A randomized porcine study of hemorrhagic shock comparing end-tidal carbon dioxide targeted and proximal systolic blood pressure targeted partial resuscitative endovascular balloon occlusion of the aorta in the mitigation of metabolic injury

Anna Stene Hurtsén[1,2,3]* , David T. McGreevy[1,3], Christina Karlsson[4], Claes G. Frostell[5], Tal M. Hörer[1,6] and Kristofer F. Nilsson[1,3]

*Correspondence:
anna.stene-hurtsen@regionorebrolan.se

[1] Department of Cardiothoracic and Vascular Surgery, Faculty of Medicine and Health, Örebro University, Örebro, Sweden
[2] Centre for Clinical Research and Education, County Council of Värmland, Karlstad, Sweden
[3] School of Medical Sciences, Örebro University, Örebro, Sweden
[4] School of Health Sciences, Örebro University, Örebro, Sweden
[5] Anesthesiology and Intensive Care, Department of Clinical Sciences, Karolinska Institute at Danderyd Hospital, Stockholm, Sweden
[6] Department of Surgery, Faculty of Medicine and Health, Örebro University, Örebro, Sweden

Abstract

Background: The definition of partial resuscitative endovascular balloon occlusion of the aorta (pREBOA) is not yet determined and clinical markers of the degree of occlusion, metabolic effects and end-organ injury that are clinically monitored in real time are lacking. The aim of the study was to test the hypothesis that end-tidal carbon dioxide (ETCO$_2$) targeted pREBOA causes less metabolic disturbance compared to proximal systolic blood pressure (SBP) targeted pREBOA in a porcine model of hemorrhagic shock.

Materials and methods: Twenty anesthetized pigs (26–35 kg) were randomized to 45 min of either ETCO$_2$ targeted pREBOA (pREBOA$_{ETCO2}$, ETCO$_2$ 90–110% of values before start of occlusion, $n = 10$) or proximal SBP targeted pREBOA (pREBOA$_{SBP}$, SBP 80–100 mmHg, $n = 10$), during controlled grade IV hemorrhagic shock. Autotransfusion and reperfusion over 3 h followed. Hemodynamic and respiratory parameters, blood samples and jejunal specimens were analyzed.

Results: ETCO$_2$ was significantly higher in the pREBOA$_{ETCO2}$ group during the occlusion compared to the pREBOA$_{SBP}$ group, whereas SBP, femoral arterial mean pressure and abdominal aortic blood flow were similar. During reperfusion, arterial and mesenteric lactate, plasma creatinine and plasma troponin concentrations were higher in the pREBOA$_{SBP}$ group.

Conclusions: In a porcine model of hemorrhagic shock, ETCO$_2$ targeted pREBOA caused less metabolic disturbance and end-organ damage compared to proximal SBP targeted pREBOA, with no disadvantageous hemodynamic impact. End-tidal CO$_2$ should be investigated in clinical studies as a complementary clinical tool for mitigating ischemic–reperfusion injury when using pREBOA.

Stene Hurtsén *et al. Intensive Care Medicine Experimental*   (2023) 11:18

Page 2 of 15

Introduction

Resuscitative endovascular balloon occlusion of the aorta (REBOA) is an emerging endovascular tool in the management of life-threatening non-compressible hemorrhage [1, 2]. The aortic balloon technique originated during the Korean war [3], had a modern upsurge in the endovascular management of ruptured abdominal aortic aneurysm and is now recognized as a tool in the Endovascular Resuscitation and Trauma Management (EVTM) concept, including indications other than trauma [1, 4–9]. The aim is to decrease an ongoing bleeding but preserve cerebral and myocardial perfusion until definite hemostasis is achievable [1, 2, 8, 10, 11]. However, the supra-physiologic blood pressure proximal to the occlusion can lead to cardiac failure and worsening of traumatic brain injury [12–14]. Moreover, distal ischemia and subsequent reperfusion injury upon deflation of the balloon is challenging and highly dependent on occlusion time [1, 7, 13–17]. To overcome these complications, new techniques have been developed. Partial REBOA (pREBOA) allows a permissible blood flow over the balloon and thereby restrictively perfuse distal organs [18]. By permitting some circulation distal of the occlusion, anaerobic metabolism is restricted, thus limiting the accumulation of metabolites and subsequent metabolic acidosis, which occurs in total aortic occlusion. In addition, the ischemic burden on the kidneys and intestines is reduced compared to total occlusion [19, 20]. Partial REBOA may enable extended occlusion time and may diminish rebound hypotension upon deflation which occasionally leads to reinflation [19, 21]. The definition of "partial" is, however, not yet concluded and clinical markers of the degree of occlusion, metabolic disturbance and end-organ injury that can be easily monitored in real time are lacking. In research settings, balloon titration has been correlated with targets of proximal systolic blood pressure (SBP) alone, pressure gradient proximal to distal over the balloon, intra-balloon pressure and volume, Computer Tomography imaging or by specially designed devices to control distal blood flow [19, 20, 22–25]. None of these indices alone provide enough information about tissue perfusion and metabolic state at organ level [14, 19–21]. End-tidal carbon dioxide ($ETCO_2$) has been suggested as an indicator in the use of Preboa, since it correlates to oxygen consumption [20, 26]. End-tidal CO_2 estimates oxygen consumption, since delivered oxygen converts to carbon dioxide during cell metabolism [27]. End-tidal CO_2 is used clinically and can be continuously measured in all intubated patients, and associates to cardiac output in critically ill trauma patients [28]. It could, therefore, be a useful noninvasive indicator of changes in systemic oxygen consumption and thereby a potential marker of metabolic disturbance and eventual end-organ injury caused by pREBOA. The aim of this study was to test the hypothesis that $ETCO_2$ targeted pREBOA causes less metabolic damage (determined by arterial lactate concentrations at 1 h of reperfusion as primary outcome) and end-organ damage compared to proximal SBP targeted pREBOA in a porcine model of controlled hemorrhagic shock.

Materials and methods

Study design

An interventional study approved by the Regional Animal Ethics Committee (ID 1525-2019, Linköping Sweden March 7, 2019) was conducted at the animal research laboratory, Örebro University, Örebro, Sweden. All animal handling was in compliance with the directives of the European Parliament and the Council on the protection of animals used for scientific purposes and conduced according to the Replacement, Reduction and Refinement principles [29]. Twenty-nine Swedish country breed (Hampshire and English Yorkshire cross breed) pigs were obtained from a local farmer at 3–4 months, with a mean weight of 30 kg (26–35 kg) and a gender ratio of approximately 1:1. Each group included 10 animals based on an interim analysis of arterial lactate concentrations as primary outcome. An a priori exclusion criterium was that animals that died before conclusion of the experiment should be replaced with new animals. The study adhered to the ARRIVE guidelines as closely as feasible [30].

Surgical preparation, anesthesia, and monitoring

The animals were premedicated with azaperone and anesthetized with tiletamine/zolazepam $\pm$ propofol as previously described as previously described [20, 31]. Anesthesia was maintained by Propofol 10 mg kg^{-1} h^{-1} (Fresenius Kabi, Uppsala Sweden) and Remifentanil 40 µg kg^{-1} h^{-1} (Actavis, Dublin, Irland) using infusion pumps (Alaris GP; Cardinal Health Care, Rolle, Switzerland). At baseline, volume-controlled ventilation (Airox$^{™}$ Legendair$^{™}$, Coviden, Hampshire, UK) was set to tidal volumes 10 ml kg^{-1} and the respiratory rate was adjusted to maintain ETCO$_2$ at $5.0 \pm 0.5\%$, except during the hemorrhage and intervention periods. Body temperature (measured through the pulmonary arterial catheter) was maintained at $39 \pm 0.5\,°C$ using heat blankets.

Arterial and venous accesses for hemodynamic measurements, blood sampling, controlled hemorrhage, endovascular intervention and fluid administration were obtained in accordance with a previous study [20]. In short, arterial access was gained through the right common carotid artery (5 Fr) and the femoral artery bilaterally (10 Fr in the right, 4 Fr in the left) and venous access was gained through the external jugular vein bilaterally (10 Fr). A pulmonary arterial catheter (7.5 Fr, CCOMBO, Edwards Lifesciences, Irvine, CA, USA) was inserted through the right venous sheath. A baby feeding catheter (Nutrisafe2, Vycon, Ecouen, France) was placed into a mesenteric vein for blood sampling. A flow probe (Vascular TTFM Probe 10 mm; Medistim ASA, Oslo, Norway) was placed over the aorta at diaphragm level through laparotomy and the urinary bladder was catheterized through a cystotomy (12 Fr, Foley; Bard Limited, Crawley, UK). The abdomen was closed and Heparin 5000 IE (LEO Pharma, Malmö, Sweden) was administered. An intervention-free hour was allowed for hemodynamic stabilization before the start of the experiment.

Protocol and study groups

After stabilization, controlled hemorrhage was induced through the right femoral access (Fig. 1). Thirty percent of the calculated blood volume (66 ml kg^{-1}) was eliminated over 15 min, additional blood was withdrawn over a further 15 min, aiming for
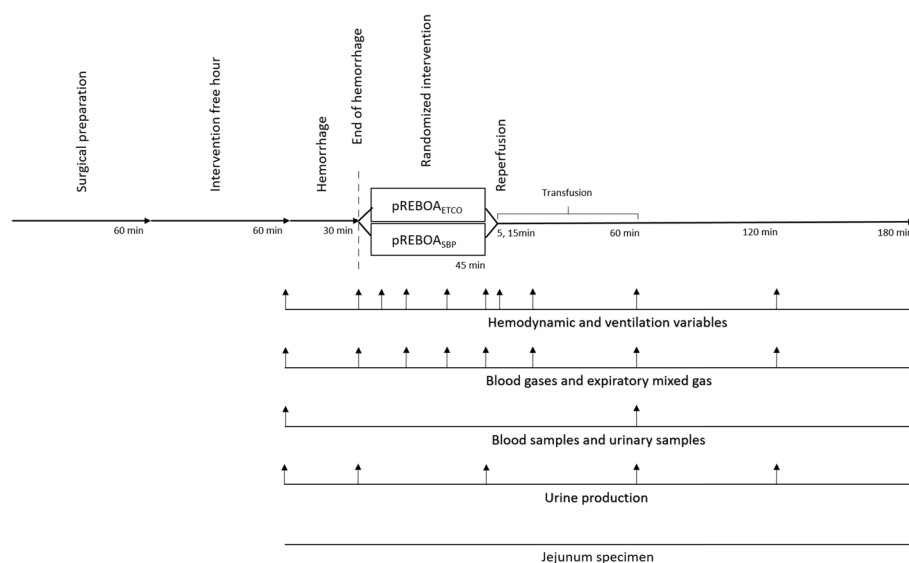
Fig. 1 Experimental protocol of partial endovascular balloon occlusion of the aorta (pREBOA) targeted by end-tidal carbon dioxide (pREBOA$_{ETCO2}$, $n=10$) and proximal systolic blood pressure (pREBOA$_{SBP}$, $n=10$) in anesthetized, mechanically ventilated pigs. Each arrowhead indicates a measurement point

a SBP of 55–60 mm Hg. The blood was collected in citrated bags (Fenwal™, Baxter, Illinois, USA) for later autologous transfusion. A REBOA catheter (Rescue Balloon; Tokai Ltd, Japan) was then inserted through the same access and advanced to the thoracic aorta (zone I) using landmarks; correct position was confirmed by blood pressure elevation and reduction of aortic blood flow. The subjects were block randomized by envelop system to either proximal SBP targeted (pREBOA$_{SBP}$) or ETCO$_2$ targeted inflation of the REBOA (pREBOA$_{ETCO2}$). The SBP target was set at 80–100 mm Hg and the ETCO$_2$ target at 90–110% of the reference value at the end of hemorrhagic. If necessary, the inflation volume was manually adjusted by 0.25 ml after every measurement point (i.e., every 15 min) to maintain the pre-set interval. After 45 min of occlusion, the balloon was gradually deflated over 5 min. Two pigs, one in each group, received Epinephrine 0.1 mg (Martindale Pharma, Romford, UK) when deflating the balloon to counteract abrupt hypotension. Fluid administration (5% glucose 1 ml kg^{-1} and Ringer's Acetate 10 ml kg^{-1}) was stopped during hemorrhage and occlusion and restarted at reperfusion in addition to autologous blood transfusion. One transfusion bag (500 ml) was transfused within 15 min, the remaining withdrawn blood was transfused over the next 45 min. Additional 20 ml glucose (30 mg ml^{-1}, Fresenius Kabi, Uppsala, Sweden) was given if glucose levels dropped below 3.0 mmol L^{-1}. If required, an extra blood gas was taken after 30 min for evaluation. Ventilation settings were fixed during hemorrhage and intervention. Respiratory frequency was adjusted after 1 h of reperfusion to achieve ETCO$_2$ at 5.0 ± 0.5%. Hemodynamic and respiratory variables were monitored on an intensive care monitor (AS/3, Datex, Helsinki, Finland), collected by MP150/Acknowledge 3.9.1 system (BIOPAC systems; Goleta, CA, USA), and data were sampled according to the protocol (Fig. 1). Airtight bags were used to collect exhaled gas for analysis of the fractions of mixed exhaled gases in the intensive care monitor (AS/3, Datex, Helsinki, Finland). Arterial, mixed-venous and mesenteric blood gases, arterial

and mesenteric blood samples, and urine samples were collected at the indicated time-points (Fig. 1). After 3 h of reperfusion, a specimen from the jejunum was collected and preserved in 4% Formaldehyde solution (Solveco AB, Rosersberg, Sweden) for blinded evaluation by a pathologist using a six-grade system, where 0 represents normal tissue and 6 represents villi without epithelium (dead villi) and crypt destruction [6, 32, 33]. Euthanasia was performed with 20 ml potassium chloride (2 mmol ml^{-1}, Braun, Danderyd, Sweden) after a bolus dose of Propofol (200 mg). Cardiac arrest was confirmed by SBP, electrocardiography and ETCO$_2$. The primary outcome variable was arterial lactate concentrations at 1 h of reperfusion.

Calculations

Oxygen consumption (VO$_2$) was calculated as (inspired fraction of oxygen − fraction of mixed exhaled oxygen) × minute ventilation (MV). Carbon dioxide production (VCO$_2$) was calculated as fraction of mixed exhaled carbon dioxide × MV. Oxygen delivery (DO$_2$) was calculated as CO × arterial content of oxygen and oxygen extraction ratio (O$_2$ER) was calculated as (arterial content of oxygen − mixed-venous content of oxygen)/arterial content of oxygen. Arterial and mixed-venous content of oxygen was calculated as Hb × oxygen saturation × 1.34 + pO$_2$ × 0.23 using values from arterial and mixed-venous blood samples, respectively.

Statistical analysis

Statistical analyses were not blinded. Normal distribution was examined using the Shapiro–Wilk test and non-normally distributed data were transformed by the logarithm (aspartate aminotransferase, troponin I and diuresis) and reanalyzed using the Shapiro–Wilk test. A linear mixed model with group, time and their interaction was used in an autoregressive model using IBM SPSS version 26 (SPSS Inc., Chicago, IL. USA). If the interaction between group and time was statistically significant, a Bonferroni-adjusted post-hoc multiple comparison analysis was performed. Mann–Whitney U test was used for statistical analysis of histologic samples. Statistical significance was considered as $p < 0.05$. Graphs were made in GraphPad prism version 8 (GraphPad Software Inc, San Diego, CA, USA). Data are presented as means with confidence intervals unless otherwise indicated.

Results

Four animals died during hemorrhage before randomization and were excluded from the study. Further two animals died during reperfusion in the pREBOA$_{SBP}$ group and one in the pREBOA$_{ETCO2}$ group. In addition, one animal in the pREBOA$_{SBP}$ group was excluded as an outlier (decided by the first and last authors due to deviation from the mean by more than two standard deviations in several variables) and one in the pREBOA$_{ETCO2}$ group due to accidental air pulmonary embolism. All excluded animals were replaced according to the pre-determined protocol, resulting in 10 animals in each group completing the study and included in the statistical analysis.
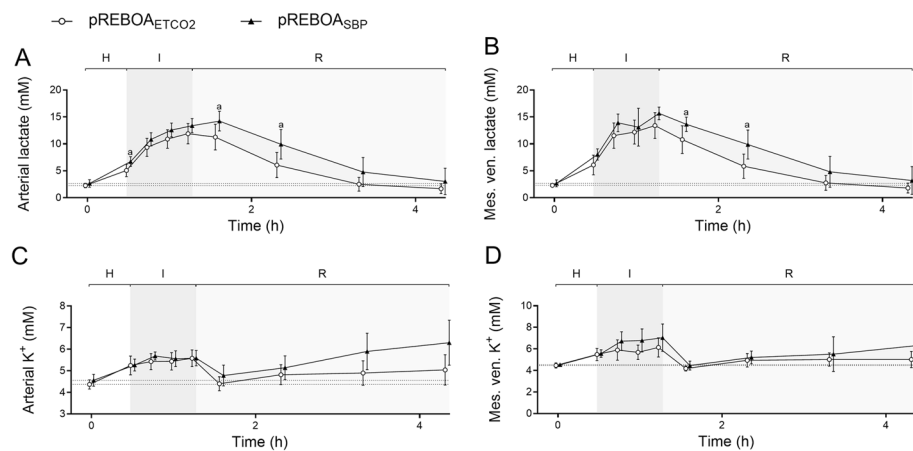
Fig. 2 End-tidal carbon dioxide (ETCO$_2$, **A**), systemic blood pressure (SBP, **B**), abdominal aortic blood flow (ABF, **C**) and femoral arterial mean pressure (FMP, **D**) in anesthetized, mechanically ventilated pigs undergoing hemorrhage (H), intervention (I) using either end-tidal carbon dioxide (ETCO$_2$) targeted partial resuscitative endovascular balloon occlusion of the aorta (pREBOA, pREBOA$_{ETCO2}$, $n=10$) and proximal systolic blood pressure targeted pREBOA (pREBOA$_{SBP}$, $n=10$) and subsequent reperfusion (R). [a] Denotes statistical difference between the groups at a given timepoint. Data are means (95% confidence interval). The dotted line represents baseline
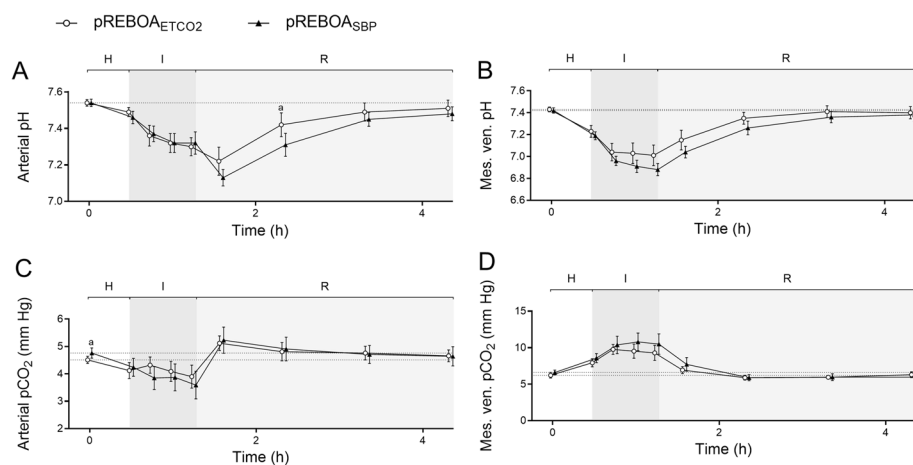

Fig. 3 Arterial and mesenteric venous pH (**A**, **B**) and pCO$_2$ (**C**, **D**) in anesthetized, mechanically ventilated pigs undergoing hemorrhage (H), intervention (I) using either end-tidal carbon dioxide (ETCO$_2$) targeted partial resuscitative endovascular balloon occlusion of the aorta (pREBOA, pREBOA$_{ETCO2}$, $n=10$) and proximal systolic blood pressure targeted pREBOA (pREBOA$_{SBP}$, $n=10$) and subsequent reperfusion (R). [a] Denotes statistical difference between the groups at given timepoint. Data are means (95% confidence interval). The dotted line represents baseline

Hemorrhage

The groups were equivalent at baseline except for arterial pCO$_2$ (Figs. 2, 3, 4, Tables 1, 2). Hemorrhage caused a hemodynamic shock state with systemic hypotension, SBP was 56 (CI 52–60) mm Hg in the pREBOA$_{ETCO2}$ group and 57 (CI 55–59) mm Hg in the pREBOA$_{SBP}$ group (Fig. 2, Table 1), which corresponded to a blood loss of 47% and 50% of total blood volume, respectively.
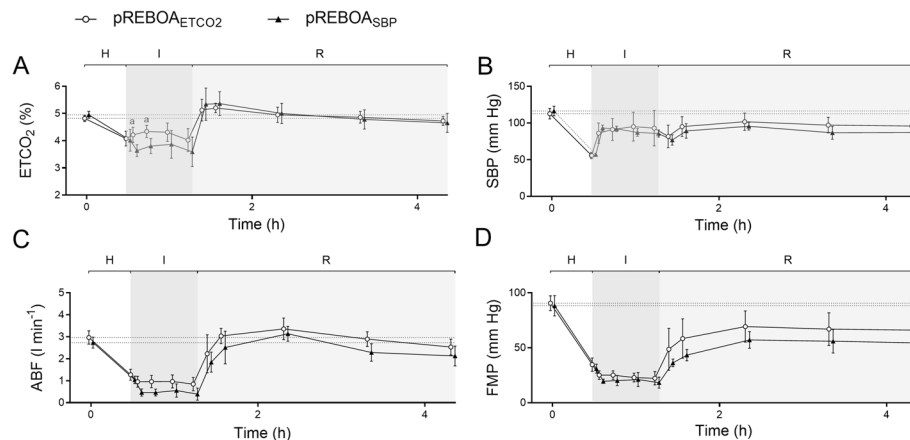
Fig. 4 Arterial and mesenteric venous lactate (**A**, **B**) and potassium (K; **C**, **D**) concentrations in anesthetized, mechanically ventilated pigs undergoing hemorrhage (H), intervention (I) by end-tidal carbon dioxide ($ETCO_2$) targeted partial resuscitative endovascular balloon occlusion of the aorta (pREBOA, $pREBOA_{ETCO2}$, $n = 10$) and proximal systolic blood pressure targeted pREBOA ($pREBOA_{SBP}$, $n = 10$) and subsequent reperfusion (R). [a] Denotes statistical difference between the groups at given timepoint. Data are means (95% confidence interval). The dotted line represents baseline

Table 1 Hemodynamics during partial resuscitative endovascular balloon occlusion of the aorta

	Baseline	Hemorrhage	15 min pREBOA	30 min pREBOA	45 min pREBOA	15 min reperfusion	60 min reperfusion	120 min reperfusion	180 min reperfusion
HR (beats min^{-1})									
$pREBOA_{ETCO2}$	117 (101–132)	163 (137–189)	175 (145–204)	192 (153–220)	181 (158–204)	173 (156–191)	165 (148–183)	143 (125–162)	140 (116–165)
$pREBOA_{SBP}$	127 (107–148)	206 (192–220)	198 (176–220)	211 (197–225)	199 (184–214)	160 (152–168)	169 (158–181)	161 (140–183)	156 (132–179)
MAP (mm Hg)									
$pREBOA_{ETCO2}$	92 (85–100)	35 (30–40)	71 (58–84)	73 (57–89)	69 (51–87)	61 (46–75)	72 (60–85)	71 (59–82)	69 (58–81)
$pREBOA_{SBP}$	94 (86–103)	34 (29–38)	66 (60–72)	58 (48–69)	62 (55–70)	44 (40–48)	59 (54–64)	59 (50–64)	58 (47–69)
CVP (mm Hg)									
$pREBOA_{ETCO2}$	7 (6–8)	4 (3–5)	5 (4–6)	5 (4–6)	5 (4–6)	7 (6–8)	7 (6–8)	6 (6–7)	7 (6–8)
$pREBOA_{SBP}$	9 (8–10)	5 (4–6)	7 (6–8)	7 (6–8)	7 (6–8)	9 (8–10)	9 (8–9)	8 (7–9)	8 (7–8)
CO (l min^{-1})									
$pREBOA_{ETCO2}$	6.1 (5.5–6.7)	2.8 (2.4–3.2)	3.0 (2.3–3.6)	3.3 (2.5–4.0)	3.2 (2.5–4.0)	4.8 (3.8–5.8)	6.0 (5.4–6.7)	5.2 (4.7–5.7)	4.6 (4.1–5.0)
$pREBOA_{SBP}$	5.2 (4.5–5.6)	2.4 (2.2–2.6)	2.8 (2.5–3.1)	2.87 (2.5–3.2)	2.9 (2.5–3.3)	4.0 (3.4–4.5)	5.8 (5.2–6.5)	4.47 (3.8–5.1)	4.0 (3.2–4.5)

Hemodynamics during end-tidal carbon dioxide ($ETCO_2$) targeted partial resuscitative endovascular balloon occlusion of the aorta (pREBOA, $pREBOA_{ETCO2}$, $n = 10$) and proximal systolic blood pressure targeted pREBOA ($pREBOA_{SBP}$, $n = 10$) in anesthetized, mechanically ventilated pigs. Data are means (95% confidence intervals)

HR: heart rate; MAP: mean arterial pressure; CVP: central venous pressure; CO: cardiac output

Oxygen consumption, O_2ER and carbon dioxide production (VCO_2) were similar in both groups (Table 2). Arterial pCO_2 followed a similar pattern as $ETCO_2$ (Fig. 3). The mesenteric pO_2 differed significantly between the groups during hemorrhage, although with a numerically small difference (Table 2).

Stene Hurtsén *et al. Intensive Care Medicine Experimental*   (2023) 11:18

Page 8 of 15

Table 2 Blood gases and blood samples during partial resuscitative endovascular balloon occlusion of the aorta

	Baseline	Hemorrhage	15 min REBOA	30 min REBOA	45 min REBOA	15 min reperfusion	60 min reperfusion	120 min reperfusion	180 min reperfusion
Arterial pO$_2$ (kPa)									
pREBOA$_{ETCO2}$	12.2 (11.7–12.6)	12.1 (11.5–12.7)	12.9 (11.9–14.0)	13.0 (11.8–14.1)	13.4 (12.2–14.5)	10.3 (9.7–10.8)	11.1 (10.5–11.6)	11.4 (10.7–12.1)	11.6 (11.0–12.3)
pREBOA$_{SBP}$	11.8 (11.1–12.6)	12.1 (10.9–13.3)	13.5 (12.1–14.8)	14.1 (13.3–14.9)	13.7 (12.4–14.9)	10.8 (9.9–11.8)	10.8 (10.2–11.4)	11.3 (10.4–12.2)	11.6 (10.9–12.3)
Mes. ven. pO$_2$ (kPa)									
pREBOA$_{ETCO2}$	5.5 (5.0–5.9)	4.1 (3.8–4.4)[a]	4.4 (4.1–4.7)	4.2 (3.9–4.5)	4.6 (4.2–5.0)	6.1 (5.9–6.4)	6.4 (6.0–6.9)	6.5 (6.2–6.9)	5.8 (5.4–6.2)
pREBOA$_{SBP}$	5.0 (4.5–5.5)	3.6 (3.3–3.9)[a]	4.2 (3.9–4.4)	4.3 (4.1–4.5)	4.4 (4.1–4.6)	6.5 (6.0–7.0)	6.9 (6.6–7.2)	6.1 (5.8–6.5)	5.7 (5.2–6.1)
VO$_2$ (ml O$_2$ min^{-1})									
pREBOA$_{ETCO2}$	93 (73–113)	66 (48–85)	54 (43–65)	56 (45–67)	52 (41–63)	84 (68–102)	80 (64–96)	77 (61–93)	72 (56–89)
pREBOA$_{SBP}$	87 (81–94)	56 (48–64)	52 (45–59)	53 (47–59)	51 (44–58)	87 (78–96)	88 (80–96)	80 (71–90)	75 (63–87)
VCO$_2$ (ml CO$_2$ min^{-1})									
pREBOA$_{ETCO2}$	108 (98–117)	82 (72–91)	88 (82–93)	86 (79–94)	81 (74–88)	112 (104–119)	101 (94–107)	93 (88–98)	90 (83–96)
pREBOA$_{SBP}$	102 (93–111)	77 (69–85)	78 (69–86)	79 (71–86)	72 (63–80)	112 (104–120)	104 (97–111)	95 (85–104)	87 (75–99)
DO$_2$ (ml/min)									
pREBOA$_{ETCO2}$	669 (577–762)	276 (222–331)	261 (186–335)	279 (195–363)	283 (192–373)	419 (276–562)	635 (517–753)	550 (479–622)	515 (438–590)
pREBOA$_{SBP}$	560 (448–672)	216 (180–252)	239 (195–283)	232 (182–282)	232 (184–281)	305 (240–370)	557 (430–683)	515 (392–637)	436 (266–605)
O$_2$ER									
pREBOA$_{ETCO2}$	38 (32–44)	76 (71–81)	64 (55–73)[a]	60 (51–68)	64 (52–76)	52 (45–59)	38 (33–43)	45 (31–59)	45 (35–54)
pREBOA$_{SBP}$	38 (32–45)	80 (79–82)	52 (42–62)[a]	57 (48–67)	55 (44–66)	58 (51–66)	45 (39–50)	45 (41–50)	50 (39–61)
Creatinine (μmol L^{-1})									
pREBOA$_{ETCO2}$	60 (55–64)						87 (76–97)[a]		93 (74–112)[a]
pREBOA$_{SBP}$	62 (56–68)						99 (91–106)[a]		117 (105–130)[a]
Troponin (ng L^{-1})									
pREBOA$_{ETCO2}$	471 (290–766)						2641 (1166–5984)[a]		5366 (1768–5366)[a]
pREBOA$_{SBP}$	513 (381–690)						7384 (3966–13,747)[a]		20,171 (8748–46,514)[a]

Table 2 (continued)

	Baseline	Hemorrhage	15 min REBOA	30 min REBOA	45 min REBOA	15 min reperfusion	60 min reperfusion	120 min reperfusion	180 min reperfusion
ALAT (μkat L^{-1})									
pREBOA$_{ETCO2}$	1.1 (0.9–1.4)						1.1 (0.9–1.4)		1.2 (0.9–1.5)
pREBOA$_{SBP}$	1.3 (1.1–1.5)						1.3 (1.1–1.5)		1.5 (1.3–1.7)
ASAT (μkat L^{-1})									
pREBOA$_{ETCO2}$	1.0 (0.8–1.3)						1.8 (1.3–2.5)		3.0 (1.8–4.9)
pREBOA$_{SBP}$	1.1 (0.9–1.4)						2.4 (2.1–2.8)		4.5 (3.1–6.4)
CK (μkat L^{-1})									
pREBOA$_{ETCO2}$	19.3 (13.7–24.8)						22.3 (17.4–27.2)		28.3 (22.1–34.4)
pREBOA$_{SBP}$	18.5 (14.9–22.1)						23.2 (18.9–27.6)		32.1 (26.2–38.0)

Arterial and mesenteric venous blood gases and arterial blood samples during end-tidal carbon dioxide (ETCO$_2$) targeted partial resuscitative endovascular balloon occlusion of the aorta (pREBOA, pREBOA$_{ETCO2}$, $n = 10$) and proximal systolic blood pressure targeted pREBOA (pREBOA$_{SBP}$ $n = 10$) in anesthetized, mechanically ventilated pigs. Data are means (95% confidence intervals); [a] denotes statistical difference between the groups

pO2: partial pressure of oxygen; Mes. ven. pO$_2$: mesenteric venous pO$_2$; VO$_2$: oxygen consumption; VCO$_2$: carbon dioxide production; DO$_2$: oxygen delivery; O$_2$ER: oxygen extraction ratio; ALAT: alanine aminotransferase; ASAT: aspartate aminotransferase; CK: creatine kinase

Aortic occlusion

In the pREBOA$_{ETCO2}$ group, ETCO$_2$ was preserved on a statistically significantly higher level during the occlusion compared to the pREBOA$_{SBP}$ group, whereas SBP was similar in both groups (Fig. 2). There were no statistically significant differences between the groups during occlusion in abdominal aortic blood flow, femoral arterial mean pressure, and metabolic markers; however, O$_2$ER was slightly lower in the pREBOA$_{SBP}$ group at 15 min of REBOA (Figs. 2, 3, Table 2).

The inflation volume of the balloon was adjusted from 2.3 (CI 1.8–2.9) ml at the start of occlusion to 2.8 (CI 2.1–3.4) ml at the end of occlusion in the pREBOA$_{ETCO2}$ group, corresponding to 3.8 (CI 3.1–4.5) ml and 3.9 (CI 2.7–5.1) ml in the pREBOA$_{SBP}$ group.

Reperfusion

There was a statistically significant difference in arterial and mesenteric lactate concentrations between the groups during reperfusion, including at 1 h of reperfusion (primary outcome, Fig. 4). Arterial and mesenteric pH and potassium concentration showed a similar pattern, although only the arterial pH was statistically significant between the groups (Fig. 3). Oxygen extraction ratio was higher in pREBOA$_{SBP}$ group during reperfusion (statistically significant at 120 min of reperfusion), in parallel to a slightly lower cardiac output in this group (not statistically significant, Tables 1 and 2).

Diuresis was absent at the end of REBOA in both groups and increased during reperfusion, with the maximal difference seen after 2 h, 55 (CI 26–118) µl kg^{-1} min^{-1} and 24 (CI 6–87) µl kg^{-1} min^{-1} in the pREBOA$_{ETCO2}$ group and pREBOA$_{SBP}$ group, respectively. The plasma creatinine and troponin concentrations were significantly lower in the pREBOA$_{ETCO2}$ group after 1 and 3 h of reperfusion (Table 2). The plasma concentrations of CK and ASAT followed a similar pattern, although not statistically significant (Table 2).

The severity of ischemic injury in the small intestinal bowel segments differed between the subjects, ranging from no detectable damage to intact villi without epithelium and with crypt destruction. The dominating score was comparable between the groups with a median (25th–75th percentiles) of 3 (1–3.75) in the pREBOA$_{ETCO2}$ group and 3.5 (1–4) in the pREBOA$_{SBP}$ group.

Discussion

This study compared the hemodynamic, metabolic and end-organ consequences of ETCO$_2$ targeted and proximal SBP targeted pREBOA in a porcine model of hemorrhagic shock. The findings demonstrated that ETCO$_2$ targeted pREBOA was as effective as proximal SBP targeted pREBOA in preserving hemodynamic stability but generated overall less metabolic insult; this included the primary outcome, arterial lactate concentrations at 1 h of reperfusion, and end-organ insults. These findings suggest a role of ETCO$_2$ as a potential clinical tool when using pREBOA and may stimulate clinical studies on the subject, as well as the use of other metabolic and organ damage markers.

When using REBOA, achieving a balance between hemorrhagic control and ischemic burden is difficult in practice and there is, as yet, no consensus concerning how to use or titrate partial occlusion. Pressure targeted balloon occlusion has been the main focus in both preclinical and clinical settings; primarily SBP targeted, but also pressure gradient

targeted occlusion [14, 19, 21, 25]. Distal blood pressure targeted occlusion has been studied by reducing the inflation volume from initial total occlusion to reach a pulsatile waveform or a target distal pressure, with a maximum of 10 mmHg being suggested [10, 19, 21, 34]. The pressure alone may, however, not represent the blood flow, since the resistance is unknown and may be dynamic [35]. In translational studies, a distal pressure increase of 10 mmHg has been suggested to correlate to a blood flow of 250–500 ml min^{-1}, depending on shock state [10]. Recent studies have focused on distal blood flow instead of pressure and new balloons have been designed to facilitate titration to a targeted distal blood flow [23, 36–42]. A distal flow of 5–10% of naïve blood flow or 250–500 ml min^{-1} has been advocated sufficient to maintain viability of the viscera. However, the exact level of blood flow required to keep the distal organs perfused remains unclear. The association between distal ischemia and pREBOA has not been extensively studied. Matsumura et al. [43] demonstrated organ perfusion during pREBOA using an advanced computer tomography technique. The majority of these techniques are, however, difficult to use clinically or lack correlation to organ metabolism.

In a previous study by our group [31], aortic blood flow correlated well to $ETCO_2$ and also to overall VO_2 and VCO_2 in a porcine study during normovolemia and during hemorrhage. As a result of hypoperfusion and maximum oxygen extraction, VO_2 becomes oxygen delivery dependent and eventually drops below the critical oxygen delivery level. This is the point of onset of anaerobic metabolism and the rise in lactate levels [27, 44], and, therefore, the reason for choosing the arterial lactate concentration as a primary outcome. In this study, the lactate concentrations were significantly higher in the proximal SBP targeted group compared to the $ETCO_2$ targeted group, indicating more anaerobic metabolism in the proximal SBP targeted group. Consequently, this group tended to be more acidotic, an aspect that affects the maintenance of vital organ function during shock. The targeted $ETCO_2$ level used in this study was based on a prior study by our group in an attempt to maintain overall aerobic metabolism [31]. The targeted SBP level was based on clinically accepted permissive hypotension in trauma situations.

In this study, $ETCO_2$ was preserved at a higher level in the $ETCO_2$ targeted pREBOA group, while the SBP did not differ significantly during occlusion. The distribution of SBP in the $ETCO_2$ targeted pREBOA group was notable and could be explained by the $ETCO_2$ being used as the predetermined regulating factor. However, the SBP remained at a generally higher level in the $ETCO_2$ targeted pREBOA group, even though it required a lower inflation volume. This could be the result of a gradual activation of the endogenous compensatory systems, such as the sympathetic nervous system and the renin–angiotensin–aldosterone system, in response to hypovolemia and occlusion of the distal organs [45]. The subjects responded diversely to hypovolemia; some responded with compensatory increased heart rate during the occlusion rather than during the hemorrhagic period, possibly due to individual responses to the anesthetic drugs, thus interfering with the balloon effect. The full mechanism of the physiological compensatory systems during pREBOA are not completely understood; however, higher angiotensin II levels during pREBOA compared to total REBOA have previously been suggested [46]. Higher systemic angiotensin II levels also concur with greater renal blood flow and glomerular filtration [46]. In clinical settings, pREBOA is currently most commonly performed as a stepwise degradation after initial total REBOA to establish clot stabilization

[21, 47]. It might, however, be physiologically favorable to start with partial occlusion and gradually inflate, if necessary, to progressively stimulate the endogenous endocrine and sympathetic system to achieve less metabolic disturbance at acceptable central blood pressure.

The insignificant difference in aortic blood flow and the statistically significantly higher global O_2ER, but lower mesenteric venous pCO_2 in the $ETCO_2$ targeted group during aortic occlusion, may indicate greater blood flow to the lower body including the intestines. Higher diuresis and significantly lower creatinine levels were also found during reperfusion in this group. This is in accordance with previous studies comparing total REBOA and permissive perfusion [48]. Likewise, the troponin levels were significantly higher in the SBP targeted group, further suggesting greater tissue damage. These results may indicate that pREBOA targeted by proximal SBP alone requires more extensive occlusion than is necessary at the expense of the perfusion of distal organs.

Limitations

This study has some limitations. No a priori statistical power analysis was performed, although an interim analysis was carried out to calculate the number of animals required. Another limitation is related to the implementation being performed on healthy pigs in a single trauma setting without ongoing bleeding. In a clinical setting, $ETCO_2$ may be influenced by underlying pulmonary disease, thoracic trauma or ongoing trauma. When constant ventilation cannot be maintained, $ETCO_2$ is difficult to interpret due to confounding factors. However, it may be possible to calculate an estimate of VCO_2 using minute ventilation and $ETCO_2$ concentration to make the interpretation independent of minute ventilation, thus allowing non-constant ventilation. In addition, this study investigated the short-term but not the long-term effects of pREBOA using the different techniques. Further studies are needed to evaluate the long-term effects on organ failure.

This study does not encourage $ETCO_2$ as a single measurement tool for pREBOA in the emergency room or prehospitally, but as a supplementary tool to other hemodynamic measurements to estimate the metabolic sequelae caused by aortic occlusion. This is, to our knowledge, the first study using a metabolic variable to target pREBOA and further studies are needed to evaluate the clinical value of $ETCO_2$ during pREBOA.

Conclusion

In a porcine model of hemorrhagic shock, $ETCO_2$ targeted pREBOA caused less metabolic disturbance and end-organ damage compared to proximal SBP targeted pREBOA, with no disadvantageous hemodynamic impact. End-tidal CO_2 should be investigated in clinical trials as a complementary clinical tool to mitigate ischemic–reperfusion injury when using pREBOA.

Abbreviations

REBOA	Resuscitative Endovascular Balloon Occlusion of the Aorta
pREBOA	Partial REBOA
$ETCO_2$	End-tidal carbon dioxide
SBP	Systolic blood pressure
$pREBOA_{ETCO2}$	$ETCO_2$ targeted pREBOA
$pREBOA_{SBP}$	Proximal SBP targeted pREBOA
EVTM	Endovascular Resuscitation and Trauma Management

VO_2	Oxygen consumption
DO_2	Oxygen delivery
O_2ER	Oxygen extraction rate
MV	Minute Volume
pCO_2	Partial pressure of CO_2
VCO_2	Carbon dioxide consumption
pO_2	Partial pressure of O_2

Availability of data and materials
The data sets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study is approved by the Regional Animal Ethics Committee (ID 1525-2019, Linköping, Sweden). Informed consent by the owner was retrieved.

Consent for publication
Not applicable.

Competing interests
No conflicts of interest related to the subject are present.

References
1. Stannard A, Eliason JL, Rasmussen TE (2011) Resuscitative endovascular balloon occlusion of the aorta (REBOA) as an adjunct for hemorrhagic shock. J Trauma 71(6):1869–1872. https://doi.org/10.1097/TA.0b013e31823fe90c
2. Hörer TM, Skoog P, Pirouzram A, Nilsson KF, Larzon T (2016) A small case series of aortic balloon occlusion in trauma: lessons learned from its use in ruptured abdominal aortic aneurysms and a brief review. Eur J Trauma Emerg Surg Off Publ Eur Trauma Soc 42(5):585–592. https://doi.org/10.1007/s00068-015-0574-0
3. Hughes CW (1954) Use of an intra-aortic balloon catheter tamponade for controlling intra-abdominal hemorrhage in man. Surgery 36(1):65–68
4. Sadeghi M, Nilsson KF, Larzon T et al (2018) The use of aortic balloon occlusion in traumatic shock: first report from the ABO trauma registry. Eur J Trauma Emerg Surg Off Publ Eur Trauma Soc 44(4):491–501. https://doi.org/10.1007/s00068-017-0813-7
5. Dogan EM, Beskow L, Calais F, Hörer TM, Axelsson B, Nilsson KF (2019) Resuscitative endovascular balloon occlusion of the aorta in experimental cardiopulmonary resuscitation: aortic occlusion level matters. Shock Augusta Ga 52(1):67–74. https://doi.org/10.1097/SHK.0000000000001236
6. Wikström MB, Smårs SM, Karlsson C, Hurtsén AS, Hörer TM, Nilsson KF (2021) A randomized porcine study of the hemodynamic and metabolic effects of combined endovascular occlusion of the vena cava and the aorta in normovolemia and in hemorrhagic shock. J Trauma Acute Care Surg. https://doi.org/10.1097/TA.0000000000003098
7. Sadeghi M, Dogan EM, Karlsson C et al (2020) Total resuscitative endovascular balloon occlusion of the aorta causes inflammatory activation and organ damage within 30 minutes of occlusion in normovolemic pigs. BMC Surg 20(1):43. https://doi.org/10.1186/s12893-020-00700-3
8. Hörer TM, Pirouzram A, Khan M et al (2021) Endovascular resuscitation and trauma management (EVTM)-practical aspects and implementation. Shock Augusta Ga 56(1S):37–41. https://doi.org/10.1097/SHK.0000000000001529
9. Hörer T (2018) Resuscitative endovascular balloon occlusion of the aorta (REBOA) and endovascular resuscitation and trauma management (EVTM): a paradigm shift regarding hemodynamic instability. Eur J Trauma Emerg Surg Off Publ Eur Trauma Soc 44(4):487–489. https://doi.org/10.1007/s00068-018-0983-y

10. Johnson MA, Neff LP, Williams TK, DuBose JJ, EVAC Study Group (2016) Partial resuscitative balloon occlusion of the aorta (P-REBOA): clinical technique and rationale. J Trauma Acute Care Surg 81:S133–S137. https://doi.org/10.1097/TA.0000000000001146

11. McGreevy DT, Abu-Zidan FM, Sadeghi M et al (2020) Feasibility and clinical outcome of reboa in patients with impending traumatic cardiac arrest. Shock Augusta Ga 54(2):218–223. https://doi.org/10.1097/SHK.0000000000001500

12. Long KN, Houston R, Watson JDB et al (2015) Functional outcome after resuscitative endovascular balloon occlusion of the aorta of the proximal and distal thoracic aorta in a swine model of controlled hemorrhage. Ann Vasc Surg 29(1):114–121. https://doi.org/10.1016/j.avsg.2014.10.004

13. Saito N, Matsumoto H, Yagi T et al (2015) Evaluation of the safety and feasibility of resuscitative endovascular balloon occlusion of the aorta. J Trauma Acute Care Surg 78(5):897–903. https://doi.org/10.1097/TA.0000000000000614. (**discussion 904**)

14. Johnson MA, Williams TK, Ferencz SAE et al (2017) The effect of resuscitative endovascular balloon occlusion of the aorta, partial aortic occlusion and aggressive blood transfusion on traumatic brain injury in a swine multiple injuries model. J Trauma Acute Care Surg 83(1):61–70. https://doi.org/10.1097/TA.0000000000001518

15. Morrison JJ, Lendrum RA, Jansen JO (2014) Resuscitative endovascular balloon occlusion of the aorta (REBOA): a bridge to definitive haemorrhage control for trauma patients in Scotland? Surg J R Coll Surg Edinb Irel 12(3):119–120. https://doi.org/10.1016/j.surge.2013.10.004

16. Russo RM, Neff LP, Johnson MA, Williams TK (2016) Emerging endovascular therapies for non-compressible torso hemorrhage. Shock Augusta Ga 46(3 Suppl 1):12–19. https://doi.org/10.1097/SHK.0000000000000641

17. Avaro JP, Mardelle V, Roch A et al (2011) Forty-minute endovascular aortic occlusion increases survival in an experimental model of uncontrolled hemorrhagic shock caused by abdominal trauma. J Trauma 71(3):720–725. https://doi.org/10.1097/TA.0b013e318221a94a. (**discussion 725-726**)

18. Hörer TM, Cajander P, Jans A, Nilsson KF (2016) A case of partial aortic balloon occlusion in an unstable multi-trauma patient. Trauma 18(2):150–154. https://doi.org/10.1177/1460408615624727

19. Russo RM, Neff LP, Lamb CM et al (2016) Partial resuscitative endovascular balloon occlusion of the aorta in swine model of hemorrhagic shock. J Am Coll Surg 223(2):359–368. https://doi.org/10.1016/j.jamcollsurg.2016.04.037

20. Sadeghi M, Hörer TM, Forsman D et al (2018) Blood pressure targeting by partial REBOA is possible in severe hemorrhagic shock in pigs and produces less circulatory, metabolic and inflammatory sequelae than total REBOA. Injury 49(12):2132–2141. https://doi.org/10.1016/j.injury.2018.09.052

21. Russo RM, Williams TK, Grayson JK et al (2016) Extending the golden hour: partial resuscitative endovascular balloon occlusion of the aorta in a highly lethal swine liver injury model. J Trauma Acute Care Surg 80(3):372–378. https://doi.org/10.1097/TA.0000000000000940. (**discussion 378-380**)

22. Matsumura Y, Matsumoto J, Kondo H et al (2017) Fewer REBOA complications with smaller devices and partial occlusion: evidence from a multicentre registry in Japan. Emerg Med J EMJ 34(12):793–799. https://doi.org/10.1136/emermed-2016-206383

23. Kauvar DS, Schechtman DW, Thomas SB et al (2019) Effect of partial and complete aortic balloon occlusion on survival and shock in a swine model of uncontrolled splenic hemorrhage with delayed resuscitation. J Trauma Acute Care Surg 87(5):1026–1034. https://doi.org/10.1097/TA.0000000000002439

24. Forte DM, Do WS, Weiss JB et al (2019) Titrate to equilibrate and not exsanguinate! Characterization and validation of a novel partial resuscitative endovascular balloon occlusion of the aorta catheter in normal and hemorrhagic shock conditions. J Trauma Acute Care Surg 87(5):1015–1025. https://doi.org/10.1097/TA.0000000000002378

25. Reva VA, Matsumura Y, Samokhvalov IM et al (2018) Defining degree of aortic occlusion for partial-REBOA: A computed tomography study on large animals. Injury 49(6):1058–1063. https://doi.org/10.1016/j.injury.2018.04.021

26. Cruz RJ, Poli de Figueiredo LF, Bras JLM, Rocha e Silva M (2004) Effects of intra-aortic balloon occlusion on intestinal perfusion, oxygen metabolism and gastric mucosal PCO2 during experimental hemorrhagic shock. Eur Surg Res Eur Chir Forsch Rech Chir Eur. 36(3):172–178. https://doi.org/10.1159/000077260

27. Guinot PG, Guilbart M, Hchikat AH et al (2016) Association between end-tidal carbon dioxide pressure and cardiac output during fluid expansion in operative patients depend on the change of oxygen extraction. Medicine (Baltimore) 95(14):e3287

28. Dunham CM, Chirichella TJ, Gruber BS et al (2013) In emergently ventilated trauma patients, low end-tidal CO2 and low cardiac output are associated and correlate with hemodynamic instability, hemorrhage, abnormal pupils, and death. BMC Anesthesiol 13(1):20. https://doi.org/10.1186/1471-2253-13-20

29. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Accessed May 23, 2019. http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32010L0063&rid=1

30. du Sert NP, Ahluwalia A, Alam S et al (2020) Reporting animal research: explanation and elaboration for the ARRIVE guidelines 2.0. PLOS Biol 18(7):e3000411. https://doi.org/10.1371/journal.pbio.3000411

31. Sadeghi M, Hurtsén AS, Tegenfalk J et al (2021) End-tidal carbon dioxide as an indicator of partial REBOA and distal organ metabolism in normovolemia and hemorrhagic shock in anesthetized pigs. Shock Augusta Ga. https://doi.org/10.1097/SHK.0000000000001807

32. Juel IS, Solligård E, Lyng O et al (2004) Intestinal injury after thoracic aortic cross-clamping in the pig. J Surg Res 117(2):283–295. https://doi.org/10.1016/j.jss.2003.10.025

33. Juel IS, Solligård E, Tvedt KE et al (2008) Post-ischaemic restituted intestinal mucosa is more resistant to further ischaemia than normal mucosa in the pig. Scand J Clin Lab Invest 68(2):106–116. https://doi.org/10.1080/00365510701534833

34. Matsumura Y, Higashi A, Izawa Y et al (2019) Distal pressure monitoring and titration with percent balloon volume: feasible management of partial resuscitative endovascular balloon occlusion of the aorta (P-REBOA). Eur J Trauma Emerg Surg Off Publ Eur Trauma Soc. https://doi.org/10.1007/s00068-019-01257-4

35. Carr MJ, Benham DA, Lee JJ et al (2021) Real-time bedside management and titration of partial resuscitative endovascular balloon occlusion of the aorta without an arterial line: good for pressure, not for flow! J Trauma Acute Care Surg 90(4):615–622. https://doi.org/10.1097/TA.0000000000003059

36. Daniel L, Christopher M, Dominic F et al (2020) Partial resuscitative endovascular balloon occlusion of the aorta via the tri-lobe balloon catheter. J Surg Res 260:20–27. https://doi.org/10.1016/j.jss.2020.11.056

37. Hoareau GL, Beyer CA, Caples CA et al (2020) Automated partial versus complete resuscitative endovascular balloon occlusion of the aorta for the management of hemorrhagic shock in a pig model of polytrauma: a randomized controlled pilot study. Mil Med. https://doi.org/10.1093/milmed/usaa217

38. Necsoiu C, Jordan BS, Choi JH et al (2020) Mitigating ischemia-reperfusion injury using a bilobed partial reboa catheter: controlled lower-body hypotension. Shock Augusta Ga. https://doi.org/10.1097/SHK.0000000000001640

39. White JM, Ronaldi AE, Polcz JE et al (2020) A new pressure-regulated, partial REBOA device achieves targeted distal perfusion. J Surg Res 256:171–179. https://doi.org/10.1016/j.jss.2020.06.042

40. Forte D, Do WS, Weiss JB et al (2020) Validation of a novel partial reboa device in a swine hemorrhagic shock model: fine tuning flow to optimize bleeding control and reperfusion injury. J Trauma Acute Care Surg. https://doi.org/10.1097/TA.0000000000002718

41. Zilberman-Rudenko J, Behrens B, McCully B et al (2020) Use of bilobed partial resuscitative endovascular balloon occlusion of the aorta is logistically superior in prolonged management of a highly lethal aortic injury. J Trauma Acute Care Surg 89(3):464–473. https://doi.org/10.1097/TA.0000000000002797

42. Madurska MJ, Jansen JO, Reva VA, Mirghani M, Morrison JJ (2017) The compatibility of computed tomography scanning and partial REBOA: a large animal pilot study. J Trauma Acute Care Surg 83(3):557–561. https://doi.org/10.1097/TA.0000000000001574

43. Matsumura Y, Higashi A, Izawa Y et al (2020) Organ ischemia during partial resuscitative endovascular balloon occlusion of the aorta: dynamic 4D computed tomography in swine. Sci Rep 10(1):5680. https://doi.org/10.1038/s41598-020-62582-y

44. Dubin A, Murias G, Estenssoro E et al (2000) End-tidal CO2 pressure determinants during hemorrhagic shock. Intensive Care Med 26(11):1619–1623

45. Hong SA, Gelman S, Henderson T (1992) Angiotensin and adrenoceptors in the hemodynamic response to aortic cross-clamping. Arch Surg 127(4):438–441. https://doi.org/10.1001/archsurg.1992.01420040084014

46. Hoareau GL, Williams TK, Davidson AJ et al (2019) Endocrine effects of simulated complete and partial aortic occlusion in a swine model of hemorrhagic shock. Mil Med 184(5–6):e298–e302. https://doi.org/10.1093/milmed/usy287

47. Russo RM, White JM, Baer DG (2021) Partial REBOA: a systematic review of the preclinical and clinical literature. J Surg Res 262:101–114. https://doi.org/10.1016/j.jss.2020.12.054

48. Williams TK, Neff LP, Johnson MA et al (2017) Automated variable aortic control versus complete aortic occlusion in a swine model of hemorrhage. J Trauma Acute Care Surg 82(4):694–703. https://doi.org/10.1097/TA.0000000000001372

Publisher's Note