 (0.19)
	3T	1.1 (0.32)	1.2 (0.15)	0.9 (0.31)	0.9 (0.23)[a]	1.3 (0.59)[a,b]
$\Delta PaCO_2$ (kPa)	NT	1.5 (0.6)	2.2 (0.6)	1.9 (0.5)	2.2 (0.7)	2.1 (0.8)
	1T	1.8 (0.4)	2.6 (0.3)	0.7 (1.1)[a]	2.1 (0.3)	2.2 (0.6)
	3T	1.5 (0.3)	2.7 (0.3)	0.6 (0.4)[a]	0.2 (0.6)[a,b]	1.0 (0.7)[a,b]

Abbreviations: BE arterial base excess, *HCO_3^-* arterial bicarbonate concentration, *NT* control animals that did not receive THAM, *$PaCO_2$* arterial carbon dioxide tension, *$\Delta PaCO_2$* arteriovenous difference in carbon dioxide tension, *PaO_2* arterial oxygen tension, *Q_s/Q_t* venous admixture obtained at inspired oxygen fraction 1.0, *SvO_2* mixed venous oxygen saturation, *1T* group that received THAM infusion for 1 h, *3T* group that received THAM infusion for 3 h, *THAM* Tris(hydroxymethyl) aminomethane, *VO_2* body oxygen consumption

The time points refer to time after start of THAM infusion or corresponding time points in the controls (NT)

[a]Value is different from NT group

[b]Value is different from 1T group

[c]Only 5 subjects represented owing to technical error

1. Permissive hypercapnia obtained by reducing the effective V_T from 6 ml/kg to 3 ml/kg decreased pH with a slow metabolic compensation during the study period and increased PVR, but it did not deteriorate CO or have other severe effects. Thus, these findings indicate that hypercapnia in this model did not compromise right heart function.
2. Buffering with THAM infused for 1 or 3 h intravenously immediately normalized pH both by reducing the increase in $PaCO_2$ and by a fast

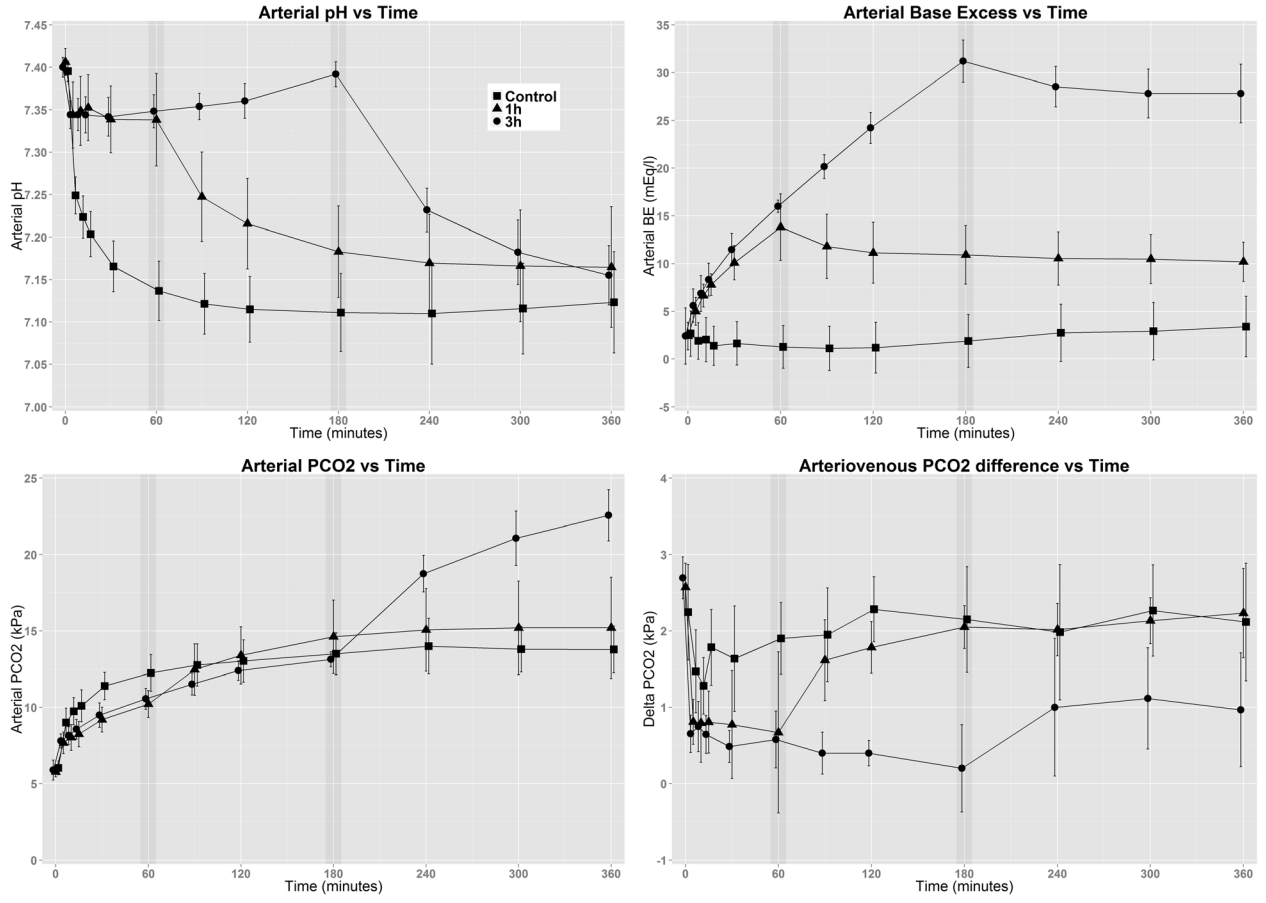

Fig. 1 Effects of Tris(hydroxymethyl)aminomethane on arterial pH, base excess, arterial carbon dioxide tension (PCO_2) and arteriovenous difference in PCO_2. The graph shows the progression over time, with the *x*-axes representing time points. Note the offset *y*-axes. The end of infusion for the 1-h and 3-h groups are marked with a *darkened vertical bar*. Values are means, and the *error bars* represent standard deviations

increase in BE. It was also associated with a decreased PVR.

3. After the end of the continuous infusion of THAM, there was a rebound increase in $PaCO_2$, and, in spite of a continued high BE, pH decreased to levels similar to those of the controls at the end of the experimental period.
4. Notwithstanding the decrease in pH and marked increase in $PaCO_2$, PVR remained low.
5. The inflammatory response in the lungs as well as the lung volumes and lung mechanics were not markedly different between the THAM groups and the controls.

We previously showed, in a porcine apneic model, that a continuous THAM infusion maintained pH at physiologically acceptable levels for at least 3 h. PVR was only slightly increased, despite a marked increase in $PaCO_2$ to a maximum of 30 kPa [23]. In that study, where we investigated whether THAM could totally replace ventilation of the lungs for a longer period, we did not discontinue the THAM infusion during the experiment. In the present study, where we studied whether THAM could be an adjunct to ventilation, pH decreased after the THAM infusion was stopped. This was due to a rebound increase in $PaCO_2$ that was severe after the prolonged THAM infusion. Despite this fact, PVR did not increase, MPAP remained low, and CO increased.

Most studies of the pulmonary circulatory effects of hypercapnia have been performed in models with hypoxia-induced pulmonary vasoconstriction (HPV) in either intact animals or isolated lung preparations where vasoconstriction was induced by lowering the inspired oxygenation concentration [29–33]. This is in contrast to our lavage model, where we kept PaO_2 high by FiO_2 1.0 and a lung recruitment maneuver followed by PEEP 10 cmH_2O. The other important differences are that, in most of the other previous studies, hypercapnia was induced by administering CO_2 in the breathing gas and manipulations of the metabolic component were done by infusion of $NaHCO_3$ or HCl [17, 34, 35]. In the majority of these studies, researchers found that both

Table 2 Hemodynamics

Parameter	Group	Baseline	Start	60 min	180 min	360 min
MPAP (mmHg)	NT	18 (3)	21 (4)	28 (4)	28 (4)	25 (5)
	1T	19 (2)	20 (3)	21 (4)[a]	23 (3)[a]	21 (2)
	3T	14 (2)[a,b]	18 (2)	19 (2)[a]	17 (2)[a,b]	18 (2)[a]
MAP (mmHg)	NT	83 (9)	72 (9)	68 (9)	67 (9)	63 (13)
	1T	95 (13)	65 (6)	67 (17)	63 (2)	60 (5)
	3T	80 (7)	62 (5)	68 (5)	68 (6)	69 (6)
CI (L/min/m^2)	NT	3.6 (0.5)	2.4 (0.3)	2.9 (0.3)	3.4 (0.7)	3.5 (1.0)
	1T	3.4 (0.7)	2.4 (0.3)	2.8 (0.4)	3.8 (0.7)	3.8 (0.8)
	3T	4.2 (1.1)	2.4 (0.4)	3.1 (0.5)	4.2 (0.7)	5.0 (0.6)[a,b]
PVR (dyn·s/m^5)	NT	317 (92)	465 (125)	572 (85)	504 (101)	450 (141)
	1T	323 (79)	448 (75)	423 (113)[a]	373 (65)[a]	329 (77)
	3T	264 (69)	481 (109)	373 (103)[a]	290 (62)[a]	255 (43)[a]
SVR (dyn·s/m^5)	NT	2240 (515)	2757 (736)	2030 (257)	1720 (297)	1566 (262)
	1T	2942 (1201)	2485 (619)	2136 (782)	1517 (283)	1431 (255)
	3T	2147 (716)	2558 (250)	2204 (208)	1725 (276)	1449 (125)

Abbreviations: CI cardiac index, *MAP* mean systemic arterial pressure, *MPAP* mean pulmonary arterial pressure, *NT* control animals that did not receive THAM, *PVR* pulmonary vascular resistance, *SVR* systemic vascular resistance, *1T* group that received THAM infusion for 1 h, *3T* group that received THAM infusion for 3 h, *THAM* Tris(hydroxymethyl)aminomethane

The time points refer to time after start of THAM infusion or corresponding time points in the controls (NT)

Values are mean (standard deviation)

[a]Value is different from NT group

[b]Value is different from 1T group

metabolic and hypercapnic acidosis increased PVR and that alkalosis, independent of whether it was metabolic or hypercapnic, reduced or normalized pulmonary vascular tone. Thus, the pH change in itself was interpreted to be the main mechanism of this effect [29, 36, 37]. Our results challenge this interpretation because PVR in the THAM groups was low despite development of a moderate to severe respiratory acidosis after the THAM infusion was stopped. Instead, BE and HCO_3^- were maintained at high levels, indicating that the metabolic component might be more important than pH alone in the regulation of pulmonary vascular tone. That THAM reduces increases in MPAP caused by hypercapnia has previously been demonstrated by Weber et al. [5]. However, they did not find any significant change in PVR induced by THAM, probably owing to simultaneous administration of cardiovascular active agents. Furthermore, they did not report any remaining metabolic effect induced by THAM after the infusion was ended.

In contrast to the pulmonary circulation, we did not find any relation between arterial BE or pH and vascular resistance in the systemic circulation. However, the reduction in SVR mirrored the increase in $PaCO_2$, suggesting that, in the systemic circulation, $PaCO_2$ per se influences the vascular tone more than BE or pH does, confirming results in previous studies [34, 38]. Although the underlying mechanism of the effect on the vasculature by respiratory or metabolic acid–base changes have not been fully elucidated, it has been suggested that intracellular pH changes may regulate the voltage-gated potassium channels and that this effect is different between the pulmonary and systemic vessels [39]. Thus, a decrease in the intracellular pH dilates systemic vessels and constricts pulmonary vessels. In addition, on the basis of our present study, we cannot exclude a direct pharmacological effect of THAM on the vascular smooth muscles. In agreement with clinical studies, the decrease in SVR caused by THAM was associated with an increase in CO and mixed venous oxygen saturation [5].

The significant increase in $PaCO_2$ after THAM infusion was completed in the THAM-treated animals compared with the controls was unexpected and, to our knowledge, has not been described before. The two THAM dosages were calculated to increase BE to balance the increased CO_2 to keep pH normal. We assumed that $PaCO_2$ would level out at a new high steady state similar to the controls when the CO_2 excretion via the lungs would again equalize the CO_2 production in the tissues. In the control animals, this new, higher steady state (at $PaCO_2$ around 15 kPa) occurred after about 120 minutes. However, in the THAM-treated animals, $PaCO_2$ showed a two-phase course. During the infusion, the increase in $PaCO_2$ followed, but, as expected, below the increase in $PaCO_2$ of the controls because THAM reduces the CO_2 content in the blood by catching protons, moving the Henderson-Hasselbalch equation to the right. However, after the

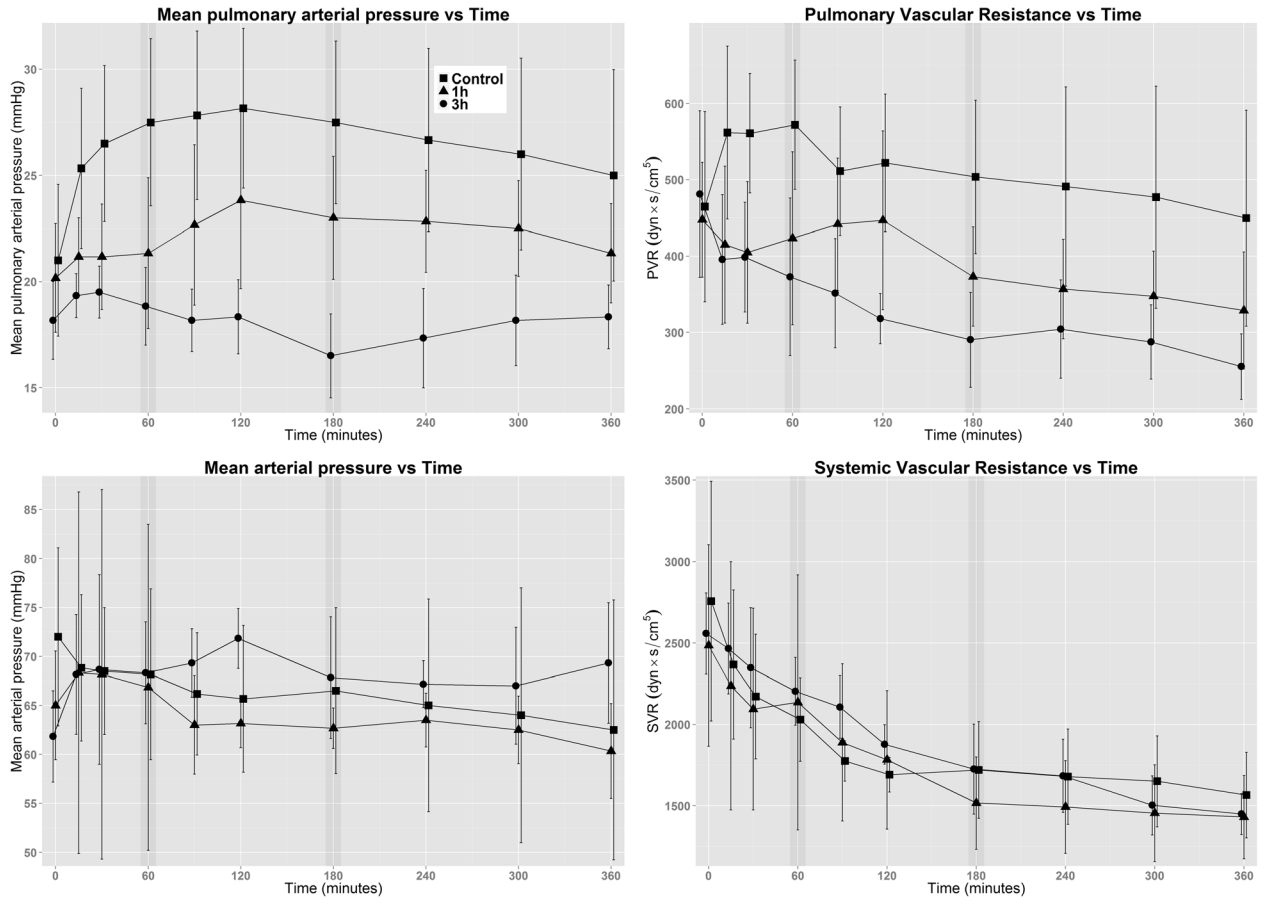

Fig. 2 Effects of Tris(hydroxymethyl)aminomethane on hemodynamics. The graph shows the progression over time, with the *x*-axes representing time points. Note the offset *y*-axes. The end of infusion for the 1-h and 3-h groups is marked with a *darkened vertical bar*. Values are means, and the represent standard deviations. *SVR* systemic vascular resistance

infusion was stopped, $PaCO_2$ increased and leveled off at a higher level than in the controls. In the animals treated with THAM for 3 h, the $PaCO_2$ increase was substantial, from a mean of 15 to 23 kPa with no clear steady state at the end of the experiment.

Three theoretical mechanisms might explain the increased $PaCO_2$ after the THAM infusion was stopped:

1. *Reduced alveolar ventilation*: However, the tidal volumes were kept constant throughout the study, and we do not have any indications in any of the groups that physiological dead space increased or that a marked increase in pulmonary shunt occurred after the end of the infusion.
2. *Increased metabolism*: However, body temperature was kept constant, and calculated oxygen consumption was unchanged; therefore, it is not plausible that CO_2 production increased.
3. *Increased CO_2 accumulation in the tissues due to reduced pulmonary excretion during the THAM infusion*: This is indicated by the significantly lower pulmonary mixed venous-arterial $PaCO_2$ gradient value during the THAM infusion in the 3-h THAM group. Thus, THAM might have sequestered CO_2 that thereafter accumulated in the tissues. Because the tension of CO_2 cannot be higher in the tissues than in the blood, the CO_2 must have been stored as HCO_3^-, probably in the intracellular compartment. However, THAM penetrates slowly into the intracellular compartment, and during the first hour most of the protonated THAM was probably excreted via the kidneys [19]. This can explain why we found only a minor (non-significant) increase in $PaCO_2$ after the infusion in the 1-h THAM infusion group, in contrast to the marked increase in the 3-h infusion group. Thus, one could speculate that the intracellular concentration of CO_2, stored as HCO_3^- together with protonated THAM, might have been very high at the end of the infusion in the 3-h group. When the infusion was stopped, the intracellular HCO_3^- was, via the effect of carbonic anhydrase, transformed into CO_2, which penetrated the cell membranes

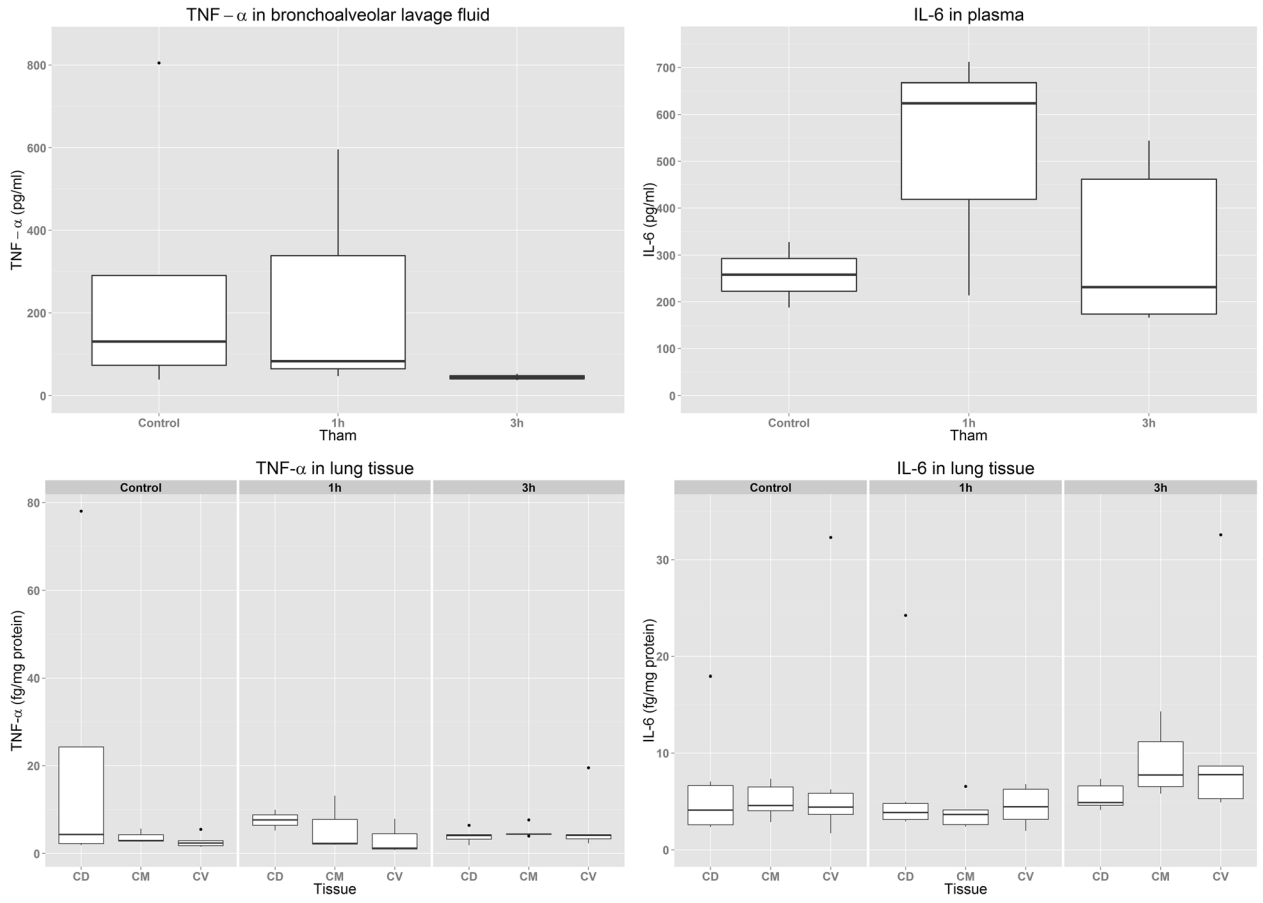

Fig. 3 Standard box-and-whisker plots of the cytokine levels stratified by amount of Tris(hydroxymethyl)aminomethane (THAM) given. Note that the two *top panels* depict tumor necrosis factor (TNF)-α in bronchoalveolar lavage fluid and interleukin (IL)-6 in plasma. *CD* dorsal, *CM* medial, *CV* ventral

Table 3 Lung mechanics

Parameter	Group	Baseline	Lung lavage	End
FRC (ml)	NT	386 (45)	224 (81)	278 (46)
	1T	435 (105)	231 (54)	287 (116)
	3T	341 (53)	158 (21)	202 (46)
Cexp (ml/cmH_2O)	NT	47 (10)	37 (9)	47 (5)
	1T	51 (19)	39 (17)	49 (17)
	3T	51 (7)	41 (2)	50 (3)

Abbreviations: Cexp compliance of the respiratory system obtained as the maximal slope of a full expiratory pressure-volume curve, *FRC* functional residual capacity, *NT* control animals that did not receive Tris(hydroxymethyl) aminomethane (THAM), *1T* group that received THAM infusion for 1 h, *3T* group that received THAM infusion for 3 h
Lung lavage values obtained 30 minutes after lung lavage; end values obtained at the end of the experiment
Values are mean (standard deviation)

easily and increased $PaCO_2$. Nevertheless, this unexpected effect of THAM needs further exploration.

THAM can have important side effects, such as hypoglycemia, hyperkalemia, and hypotension [19]. In this study, although potassium levels increased, glucose levels were normal and blood pressure was maintained.

We did not find any major differences in inflammatory markers between the groups. Only IL-6 showed a significant increase in lung tissue, whereas we could not find any difference in TNF-α or IL-1β. The time with acidosis was significantly less in the THAM groups, which indicates that, when very low V_T ventilation in addition to adequate PEEP level are used, the potential specific anti-inflammatory effect of hypercapnic acidosis is minor and that THAM is probably safe to administer in this regard. This notion is further strengthened by the fact that lung mechanics and FRC recovered to a similar degree after lavage in all groups. Our findings are in accord with those reported by Caples et al., who found that buffering with THAM ameliorated

Table 4 Electrolytes

Parameter	Group	Start	60 min	180 min	360 min
$[Na^+]$ (mmol/L)	NT	134 (1)	134 (1)	132 (2)	130 (2)
	1T	134 (2)	129 (2)[a]	134 (1)	135 (1)[a]
	3T	132 (2)[a,b]	125 (2)[a,b]	125 (2)[a,b]	138 (3)[a,b]
$[K^+]$ (mmol/L)	NT	4.3 (0.2)	4.6 (0.2)	5.2 (0.4)	5.5 (0.6)
	1T	4.5 (0.2)	5.1 (0.3)[a]	5.0 (0.3)	5.2 (0.4)
	3T	4.3 (0.3)	5.0 (0.3)	5.9 (0.3)[a,b]	4.8 (0.5)
$[Ca^{2+}]$ (mmol/L)	NT	1.43 (0.17)	1.51 (0.16)	1.35 (0.11)	1.29 (0.11)
	1T	1.43 (0.14)	1.33 (0.19)[a]	1.36 (0.14)	1.28 (0.13)
	3T	1.25 (0.06)[b]	1.21 (0.04)[a]	1.01 (0.06)[a,b]	1.09 (0.05)[a,b]
$[Cl^-]$ (mmol/L)	NT	101 (2)	99 (1)	98 (2)	93 (3)
	1T	101 (1)	98 (1)	97 (3)	93 (2)
	3T	104 (3)	99 (1)	96 (2)	94 (2)

Abbreviations: *$[Ca^{2+}]$* arterial calcium concentration, *$[Cl^-]$* arterial chloride concentration, *$[K^+]$* arterial potassium concentration, *$[Na^+]$* arterial sodium concentration, *NT* control animals that did not receive THAM, *1T* group that received THAM infusion for 1 h, *3T* group that received THAM infusion for 3 h, *THAM* Tris(hydroxymethyl)aminomethane

The time points refer to time after start of THAM infusion or corresponding time points in the controls (NT)

Values are mean (standard deviation)

[a]Value is different from NT group

[b]Value is different from 1T group

ventilation-induced lung cell injuries in isolated rat lungs [21].

The possible clinical implication of our study is that THAM might be useful in cases of high PVR complicating permissive hypercapnia by reducing the pulmonary vascular tone. However, this has to be done very cautiously, particularly because the mechanism of the rebound effect of $PaCO_2$ is unknown. Thus, the dose and infusion time should be restricted, and $PaCO_2$ and potassium levels should be carefully monitored, as should PaO_2, which might decrease owing to inhibition of HPV.

This study has the following inherent limitations of all animal models, and the results cannot be assumed to be fully valid in patients:

1. Although pigs have physiology very similar to that of as humans, their immunologic responses, as well as their response to THAM, might be different from those of humans.
2. The effect on the pulmonary vasculature might be more prominent in pigs because pigs have a strong HPV [40].
3. We used an open lung approach, the animals were never hypoxemic, and inhibition of HPV may deteriorate oxygenation importantly in ARDS [37].
4. We used FiO_2 1.0, which might have augmented the pulmonary vasodilatory effect associated with THAM.
5. Although THAM was infused in a large vein, its high osmolality may have caused endothelial damage. However, the osmolality is less than the commonly used concentration of hypertonic saline and amino acid solutions used for parenteral nutrition.
6. Possible toxic side effects were not addressed.
7. The number of animals used, as well as the observation period, was limited.

Conclusions

This experimental study of permissive hypercapnia in a porcine lung lavage model shows that intravenous infusion of THAM increased BE and bicarbonate concentration, normalized pH, and decreased $PaCO_2$ during the infusion. After a prolonged infusion, however, pH decreased to values similar to those in controls owing to a rebound $PaCO_2$ increase. Despite a similar low pH and a higher $PaCO_2$ compared with controls, the PVR remained low in the THAM group. No major signs of augmentation of lung injury by THAM were found. These findings suggest that CO_2 removal from the lungs is hampered during THAM infusion and that the metabolic component (i.e., BE, bicarbonate) has an important influence on the pulmonary vascular tone, indicating the potential use of THAM in situations with hypercapnia-induced pulmonary hypertension and increased PVR.

Key messages

- Increased pulmonary vascular resistance caused by respiratory acidosis was countered with THAM.
- After a high-dose infusion of THAM, $PaCO_2$ rebounded to a higher-than-expected level.
- THAM did not importantly affect the inflammatory response.

Abbreviations
1T: 1-h infusion of THAM; 3T: 3-h infusion of THAM; ANOVA: Analysis of variance; ARDS: Acute respiratory distress syndrome; BE: Base excess; CD: Dorsal; Cexp: compliance of the respiratory system; CI: Cardiac index; CM: Medial; CO: Cardiac output; CV: Ventral; CVP: Central venous pressure; ELISA: Enzyme-linked immunosorbent assay; FiO_2: Fraction of inspired oxygen; FRC: Functional residual capacity; HCO_3^-: Bicarbonate; HPV: Hypoxic pulmonary vasoconstriction; I:E: Inspiratory/expiratory ratio; IL: Interleukin; MAP: Mean arterial blood pressure; MPAP: Mean pulmonary arterial pressure; $NaHCO_3$: Sodium bicarbonate; ns: Non-significant; NT: Controls (i.e., no THAM infusion); $PaCO_2$: Arterial carbon dioxide tension; PaO_2: Arterial oxygen tension; PEEP: Positive end-expiratory pressure; PV: Pressure-volume; PVR: Pulmonary vascular resistance; Q_s/Q_t: Shunt fraction; RR: Respiratory rate; SvO_2: Mixed venous oxygen saturation; SVR: Systemic vascular resistance; THAM: Tris(hydroxymethyl)aminomethane; TNF-α: Tumor necrosis factor α; VILI: Ventilator-induced lung injury; VO_2: body oxygen consumption; V_T: Tidal volume; $\Delta PaCO_2$: Arteriovenous difference in carbon dioxide tension.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
SH did the main body of experimental work and statistics and participated in manuscript preparation throughout all phases. JBB participated in of experiment planning and manuscript revisions. FS-S participated in experiment planning and manuscript revisions. KA performed immunoassays and participated in manuscript revisions. JE participated in experimental work and manuscript preparation. GH participated in experiment planning and manuscript revisions. AL participated in planning, experimental work, and manuscript preparation and revisions. All authors read and approved the final manuscript.

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References
1. The Acute Respiratory Distress Syndrome Network. Ventilation with lower tidal Volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. N Engl J Med. 2000;342:1301–8.
2. Futier E, Constantin JM, Paugam-Burtz C, Pascal J, Eurin M, Neuschwander A, et al. A trial of intraoperative low-tidal-volume ventilation in abdominal surgery. N Engl J Med. 2013;369:428–37.
3. Terragni PP, Rosboch G, Tealdi A, Corno E, Menaldo E, Davini O, et al. Tidal hyperinflation during low tidal volume ventilation in acute respiratory distress syndrome. Am J Respir Crit Care Med. 2007;175:160–6.
4. Masterson C, Otulakowski G, Kavanagh BP. Hypercapnia. Curr Opin Crit Care. 2015;21:7–12.
5. Weber T, Tschernich H, Sitzwohl C, Ullrich R, Germann P, Zimpfer M, et al. Tromethamine buffer modifies the depressant effect of permissive hypercapnia on myocardial contractility in patients with acute respiratory distress syndrome. Am J Respir Crit Care Med. 2000;162:1361–5.
6. Curley GF, Laffey JG. Acidosis in the critically ill - balancing risks and benefits to optimize outcome. Crit Care. 2014;18:129.
7. Ijland MM, Heunks LM, van der Hoeven JG. Bench-to-bedside review: hypercapnic acidosis in lung injury – from "permissive" to "therapeutic". Crit Care. 2010;14:237.
8. Osman D, Monnet X, Castelain V, Anguel N, Warszawski J, Teboul JL, et al. Incidence and prognostic value of right ventricular failure in acute respiratory distress syndrome. Intensive Care Med. 2009;35:69–76.
9. Dessap AM, Charron C, Devaquet J, Aboab J, Jardin F, Brochard L, et al. Impact of acute hypercapnia and augmented positive end-expiratory pressure on right ventricle function in severe acute respiratory distress syndrome. Intensive Care Med. 2009;35:1850–8.
10. Larsson A. Elimination of apparatus dead space – a simple method for improving CO_2 removal without increasing airway pressure. Acta Anaesthesiol Scand. 1992;36:796–9.
11. De Robertis E, Uttman L, Jonson B. Re-inspiration of CO_2 from ventilator circuit: effects of circuit flushing and aspiration of dead space up to high respiratory rate. Crit Care. 2010;14:R73.
12. Devaquet J, Jonson B, Niklason L, Si Larbi AG, Uttman L, Aboab J, et al. Effects of inspiratory pause on CO_2 elimination and arterial PCO_2 in acute lung injury. J Appl Physiol. 2008;105:1944–9.
13. Nahum A, Ravenscraft SA, Nakos G, Adams AB, Burke WC, Marini JJ. Effect of catheter flow direction on CO_2 removal during tracheal gas insufflation in dogs. J Appl Physiol. 1993;75:1238–46.
14. Ferguson ND, Cook DJ, Guyatt GH, Mehta S, Hand L, Austin P, et al. High-frequency oscillation in early acute respiratory distress syndrome. N Engl J Med. 2013;368:795–805.
15. Young D, Lamb SE, Shah S, MacKenzie I, Tunnicliffe W, Lall R, et al. High-frequency oscillation for acute respiratory distress syndrome. N Engl J Med. 2013;368:806–13.
16. Bein T, Weber-Carstens S, Goldmann A, Müller T, Staudinger T, Brederlau J, et al. Lower tidal volume strategy (≈3 ml/kg) combined with extracorporeal CO_2 removal versus "conventional" protective ventilation (6 ml/kg) in severe ARDS: the prospective randomized Xtravent-study. Intensive Care Med. 2013;39:847–56.
17. Cardenas Jr VJ, Zwischenberger JB, Tao W, Nguyen PD, Schroeder T, Traber LD, et al. Correction of blood pH attenuates changes in hemodynamics and organ blood flow during permissive hypercapnia. Crit Care Med. 1996;24:827–34.
18. Simma B, Kirpalani H. Sodium bicarbonate–the swings and roundabouts will not stop without randomized evidence. Crit Care Med. 2013;41:2242–3.
19. Nahas GG, Sutin KM, Fermon C, Streat S, Wiklund L, Wahlander S, et al. Guidelines for the treatment of acidaemia with THAM. Drugs. 1998;55:191–224.
20. Holmdahl MH, Wiklund L, Wetterberg T, Streat S, Wahlander S, Sutin K, et al. The place of THAM in the management of acidemia in clinical practice. Acta Anaesthesiol Scand. 2000;44:524–7.
21. Caples SM, Rasmussen DL, Lee WY, Wolfert MZ, Hubmayr RD. Impact of buffering hypercapnic acidosis on cell wounding in ventilator-injured rat lungs. Am J Physiol Lung Cell Mol Physiol. 2009;296:L140–4.
22. Kallet RH, Jasmer RM, Luce JM, Lin LH, Marks JD. The treatment of acidosis in acute lung injury with Tris-hydroxymethyl aminomethane (THAM). Am J Respir Crit Care Med. 2000;161:1149–53.
23. Höstman S, Engström J, Hedenstierna G, Larsson A. Intensive buffering can keep pH above 7.2 for over 4 h during apnea: an experimental porcine study. Acta Anaesthesiol Scand. 2013;57:63–70.
24. Brody S, Comfort JE, Matthews JS. Growth and development, with special reference to domestic animals: XI. Further investigations on surface area with special reference to its significance in energy metabolism. Bulletin 115. Columbia, MO: University of Missouri Agricultural Experiment Station; 1928.
25. Thorsteinsson A, Larsson A, Jonmarker C, Werner O. Pressure-volume relations of the respiratory system in healthy children. Am J Respir Crit Care Med. 1994;150:421–30.
26. Jonmarker C, Jansson L, Jonson B. Measurement of functional residual capacity by sulfur hexafluoride washout. Anesthesiology. 1985;63:89–95.
27. Larsson A, Linnarsson D, Jonmarker C, Jonson B, Larsson H, Werner O. Measurement of lung volume by sulfur hexafluoride washout during spontaneous and controlled ventilation: further development of a method. Anesthesiology. 1987;67:543–50.
28. R Core Team. R: A Language and Environment for Statistical Computing. Vienna: R Foundation for Statistical Computing; 2014.
29. Schreiber MD, Heymann MA, Soifer SJ. Increased arterial pH, not decreased $PaCO_2$, attenuates hypoxia-induced pulmonary vasoconstriction in newborn lambs. Pediatr Res. 1986;20:113–7.
30. Gordon JB, Martinez FR, Keller PA, Tod ML, Madden JA. Differing effects of acute and prolonged alkalosis on hypoxic pulmonary vasoconstriction. Am Rev Respir Dis. 1993;148:1651–6.

31. Ketabchi F, Egemnazarov B, Schermuly RT, Ghofrani HA, Seeger W, Grimminger F, et al. Effects of hypercapnia with and without acidosis on hypoxic pulmonary vasoconstriction. Am J Physiol Lung Cell Mol Physiol. 2009;297:L977–83.
32. Marshall C, Lindgren L, Marshall BE. Metabolic and respiratory hydrogen ion effects on hypoxic pulmonary vasoconstriction. J Appl Physiol. 1984;57:545–50.
33. Brimioulle S, Lejeune P, Vachiery JL, Leeman M, Melot C, Naeije R. Effects of acidosis and alkalosis on hypoxic pulmonary vasoconstriction in dogs. Am J Physiol. 1990;258:H347–53.
34. Stengl M, Ledvinova L, Chvojka J, Benes J, Jarkovska D, Holas J, et al. Effects of clinically relevant acute hypercapnic and metabolic acidosis on the cardiovascular system: an experimental porcine study. Crit Care. 2013;17:R303.
35. Lyrene RK, Welch KA, Godoy G, Philips JB. Alkalosis attenuates hypoxic pulmonary vasoconstriction in neonatal lambs. Pediatr Res. 1985;19:1268–71.
36. Benumof JL, Wahrenbrock EA. Blunted hypoxic pulmonary vasoconstriction by increased lung vascular pressures. J Appl Physiol. 1975;38:846–50.
37. Brimioulle S, Julien V, Gust R, Kozlowski JK, Naeije R, Schuster DP. Importance of hypoxic vasoconstriction in maintaining oxygenation during acute lung injury. Crit Care Med. 2002;30:874–80.
38. Wetterberg T, Sjöberg T, Steen S. Effects of buffering in hypercapnia and hypercapnic hypoxemia. Acta Anaesthesiol Scand. 1993;37:343–9.
39. Berger MG, Vandier C, Bonnet P, Jackson WF, Rusch NJ. Intracellular acidosis differentially regulates KV channels in coronary and pulmonary vascular muscle. Am J Physiol. 1998;275:H1351–9.
40. Elliott AR, Steffey EP, Jarvis KA, Marshall BE. Unilateral hypoxic pulmonary vasoconstriction in the dog, pony and miniature swine. Respir Physiol. 1991;85:355–69.