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# Endocrine Effects of Simulated Complete and Partial Aortic Occlusion in a Swine Model of Hemorrhagic Shock

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**ABSTRACT** Introduction: Low distal aortic flow via partial aortic occlusion (AO) may mitigate ischemia induced by resuscitative endovascular balloon occlusion of the aorta (REBOA). We compared endocrine effects of a novel simulated partial AO strategy, endovascular variable aortic control (EVAC), with simulated REBOA in a swine model. Materials and methods: Aortic flow in 20 swine was routed from the supraceliac aorta through an automated extracorporeal circuit. Following liver injury-induced hemorrhagic shock, animals were randomized to control (unregulated distal flow), simulated REBOA (no flow, complete AO), or simulated EVAC (distal flow of 100–300 mL/min after 20 minutes of complete AO). After 90 minutes, damage control surgery, resuscitation, and full flow restoration ensued. Critical care was continued for 4.5 hours or until death. Results: Serum angiotensin II concentration was higher in the simulated EVAC (4,769 ± 624 pg/mL) than the simulated REBOA group (2649 ± 429) ($p = 0.01$) at 180 minutes. There was no detectable difference in serum renin [simulated REBOA: 231.3 (227.9–261.4) pg/mL; simulated EVAC: 294.1 (231.2–390.7) pg/mL; $p = 0.27$], aldosterone [simulated EVAC: 629 (454–1098), simulated REBOA: 777 (575–1079) pg/mL, $p = 0.53$], or cortisol (simulated EVAC: 141 ± 12, simulated REBOA: 127 ± 9 ng/mL, $p = 0.34$) concentrations between groups. Conclusions: Simulated EVAC was associated with higher serum angiotensin II, which may have contributed to previously reported cardiovascular benefits. Future studies should evaluate the renal effects of EVAC and the concomitant therapeutic use of angiotensin II.

## INTRODUCTION

Traumatic non-compressible torso hemorrhage (NCTH) is associated with significant morbidity and is a leading cause of preventable death on the battlefield.[1,2] Resuscitative endovascular balloon occlusion of the aorta (REBOA) has emerged as an alternative to resuscitative thoracotomy for the treatment of patients with NCTH. REBOA is an endovascular technique that allows occlusion of aortic flow via the insertion of a balloon-tipped catheter at various levels of the aorta. The balloon is then inflated to prevent downstream blood flow and augment perfusion to the heart, lungs, and brain.

The benefits of REBOA are offset by the profound ischemia that occurs distal to the point of occlusion. Research efforts have focused on an automated version of partial REBOA termed endovascular variable aortic control (EVAC) as a novel resuscitative strategy.[3] During EVAC, the balloon volume is precisely regulated to allow a small amount of blood flow to distal vascular beds to minimize ischemia and limit hemorrhage while still sustaining proximal arterial blood pressure.[4,5]

Although prior studies[6] have demonstrated cardiovascular benefits along with reduced isotonic crystalloids requirements following simulated EVAC when compared to simulated complete REBOA, the mechanisms for these effects have not been elucidated. Here we propose to investigate endocrine responses as a possible contributor to simulated EVAC responses by analyzing frozen serum samples from a previous experiment.[6] First, vascular tone and microcirculation are influenced by the renin, angiotensin, aldosterone system (RAAS).[7] Angiotensin II exerts direct arterial and venous vasopressor effects,[8–10] participates in the control of inflammation,[11] renal hemodynamics,[12,13] and mitochondrial function.[8] For example, in patients undergoing lung, cardiovascular, or orthopedic surgery RAAS inhibition is associated with hypotension and a higher incidence of acute

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kidney injury (AKI).[14–16] Second, REBOA may lead to depressed corticosteroid production and reduced biological effects. Cortisol has pleiotropic effects on the cardiovascular system and on the inflammatory cascade. Absolute or relative corticosteroid deficiency is associated with vasodilatory shock in septic patients (critical illness-related corticosteroid insufficiency or CIRCI). In a cohort of septic shock patients, survivors treated with corticosteroids showed a reduction in cardiovascular SOFA (sequential organ failure assessment) score, underlying the importance of serum cortisol levels on vascular function.[17] Although less frequently described, CIRCI has also been reported in trauma patients.[18] Endocrine responses to aortic occlusion (AO) achieved via REBOA or EVAC following traumatic exsanguination are unknown.

Considering our previous observations of the beneficial cardiovascular effects of simulated EVAC, we hypothesized that simulated EVAC would be associated with RAAS activation and higher serum cortisol concentrations when compared to simulated REBOA.

## METHODS

This study was approved by the Institutional Animal Care and Use Committee at David Grant USAF Medical Center, Travis Air Force Base, CA. All animal care and use was in compliance with the Guide for the Care and Use of Laboratory Animals in a facility accredited by AAALAC. Serum samples utilized for this report were acquired during a previously published experiment.[6]

### Animal Preparation

The experiment was performed as previously described.[6] Twenty Yorkshire-cross pigs were acclimated for at least 10 days. After an 8–12 hour fast with free access to water, they were anesthetized with an intramuscular injection of 6.6 mg/kg tiletamine/zolazepam followed by isoflurane mask induction. After endotracheal intubation, animals were maintained under anesthesia with isoflurane mixed in 100% oxygen. Mechanical ventilation was regulated to maintain end-tidal $CO_2$ between 35 and 45 mmHg. Body temperature was maintained between 35–37°C using warmers. Intravenous 0.9% saline (5 mL/kg/hour) and norepinephrine were administered to establish a baseline mean arterial pressure (MAP) between 65–75 mmHg. A splenectomy was performed via a ventral midline laparotomy. Aortic cannulas were inserted into the supraceliac aorta and blood was diverted through a previously described extracorporeal circuit capable of precise control of distal aortic blood flow.[6] Complete occlusion of the circuit was used to simulate complete REBOA and partial flow of 150–300 ml/min was used to simulate EVAC.

### Experimental Phase

The liver was transected 2 cm to the left of Cantlie's line. A custom liver tourniquet was applied to prevent blood loss.[19] Hemorrhage was induced by release of the liver tourniquet. Animals were then randomized to either simulated REBOA or EVAC ($N = 10$ in each group). After 90 seconds of hemorrhage, complete AO was performed for 20 minutes. Starting at T20, simulated EVAC animals had circuit flow reintroduced at 150 mL/min. If proximal blood pressure was maintained ≥70 mmHg at T40, flow was then increased to 300 mL/min. Animals in the simulated REBOA group had complete AO during the entire intervention period. Two 500 mL boluses of hetastarch were given at T40 and T70 if the proximal MAP fell below 70 mmHg.

At T90, the liver tourniquet was re-applied for hemorrhage control. Blood loss was quantified and animals received matching intravenous volumes of heterologous blood over 10 minutes. At T100, blood flow was reinstituted to full native flow. At T100, computer-controlled hemodynamic analysis was used to guide fluid administration in all groups, with 500 mL boluses of 0.9% saline solution administered after 2 minutes of sustained hypotension (MAP < 60 mmHg). All animals were euthanized at T360.

### Data Collection

Renin (R&D Systems, Minneapolis, MN, USA), angiotensin II (Enzo Life Sciences, Farmingdale, NY, USA), aldosterone (Enzo Life Sciences, Farmingdale, NY, USA), and cortisol (R&D Systems, Minneapolis, MN, USA) concentrations were measured at T0 and T180 using porcine enzyme-linked immunoserologic assay (ELISA) kits.

### Statistical Analysis

Data analysis was performed with STATA version 15.0. The resource equation method was used to estimate sample size. A post-hoc power analysis based on angiotensin II results showed that the sample size of eight animals per group had a 74% power to detect an effect size of 1.40 assuming a 5% significance level and a two-sided test. Continuous data were assessed for normality and results are reported as mean ± standard error of the mean or median (interquartile range) for normally or non-normally distributed data, respectively. Between groups differences were analyzed with the Student *t*-test or Mann–Whitney rank sum test, as appropriate. Within groups comparisons were performed with paired *t*-test or Wilcoxon signed rank test, as appropriate. Statistical significance was set at 0.05.

## RESULTS

There was no detectable difference in serum renin concentrations at T0 [simulated REBOA: 207.2 (176.3−436.8) pg/mL; $N = 5$; simulated EVAC: 345 (215.7−454.7) pg/mL; $N = 6$; $p = 0.36$] or T180 [simulated REBOA: 231.3 (227.9−261.4) pg/mL; $N = 5$; simulated EVAC: 294.1 (231.2−390.7) pg/mL; $N = 6$; $p = 0.27$]. While there was no significant difference between the two groups at baseline (3,272 ± 487 and 3,590 ± 727 pg/mL in the simulated EVAC and simulated REBOA groups, respectively) ($p = 0.72$), angiotensin II

concentration was significantly higher in the simulated EVAC group (4,769 ± 624 pg/mL; $N = 8$) when compared to the simulated REBOA group (2,649 ± 429 pg/mL; $N = 8$) ($p = 0.01$) at T180 (Fig. 1). There was no difference in aldosterone [T0: simulated EVAC: 624 (400–1064), simulated REBOA: 676 (413–745) pg/mL, $p = 0.67$; T180: simulated EVAC: 629 (454–1,098), simulated REBOA: 777 (575–1,079) pg/mL, $p = 0.53$; $N = 8$ in each group] or cortisol (T0: simulated EVAC: 130 ± 7, simulated REBOA: 128 ± 7 ng/mL, $p = 0.88$; T180: simulated EVAC: 141 ± 12, simulated REBOA: 127 ± 9 ng/mL, $p = 0.34$; $N = 8$ in each groups) concentrations between groups.

## DISCUSSION

We sought to characterize endocrine responses following simulated aortic occlusion in a porcine model of NCTH. We demonstrated an increased serum angiotensin II concentration and no difference in serum renin, aldosterone, or cortisol in simulated EVAC when compared to simulated REBOA.

Angiotensin II has pleiotropic effects, including control of arterial and venous vasomotor tone. Previous studies have shown that renin activity is increased following aortic cross-clamping, with angiotensin II production potentially compromising renal hemodynamics.[20,21] Conversely, ovine septic shock studies have shown that angiotensin II administration has beneficial effects, with an improvement in creatinine clearance and mitochondrial function despite a decrease in renal blood flow.[12,13] In trauma patients, while serum vasopressin and epinephrine increased, there was no difference in serum angiotensin when compared to non-injured control.[22] Angiotensin II deficiency may therefore contribute to trauma-associated vasodilatory shock. Our findings suggest that the RAAS activation following complete and partial REBOA is complex. We have previously demonstrated that complete occlusion is followed by sustained decreased renal blood flow when compared to permissive distal perfusion[6]; here we establish that this coincides with a lower concentration of angiotensin II. The increase in renal blood flow in the face of increased angiotensin II may be related to vasodilation in the distal vasculature following aortic occlusive procedures. While angiotensin II may lead to renal vasoconstriction, systemic vasoconstriction of arterial and venous beds may increase aortic blood flow, leading to increased glomerular filtration rate. Angiotensin II may also have direct effects on renal hemodynamics leading to glomerular filtration rate (GFR) modulation and renal blood flow redistribution within the parenchyma.[23,24] For example, angiotensin II potentiates the tubuloglomerular feedback mechanism whereby a decreased tubular chloride flow induces an increase in GFR.[25] The increase in angiotensin II concentration we established may therefore explain our previously reported higher urine output despite lower isotonic crystalloid requirement in the simulated EVAC group.[6] Our observation of a higher angiotensin II concentration along with reduced renal damage in the EVAC group[6] aligns with previous reports of the beneficial renal effects of the therapeutic use of angiotensin II.[26] Patients with vasodilatory shock refractory to conventional vasopressor therapy treated with angiotensin II had a lower incidence of dependence on renal replacement therapy 1 week after the beginning of the study when compared to those who received placebo.[26] The therapeutic effects of angiotensin II in NCTH and REBOA remain to be elucidated.

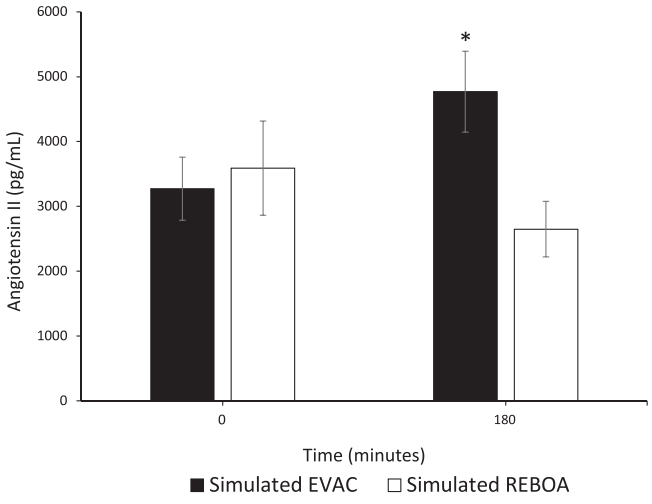


**FIGURE 1.** Mean (± standard error of the mean) angiotensin II concentration between the simulated REBOA and EVAC groups. *$p = 0.01$.

There was no difference in serum renin concentration between groups or over time. The angiotensin concentration might have been increased because only a small change in renin activity might be required to convert angiotensinogen to angiotensin. Angiotensin may also have been released via renin-independent mechanisms[27] or from local production of renin.[28] Since tissue production of angiotensin independent of systemic renin release has also been discussed,[29,30] local quantification of tissue angiotensin concentration might be of interest in future investigations. Finally, the lack of difference in renin concentration might have been the result of blood shunting.

The concept of EVAC has emerged to minimize both the ischemic and oxidative stress burden associated with complete REBOA.[3,31] During controlled flow states, the aortic balloon is partially deflated to permit controlled distal aortic flow to maintain distal organ viability. This approach favors distal clot formation and stabilization followed by a low flow state that balances ongoing hemorrhage and clot destabilization with both distal perfusion and off-loading of the supraphysiologic blood pressure observed proximal to the occlusion point. We have hypothesized that the renoprotective effects of simulated EVAC are the result of both minimizing renal ischemia with sustained but minimal perfusion as well as beneficial systemic effects. In a previous study, we have demonstrated that even in the face of maintained proximal blood pressure and reduced fluid requirements (without the need for additional colloidal support),[6] animals

within the simulated EVAC group have increased renal blood flow during the critical care phase. While this is most likely a multifactorial observation, EVAC may sustain selective perfusion of visceral organs following severe ischemia–reperfusion injuries.

We did not find a difference in serum cortisol concentration between the two groups. Cortisol has pleiotropic effects on the body that includes regulation of vasomotor tone. Although we originally hypothesized that simulated EVAC would result in a larger increase in cortisol concentration when compared to simulated REBOA, the duration of our experiment might not have been sufficient for changes in cortisol to be manifested. Serum cortisol increased over a 96-hour period in patients with acute traumatic hemorrhagic shock; the most significant rise was observed after 24 hours of hospitalization.[32] Serum cortisol levels significantly decreased over time in patients who did not survive.[32] Lastly, future studies should evaluate the response to adrenocorticotropic hormone (ACTH) stimulation rather than isolated serum cortisol concentration, similar to sepsis and trauma patients.[17,18]

Our study presents several limitations. First, a longer protocol may have allowed detection of differences in serum cortisol, renin, or aldosterone concentrations between groups. Second, this manuscript is a follow-up study to a previously published manuscript and serum samples were therefore not available from all animals, which explains why ELISA assays were performed on less than 10 animals in each group. Finally, this report utilized simulated EVAC and REBOA to achieve a tightly controlled aortic flow. As EVAC matures to a catheter-based intervention, endocrine responses should be re-evaluated.

## CONCLUSION

Simulated EVAC was associated with higher serum angiotensin II, which may be associated with improved systemic and local tissue perfusion. Future studies should focus on activation of the RAAS as well as the hypothalamo-adrenocortical axis. Specifically, comparing EVAC and REBOA in traumatic NCTH along with evaluating the therapeutic use of angiotensin II might benefit battlefield medicine and trauma response.

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## CONFLICTS OF INTEREST

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