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

Different methods to reduce PaCO₂ during low V₉ 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 V₉ using high-frequency oscillation has not been shown to be beneficial and might even increase mortality in ARDS [14, 15].

Other methods are extracorporeal CO₂ removal using an arteriovenous or venovenous approach with a low blood flow through a small membrane oxygenator/CO₂ removal. However, this method has not yet been shown to improve clinical outcome [16]. Thus, in many situations with low V₉ ventilation, hypercapnic acidosis is unavoidable.

Because the acidosis caused by the increased PaCO₂ may be the main reason for the side effects of "permissive hypercapnia," it has been speculated whether treatment with sodium bicarbonate (NaHCO₃) would be useful [1, 17, 18]. In fact, in order for NaHCO₃ to work as a buffer, it has to generate CO₂, which in turn has to be removed via the lungs increasing ventilatory demands [19]. Because the problem with low V₉ ventilation is that the CO₂ excretion via the lungs is impaired, NaHCO₃ 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 CO₂, could be a more logical choice in cases of excessive CO₂ 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 V₉ 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 Hedensterna 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 V₉ 8 ml kg⁻¹, inspiratory/expiratory ratio (I:E) 1:1, fraction of inspired oxygen (FiO₂) 0.5, RR 25 cycles/min, and PEEP 5 cmH₂O. Just before the tracheotomy, 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 cmH₂O, and PEEP 20 cmH₂O 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 described 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 PaCO₂ 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 HCO₃⁻, we could find the mEq/L amount of buffer needed as the difference between this value and normal bicarbonate (HCO₃⁻), 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, FIO₂ 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 (PaO₂), PaCO₂, 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 cmH₂O, and an airway pressure-volume (PV) loop was obtained by slow insufflation to 40 cmH₂O 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 °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 cmH₂O, V₁ 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 V₁ 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 V₁ 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 CaCl₂, 1 mM MgCl₂, 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 3000×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 a consistent 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$^2$, $p = 0.008$). 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:
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

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### 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)\(^ab\) | 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)\(^ab\) |
| 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) | 598.6 (6.3) | 494.9 (9.0) | 533.5 (5.0) |
|                            | 3T    | 55.5 (15.5) | 56.0 (4.6) | 49.7 (4.1) | 32.6 (7.6) | 30.0 (7.7)\(^ab\) |

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)\(^ab\) | 27.8 (3.1)\(^ab\) |

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)\(^ab\) | 57.2 (2.8)\(^ab\) |

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)\(^a\) | 98 (0.2)\(^a\) |
|                            | 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\(_i\)/Q\(_l\) (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)\(^ab\) |

\( \Delta \text{PaCO}_2 \) (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)\(^ab\) | 1.0 (0.7)\(^ab\) |

Abbreviations: BE arterial base excess, HCO\(_3\) arterial bicarbonate concentration, NT control animals that did not receive THAM, PaCO\(_2\) arterial carbon dioxide tension, \( \Delta \text{PaCO}_2 \) arteriovenous difference in carbon dioxide tension, PaO\(_2\) arterial oxygen tension, Q\(_i\)/Q\(_l\) 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
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$_2$O. 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

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**Fig. 1** Effects of Tris(hydroxymethyl)aminomethane on arterial pH, base excess, arterial carbon dioxide tension (PaCO$_2$) 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.
metabolic and hypercapnic acidosis increased PVR and
that alkalosis, independent of whether it was metabolic
or hypercapnic, reduced or normalized pulmonary vas-
tular tone. Thus, the pH change in itself was interpreted
as 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$_3^-$ remained
at high levels, indicating that the metabolic com-
ponent 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]. How-
ever, they did not find any significant change in PVR
induced by THAM, probably owing to simultaneous
administration of cardiovascular active agents. Further-
more, 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 re-
duction in SVR mirrored the increase in PaCO$_2$, suggest-
ing that, in the systemic circulation, PaCO$_2$ per se influ-
ences 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 vascula-
ture 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 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

| 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)$^{ab}$ | 18 (2) | 19 (2)$^a$ | 17 (2)$^{ab}$ | 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)$^{ab}$ |
| 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
$^{ab}$Value is different from 1T group
infusion was stopped, PaCO₂ increased and leveled off at a higher level than in the controls. In the animals treated with THAM for 3 h, the PaCO₂ 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 PaCO₂ 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 CO₂ production increased.

3. Increased CO₂ accumulation in the tissues due to reduced pulmonary excretion during the THAM infusion: This is indicated by the significantly lower pulmonary mixed venous-arterial PaCO₂ gradient value during the THAM infusion in the 3-h THAM group. Thus, THAM might have sequestered CO₂ that thereafter accumulated in the tissues. Because the tension of CO₂ cannot be higher in the tissues than in the blood, the CO₂ must have been stored as HCO₃⁻, 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 PaCO₂ 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 CO₂, stored as HCO₃⁻ 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 HCO₃⁻ was, via the effect of carbonic anhydrase, transformed into CO₂, which penetrated the cell membranes.
easily and increased \( \text{PaCO}_2 \). 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-\( \alpha \) or IL-1\( \beta \). 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

![Fig. 3](image)

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\(2\)O) | 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)
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 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 hypoxic, 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 inhibition of HPV. 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.

### Table 4

| 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)$^{ab}$ | 125 (2)$^{ab}$ | 125 (2)$^{ab}$ | 138 (3)$^{ab}$ |
| [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)$^{ab}$ | 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)$^{ab}$ | 1.09 (0.05)$^{ab}$ |
| [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
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; Cepx: compliance of the respiratory system; CI: Cardiac index; CM: Medi-al; CO: Cardiac output; CV: Ventral; CVP: Central venous pressure; ELISA: Enzyme-linked immunosorbent assay; FIO2: Fraction of inspired oxygen; FRC: Functional residual capacity; HCO3-: Bicarbonate; HPV: Hypoxic pulmonary vasoconstriction; IE: Inspiratory/expiratory ratio; IL: Interleukin; MAP: Mean arterial blood pressure; MPAP: Mean pulmonary arterial pressure; NaHCO3: Sodium bicarbonate; ns: Non-significant; NT: Controls (i.e., no THAM infusion); PaCO2: Arterial carbon dioxide tension; PaO2: Arterial oxygen tension; PEEP: Positive end-expiratory pressure; PV: Pressure-volume; PVr: Pulmonary vascular resistance; Q/O2: Shunt fraction; RR: Respiratory rate; SVo2: Mixed venous oxygen saturation; SVR: Systemic vascular resistance; THAM: Tris(hydroxymethyl)aminomethane; TNF-α: Tumor necrosis factor α; VILI: Ventilator-induced lung injury; VO2: body oxygen consumption; Vt: Tidal volume; ΔPaCO2: 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 immunoonassays 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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