Objective 3: Preclinical animal testing of next generation pREBOA-PRO™.

pREBOA-PRO™ is designed to permit precise management of the blood pressure above and/or below the balloon, in order to (1) provide adequate perfusion of the heart and brain; (2) stop lower-body bleeding and (3) permit some perfusion of vital organs such as the kidneys, gut, and liver. This study aims to generate physiologic data on pREBOA (inflated in aortic zone I) following uncontrolled torso hemorrhage, in comparison with full REBOA and positive control (repair the arteriotomy using vascular shunt) with the ultimate goal of maintaining survival while improving outcomes for the distal organs.

METHODS

Figure 1 shows the experiment timeline.

Preparation and instrumentation phase: Briefly, Yorkshire swine (70-90 kg; N=14) were sedated, anesthetized and endotracheally intubated. Laparotomy, splenectomy and cystotomy were then performed. Electrocardiogram (ECG) was recorded using a 5-lead monitoring system (ADinstruments, Inc). The left common carotid artery and left femoral artery were cannulated for blood pressure measurement (5-Fr solid-state pressure catheter; Transonic, Inc). A 7-Fr introducer sheath was placed in the infrarenal aorta for REBOA catheter insertion. pREBOA-PRO™ catheter was advanced for zone 1 aortic deployment. Distal aortic flow rate was measured by a 12-mm flow probe (Transonic, Inc) placed at the aorta distal to REBOA catheter insertion, whereas proximal flow rate was measured at the right common carotid artery using a 4-mm flow probe (Transonic, Inc). Blood samples were collected from the brachial artery at five time points: pre-injury baseline, end of hemorrhage, end of occlusion, during ICU phase and at the end of the protocol, for complete blood count (CBC) analysis and arterial blood gases/electrolytes analysis. Serum and plasma were stored in -80^{o}C for subsequent protein analyses.

Injury phase: After instrumentation and the10-min baseline recording, the animals were subjected to uncontrolled hemorrhage for 10 minutes via arteriotomy at the external iliac artery. Shed blood in the intraperitoneal cavity was removed using a suction pump.

Intervention phase: The animals were randomly assigned into three groups – full REBOA (FR), partial REBOA (PR) and positive control (PC). In the FR group (n=6), the aorta was completely occluded using the pREBOA-PRO™ catheter for 90 minutes. Animals in PR group (n=4) received a 10-min complete occlusion of aorta, followed by 80-min partial occlusion with the target distal mean arterial pressure (MAP) of 30±5 mmHg mmHg. Instead of aortic occlusion, a temporary vascular shunt was used in the PC group (n=4) for hemorrhage control and restoring blood flow at the external iliac artery after 10-min uncontrolled hemorrhage.

Resuscitation phase: Whole blood transfusion and bolus infusion of saline were initiated 10 minutes before deflating the REBOA balloon. Pressor and calcium chloride were provided as needed. A vascular shunt was placed at the external iliac artery for revascularization.

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14. ABSTRACT

pREBOA-PRO™ is designed to permit precise management of the blood pressure above and/or below the balloon, in order to (1) provide adequate perfusion of the heart and brain; (2) stop lower-body bleeding and (3) permit some perfusion of vital organs such as the kidneys, gut, and liver. This study aims to generate physiologic data on pREBOA (inflated in aortic zone I) following uncontrolled torso hemorrhage, in comparison with full REBOA and positive control (repair the arteriotomy using vascular shunt) with the ultimate goal of maintaining survival while improving outcomes for the distal organs.

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Intensive care unit (ICU) phase: Once the resuscitation phase was underway the REBOA balloon was then completely deflated. Target MAP was maintained at 55 mmHg with fluids (saline or lactated Ringer's solution) and pressor. The animals were monitored for 2.5 hours.

All physiological parameters were acquired using PowerLab data acquisition system (ADinstruments, Inc) continuously throughout the protocol. Data was analyzed using LabChart software and averaged over 1 minute every 1 minute. Data is presented as mean± standard error of means (SEM).

Euthanasia and sample collection: At the end of the ICU phase, the animals were humanely euthanized. Tissue samples from the lung, heart, liver, small bowel, kidney and hindlimb muscle were collected for histology analysis.

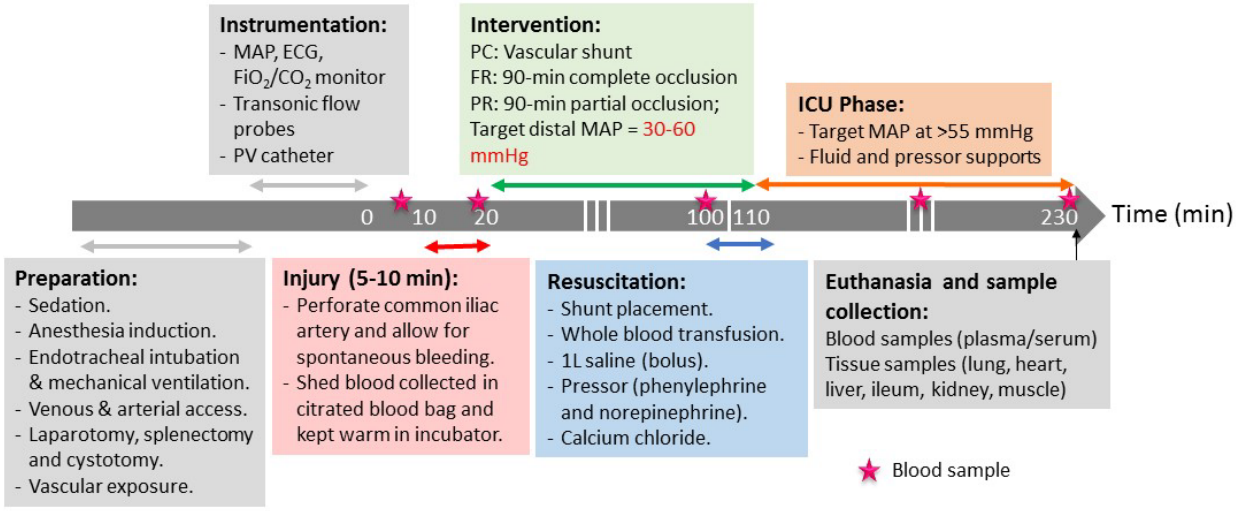

Figure 1. Experiment timeline of *in vivo* testing

RESULTS

1. Consistent target distal MAP was achieved by pREBOA-PRO™ following uncontrolled hemorrhage.

In the PR group, target distal MAP (30±5 mmHg) was achieved by partially deflating the balloon from full occlusion (Figure 1). The distal MAP in the FR group remained at 26.71±0.09 mmHg during full occlusion. Gradual increase in distal MAP was observed in both PR and FR groups during the resuscitation phase (whole blood transfusion, saline infusion, balloon deflation). Distal MAP was maintained at >55 mmHg with fluids and pressor support during the ICU phase (Figure 2, top graph).

Similar to the distal MAP, proximal MAP measured at the common aortic artery reduced during the hemorrhage phase. Complete occlusion led to an abrupt increase in proximal MAP in both REBOA groups (Figure 2, bottom graph). While the balloon was partially deflated in the PR group to allow for permissive distal perfusion, the proximal MAP dropped accordingly from

110.37±3.55 mmHg to 79.06±0.85 mmHg during the 90-min partial occlusion. A slight increase in proximal MAP was observed in the FR during the resuscitation phase, possibly due to the whole blood transfusion and bolus infusion of saline. Such effect may be dampened by the partial perfusion thus it was not detected in the PR group.

In the PC group, placement of vascular shunt prevented further reduction in distal and proximal MAP after uncontrolled hemorrhage, which remained at a level at 57.45±0.41 mmHg during the sham occlusion and ICU phases (Figure 2, top and bottom graphs, closed square).

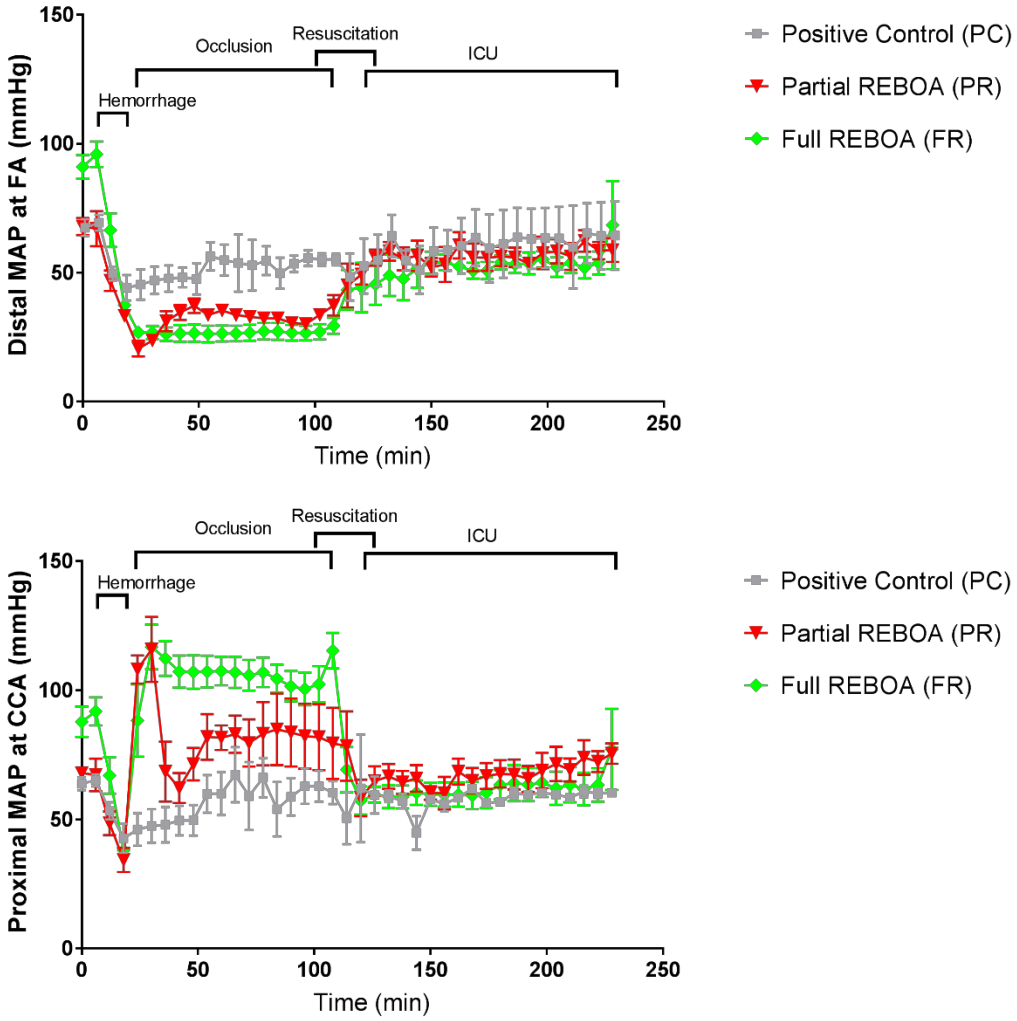

Figure 2. Mean arterial pressure (MAP) measured at the femoral artery (Top graph; FA; distal) and common carotid artery (Bottom graph; CCA; proximal)

2. Steady and precise control of distal aortic flow using pREBOA-PRO™

Figure 3 shows the flow rates measured at the infrarenal aorta (distal) and carotid artery (proximal) over time. During the hemorrhage phase, both distal and proximal aortic flow rates reduced. Distal aortic flow dropped to zero when the balloon was fully inflated. It remained zero

in the FR group whereas in the PR group, the flow rate remained at 243.92±5.03 ml/min (corresponding to the target distal MAP of 30±5 mmHg) allowing for permissive distal perfusion. Balloon deflation (i.e. reperfusion) induced a rapid spike in distal aortic flow rate in the FR group during resuscitation phase. The PR group, on the contrary, exhibited a smooth transition in distal aortic flow from partial occlusion to complete deflation of the balloon (622.23±5.34 ml/min). In the ICU phase, distal aortic flow rate in all groups remained steady (distal MAP was maintained by fluids and pressor support), in which the PR group was higher (617.71±5.89 ml/min) compared to the FR (512.82±11.48 ml/min) and the PC groups (378.45±5.99 ml/min).

Proximal flow rate exhibited a similar trend as the proximal MAP measured at the common carotid artery. It dropped during hemorrhage and then increased at complete aortic occlusion, indicating an increase in proximal perfusion induced by REBOA. In the PR group, the proximal flow rate dropped back to baseline level (537.24±7.25 ml/min) during partial occlusion. Balloon deflation/reperfusion further reduced the proximal flow rate in both REBOA groups. The proximal flow rates were comparable among the three groups during the ICU phase.

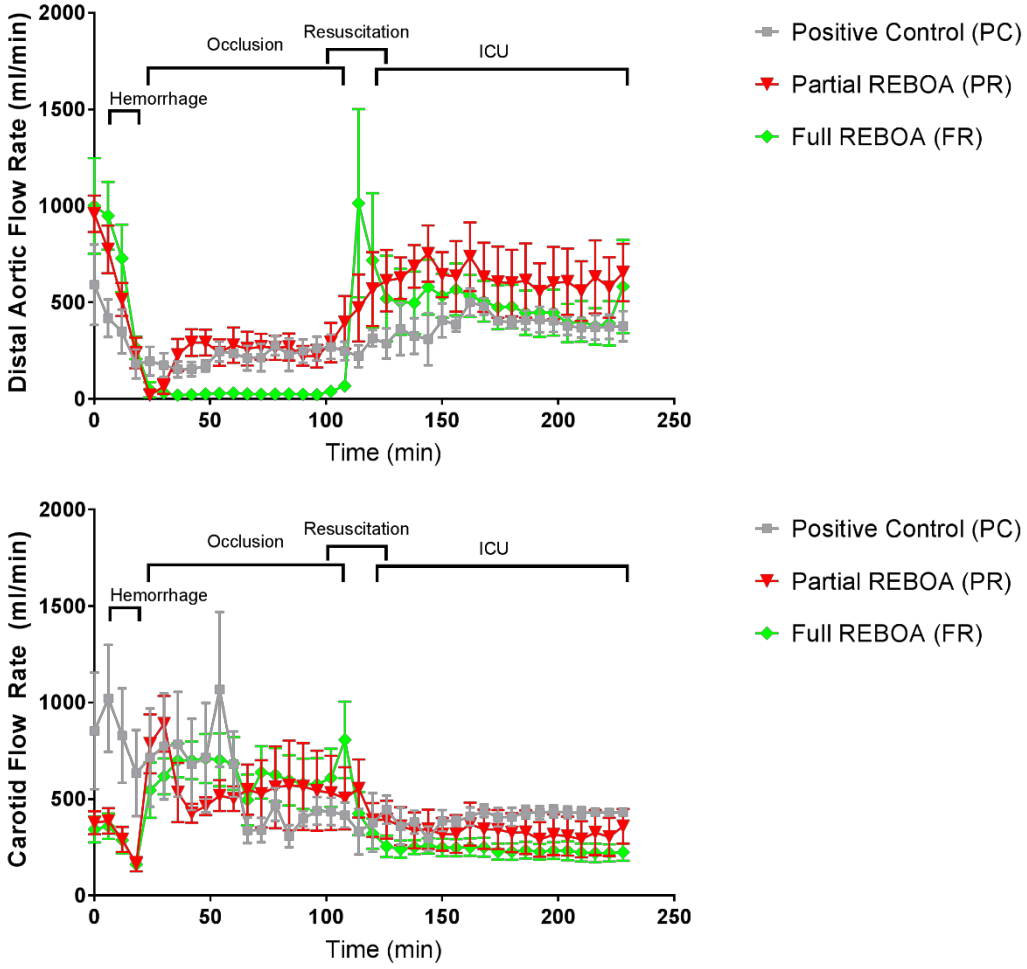

Figure 3. Flow rates measured at the infrarenal aorta (Top graph; distal) and carotid artery (Bottom graph; proximal)

3. Elevated monocyte count after REBOA

Complete blood count (CBC) test revealed an increase in circulatory monocyte percentage in the PR and FR groups at the end of aortic occlusion, which remained elevated at the end of the protocol (Figure 4, lower right graph). Such an increase may be associated with extremity ischemia induced by lack of blood flow. Other parameters including white blood cell (WBC), red blood cell (RBC), neutrophil, lymphocyte and hematocrit (HCT) were not changed dependent on treatment group..

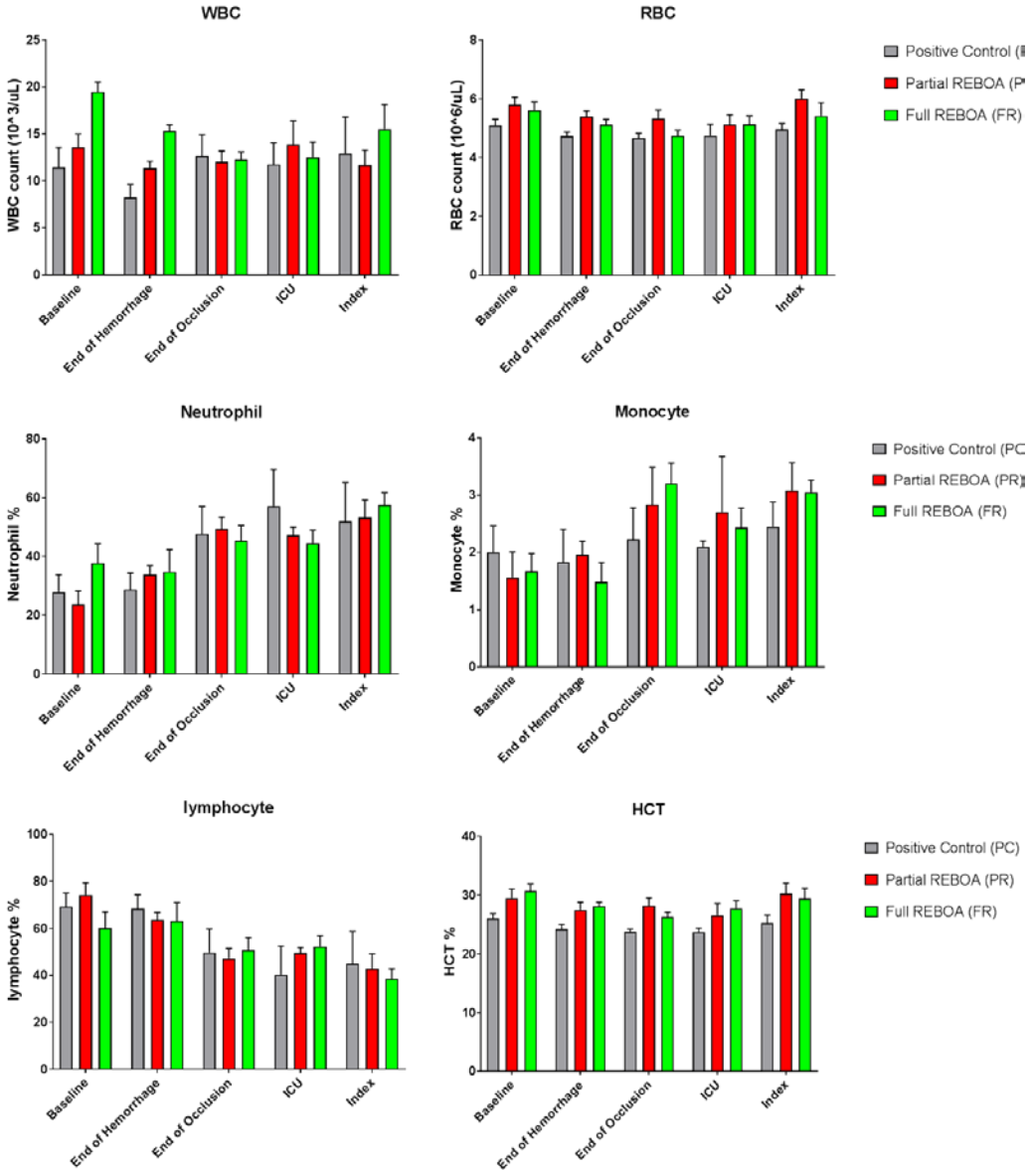

Figure 4. Complete blood count at the specified timepoints. WBC = White blood cell; RBC = Red blood cell; HCT = Hematocrit.

4. Blood pH was lowered following REBOA

As shown in Figure 5 (upper left graph), blood pH level was lowered following aortic occlusion in both REBOA groups (PR = 7.23±0.04; FR = 7.23±0.06) compared to the pre-occlusion level (PR = 7.40±0.05; FR = 7.46±0.04). It remained low until the end of the protocol, suggesting persistent acidosis induced by 90-min REBOA (both complete and partial occlusion). Partial pressure of carbon dioxide (pCO2) slightly increased after aortic occlusion but returned to baseline level at the ICU phase and at the end of the protocol. No specific changes were detected in the pO2 level. Total hemoglobin (tHb) returned to the pre-injury baseline level (baseline PR = 9.78±0.42 g/dL; baseline FR = 10.60±0.25 g/dL) at the ICU phase (PR = 9.46±0.65 g/dL; FR = 10.80±0.00), possibly due to the whole blood transfusion during the resuscitation phase. The tHb level remained high at the end of the protocol.

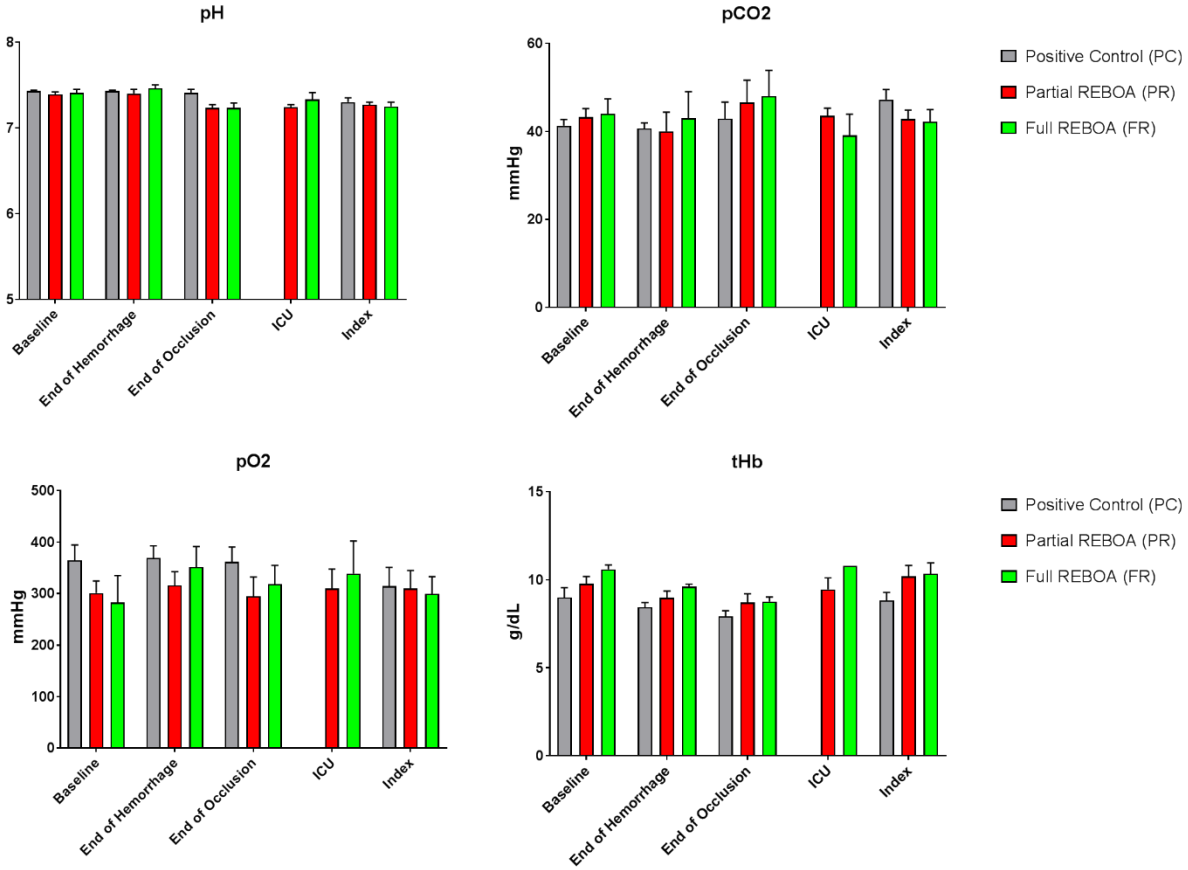

Figure 5. Blood gas analysis at the specified timepoints. pCO2 = partial pressure of carbon dioxide; pO2 = partial pressure of oxygen; tHb = total hemoglobin.

5. REBOA induced changes in potassium, glucose and lactate levels

In both REBOA groups, blood potassium levels were elevated after aortic occlusion (Figure 6, upper left graph), which may result from decreased renal excretion and potassium release from damaged cells. Glucose levels showed a distinctive pattern of change in the PR group (Figure 6, bottom left graph), in which blood glucose was increased at the end of the aortic occlusion and then returned to baseline level. On the other hand, the glucose level exhibited a decreasing trend in the FR group. Figure 6, lower right graph, shows that blood lactate level increased in both FR (6.32±0.90 mmol/L vs baseline level = 2.24±0.29 mmol/L) and PR groups (6.44±0.88 mmol/L vs baseline level = 2.22±0.20 mmol/L) following REBOA. These results indicate occlusion-induced lactate release which explains the acidosis (low pH) as mentioned in the previous section (Section 4, Figure 5) . The lactate level remained high at the end of the protocol. No changes were detected in the sodium, calcium and chloride levels following hemorrhage or REBOA.

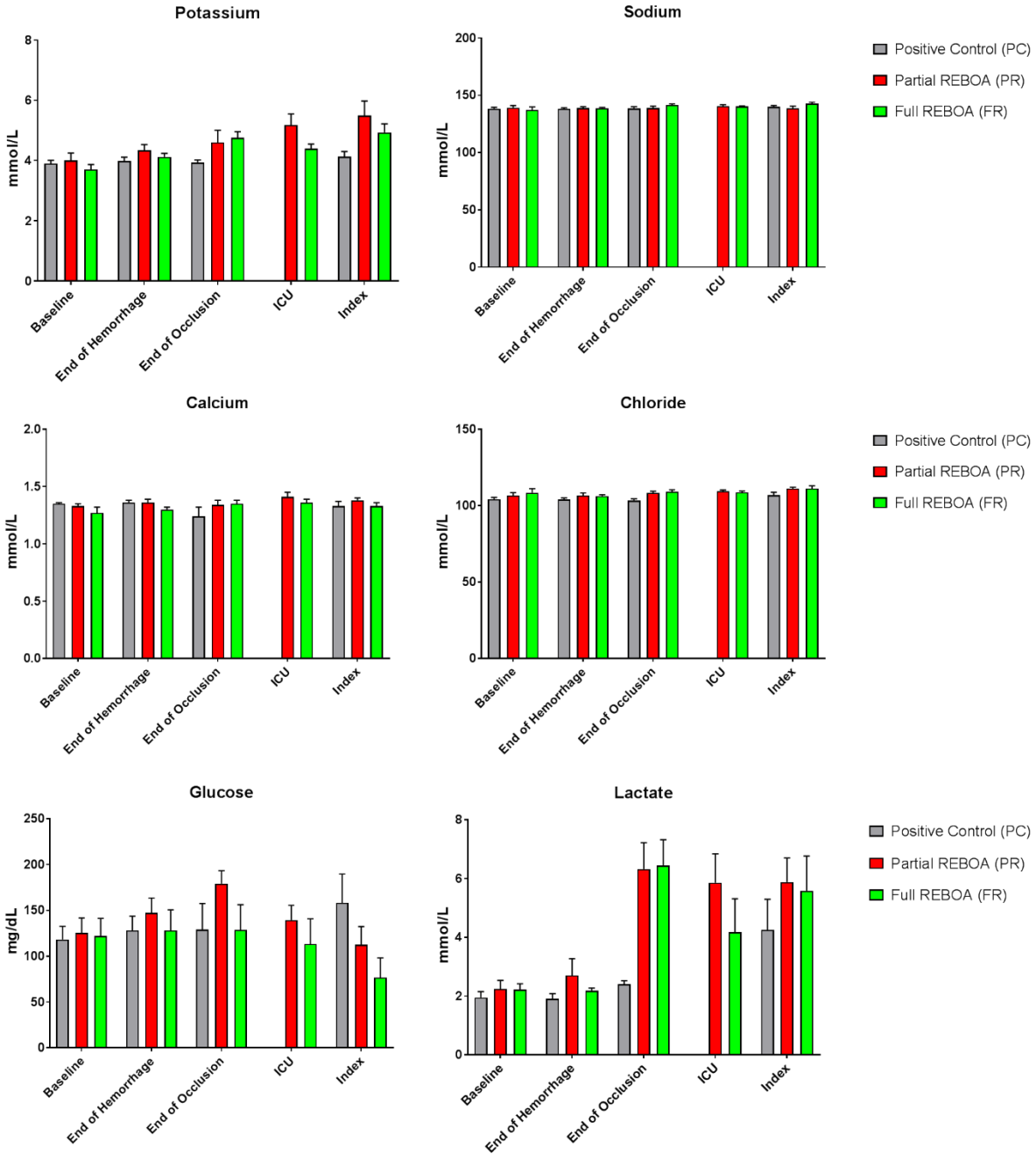

Figure 6. Electrolyte analyses at the specified timepoints.

SUMMARY

This study characterized the physiological changes following partial and complete occlusion using the pREBOA-PRO™ catheter, with the positive control group that received vascular shunt without REBOA intervention. Our data suggested that pREBOA-PRO™ was able to control the distal MAP precisely by regulating the balloon inflation/deflation. Critically, it provides a steady distal aortic perfusion, allowing for prolonged partial aortic occlusion and balancing between

hemorrhage control and distal ischemia. However, some acute complications associated with REBOA such as lactic acidosis, hyperkalemia (high potassium level) were still evident in animals subjected to partial or full REBOA. Histology analyses for ischemic tissue damages are underway.