Published in final edited form as:

J Trauma Acute Care Surg. 2019 July ; 87(1): S101–S109. doi:10.1097/TA.0000000000002311.

Indices of Muscle and Liver Dysfunction after Surviving Hemorrhage and Prolonged Hypotension

Carmen Hinojosa-Laborde, PhD¹, Robert E. Shade, PhD², Patrice A. Frost, DVM², John W. Dutton, DVM², Gary W. Muniz¹, Ian L. Hudson, DO¹, Robert Carter III, PhD³, Kathy L. Ryan, PhD¹

¹US Army Institute of Surgical Research, Fort Sam Houston, Texas

²Texas Biomedical Research Institute, San Antonio, Texas

³Program Executive Office Simulation, Training and Instrumentation, Orlando, Florida

Abstract

Background—This study determined the long-term effects of prolonged hypotension (PH) on liver, muscle, and kidney dysfunction. The hypothesis was that longer duration of PH after hemorrhage will result in greater organ dysfunction.

Methods—Baboons were sedated and hemorrhaged (30% blood volume). Systolic blood pressure > 80 mmHg was maintained for 1 hour (1hr-PH; n=5), 2 hours (2hr-PH; n=5), or 3 hours (3hr-PH; n=5). After PH, hemorrhage volume was replaced. Animals were recovered and monitored for 21 days. Control animals were hemorrhaged and immediately resuscitated (0hr-PH, n=3). Data are Mean ± Standard Deviation, and analyzed by 2-way repeated measures ANOVA and Holm-Sidak test.

Results—Hemorrhage resulted in mild hypotension. Minimal resuscitation was required during the hypotensive phase, and survival rate was 100%. Significant increases ($P < 0.001$) in Alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine phosphokinase (CPK), and lactate dehydrogenase (LDH) occurred on Day 1 after PH, and were significantly greater ($P < 0.001$) in the 2hr and 3hr PH groups than the 0hr PH group. Maximum ALT levels (U/L) were 140 ± 56 (0hr-PH), 170 ± 130 (1hr-PH), 322 ± 241 (2hr-PH), and 387 ± 167 (3hr-PH). Maximum AST levels (U/L) were 218 ± 44 (0hr-PH), 354 ± 219 (1hr-PH), 515 ± 424 (2hr-PH), and 711 ± 278 (3hr-PH). Maximum CPK values (U/L) were 7834 ± 3681 (0hr-PH), 24336 ± 22268 (1hr-PH), 50494 ± 67653 (2hr-PH), and 59857 ± 32408 (3hr-PH). Maximum LDH values (U/L) were 890 ± 396 (0hr-PH), 2055 ± 1520 (1hr-PH), 3992 ± 4895 (2hr-PH), and 4771 ± 1884 (3hr-PH). Plasma creatinine and blood urea nitrogen were unaffected by PH ($P > 0.10$).

Corresponding Author: Carmen Hinojosa-Laborde, Ph.D., Research Physiologist, DB-III, US Army Institute of Surgical Research, 3698 Chambers Pass, JBSA Fort Sam Houston, TX 78234, Phone: (210) 539-7209, FAX: (210) 539-2513, carmen.hinojosa-laborde.civ@mail.mil.

Author Contributions: All authors contributed to study implementation and writing of the article. RC and CH-L conceived and designed the study. RES, PAF, JWD, GWM, and CH-L performed experiments, collected and analyzed data. KLR, ILH, RES, PAF, JWD, and CH-L participated in the interpretation and preparation of the article and figures.

Conflict of Interest: The authors declare no conflict of interest.

Disclaimer: The opinions or assertions contained herein are the private views of the authors, and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

Conclusions—These results indicate that PH up to 3 hours in duration results in transient liver and muscle dysfunction that was most severe after 2 and 3hr PH. PH produced minimal effects on the kidney.

Level of Evidence—Level of evidence not required for basic science research

Study Type—Basic science research

Keywords

prolonged field care; hemorrhage; hypotension; resuscitation; organ dysfunction

Background

The Tactical Combat Casualty Care guidelines for prehospital care management of hemorrhage¹ are based on the concept of permissive hypotensive resuscitation which focuses on preventing hemorrhagic shock by maintaining a hypotensive level of blood pressure, but one sufficient to adequately perfuse vital organs. While the need for resuscitation during hemorrhage was described as early as World War I², the concept of hypotensive resuscitation was developed from clinical observations in military and civilian casualties^{3–6} which suggested that lower blood pressure during resuscitation improved survivability. The strategy for hypotensive resuscitation has been established based on findings from animal studies^{7–11} that provide evidence for optimal resuscitation fluids, and optimal blood pressure to attain during fluid resuscitation without provoking further bleeding. The success of hypotensive resuscitation has been confirmed in prospective clinical studies where decreased morbidity and mortality was demonstrated in patients receiving hypotensive resuscitation during surgery^{12–14}.

According to current guidelines, the casualty should be resuscitated with fresh whole blood or blood components until a palpable radial pulse or systolic pressure of 80–90 mm Hg is attained¹. Hypotensive resuscitation, as part of prehospital care, is maintained until the casualty is transported to a surgical unit which has usually been within the “Golden Hour” of prehospital care¹⁵. However, future military conflicts may occur in environments in which medical evacuations within an hour of injury are not possible^{16, 17}. In these circumstances, the prehospital treatment of casualties may extend significantly beyond one hour, and traditional role 1 providers will be required to deliver prolonged field care for casualties. During prolonged field care, hypotensive resuscitation in hemorrhaging patients may be required for longer periods of time.

While hypotensive resuscitation has been associated with improvements in survival¹⁸, the long-term effects on organ injury after recovery from hemorrhage and hypotension are unknown. The purpose of this study was to determine the long-term effects of hemorrhage followed by hypotension held for 0, 1, 2 and 3 hours on standard blood clinical chemistries up to 21 days after hemorrhage and hypotension. This study tested the hypothesis that longer duration of hypotension after hemorrhage will result in greater organ dysfunction.

Methods

Animals

The protocol was approved by the Institutional Animal Care and Use Committee of the Texas Biomedical Research Institute, San Antonio, TX. Research was conducted in compliance with the Animal Welfare Act, the implementing Animal Welfare Regulations, and the principles of *The Guide for the Care and Use of Laboratory Animals*, National Research Council. The facility where this research was conducted is fully accredited by AAALAC International.

Adult male baboons (9 ± 3 years old, 30 ± 5 kg body weight) were housed in individual cages in a room with other individually housed baboons beginning 2 weeks prior to experiments and ending one week after study termination. The baboons had access to water ad libitum, and were fed twice a day (5LE0, LabDiet, PMI, St. Louis, MO). Experiments were started when the baboons were accustomed to housing conditions.

Surgical Procedures

On the day of the experiment, the baboons were sedated with ketamine (8–10 mg/kg, im). Sedation was maintained during the experiment with continuous rate infusions of ketamine (10–15 mg/kg/h, iv) and midazolam (0.2 – 0.4 mg/kg/h, iv) via a cephalic vein catheter. The animals were intubated to maintain an open airway, but allowed to breathe spontaneously during the experiments. The animals breathed medical grade air supplemented with 40% oxygen.

Catheter introducer sheaths (5 Fr) were placed percutaneously in the femoral artery and vein for blood withdrawal and return, respectively. Arterial pressure and pulse pressure were measured via a Millar solid state catheter inserted via the catheter sheath in the femoral artery at the level of the thoracic aorta. ECG leads were attached to monitor heart rate.

Hemorrhage

After the placement of vascular catheters and ECG leads, systolic arterial pressure (SAP), pulse pressure, and heart rate (HR) were recorded and monitored throughout the experiment. Baseline values were recorded for 30 minutes prior to the procedure. The hemorrhage phase of the experiment consisted of removing blood from the arterial catheter until 30% of total blood volume was removed, or until a SAP of 70 mm Hg was attained. The maximum allowed blood volume loss was calculated for each animal based on an estimated total blood volume of 71 ml/kg in baboons¹⁹. The first half of the hemorrhage blood volume was removed at a fast rate (30 ml/min), and the second half of the blood volume was removed at a slow rate (15 ml/min) to mimic the bleeding pattern associated with trauma. Hemorrhage was completed during a period of 30–45 minutes, and the animal was held in this condition for an additional 10 minutes. Hemorrhaged blood volume was collected into sterile blood donation bags containing citrate phosphate dextrose. Shed blood were held at room temperature and rocked continuously before transfusion. During the transfusion process, blood was filtered and warmed prior to infusion into the femoral vein.

Hypotension

SAP was monitored after hemorrhage to ensure that cardiovascular compensatory mechanisms restored SAP to a level that was greater than 80 mmHg. If SAP was less than 80 mmHg, then a portion of the hemorrhaged blood volume was returned to the animal to restore SAP to a minimum of 80 mmHg. Animals were grouped according to the duration of prolonged hypotension (PH) which was 1 (1hr PH, n=5), 2 (2hr PH, n=5) or 3 (3hr PH, n=5) hours. Control animals were hemorrhaged, but not exposed to a hypotension period after hemorrhage (0hr PH, n=3). Removed blood was immediately transfused.

Transfusion and Recovery

The hemorrhaged blood volume was returned to the animal via the femoral vein catheter (30 ml/min). Autologous blood was passed through a transfusion filter (20 microns) to remove micro clots in the blood prior to infusion into the femoral vein catheter. The animals were monitored for an additional 20 minutes after the transfusion. At the completion of the experiment, animals were given 0.9% saline (500mL IV at approximately 20mL/kg/hr, and 1000mL SQ on the dorsum via a drip set). The catheters were removed and pressure was held at the catheter insertion sites until no blood appeared at the wound site. The animals were returned to their home cage, and monitored for 3 weeks after the procedure to assess the long-term effects of hemorrhage and PH. All animals survived and were returned to their outdoor group housing enclosures after the study.

Blood samples

On the day of the hemorrhage and hypotension procedure, blood samples (8 ml/sample) were collected from the arterial catheter during baseline, at the end of hemorrhage, every 30 minutes during hypotension, and during recovery. During the 3 weeks after the experiment, blood samples were collected percutaneously from the femoral vein on days 1, 3, 7, 14 and 21 after the experiment while the animal was sedated with ketamine (8–10 mg/kg, im). Blood samples were analyzed for standard blood chemistry, complete blood counts, and coagulation profiles by the Clinical Pathology Laboratory at the Texas Biomedical Research Institute. Species and sex-specific normal ranges for blood components have been established for non-human primates by the Texas Biomedical Research Institute.

Data Analysis

Data were analyzed with 2 way repeated measures ANOVA from baseline to 21 days after hemorrhage and hypotension to identify significant ($P<0.05$) group or time effects. Multiple comparisons versus the control groups were conducted using the Holm-Sidak method. Data are expressed as Mean $\pm$ Standard Deviation.

Results

Hemorrhage and Prolonged Hypotension

The average blood volume removed during hemorrhage was 612 ± 102 ml of blood. Figure 1 displays the SAP (1A), pulse pressure (1B), and HR (1C) values recorded during the experiment in the four groups of animals. Baseline mean values of SAP (128 ± 6 , 147 ± 20 ,

130±14, 143±17 mmHg, $P=0.60$), pulse pressure 51±6, 61±7, 50±5, 59±5 mmHg, $P=0.11$), and HR (89±7, 81±7, 87±15, 78±10 beats/min, $P=0.43$) in 0hr, 1hr, 2hr, and 3hr PH groups were not different between groups. During the hemorrhage phase SAP (Figure 1A) decreased significantly in all groups ($P<0.001$). SAP at the end of hemorrhage was not statistically different between groups ($P=0.12$), but was lower in the 0hr PH (69 ± 18 mmHg) and 2hr PH (66 ± 13 mmHg) groups than the 1hr PH (93 ± 32) and 3hr PH (97 ± 25 mmHg) groups. At the end of each hypotension phase, SAP was similar in the 1hr, 2hr, and 3hr PH groups, 107 ± 18, 108 ± 12, and 101 ± 27 mmHg, respectively ($P=0.85$).

Pulse pressure (Figure 1B) at baseline was similar in all four groups and decreased to similar levels during hemorrhage ($P=0.28$). At the end of the hypotension phases, the 1hr, 2hr, and 3hr PH groups attained similar ($P=0.96$) pulse pressures of 38 ± 7, 37 ± 4, and 37 ± 10 mmHg, respectively. Similarly, HR (Figure 1C) was not different between groups throughout the experiment ($P=0.75$). HR increased significantly ($P<0.001$) from baseline in response to hemorrhage and remained elevated during the hypotension phase.

The changes in blood indices of metabolic stress on the day of the hemorrhage and hypotension are shown in Figure 2. pH was below the normal range, while bicarbonate (HCO_3) was above the normal range in all groups throughout the experiment (Figure 2A and 2B). pH and HCO_3 did not change during the experiment ($P>0.05$). Lactate (Figure 2C) increased significantly from baseline during hemorrhage and hypotension in the 1hr, 2hr and 3hr PH groups, and remained elevated at the end of the experiment ($P<0.05$). Lactate levels in all groups were within the normal range throughout the experiment.

PH Duration Effects on Blood Cell Counts and Coagulation Enzymes

The long-term effects of hemorrhage and hypotension were monitored via blood samples obtained at baseline, and 1, 3, 7, 14, and 21 days after the experiment. The effects on blood cell counts (hematocrit, hemoglobin, white blood cell (WBC), red blood cell (RBC), and platelets counts), and coagulation properties (fibrinogen, prothrombin time (PT) and partial thromboplastin time (PTT)) are shown in Table 1. There were no differences ($P>0.20$) in responses in the 1hr, 2hr, or 3hr PH groups when compared to the control, 0hr PH group. However, hemorrhage and hypotension had significant effects on these parameters. Specifically, decreases in hematocrit, hemoglobin, and RBC were observed in all groups on Day 1 and Day 3 after hemorrhage and PH ($P<0.001$). WBC increased on Day 1 in the 1hr, 2hr, and 3hr PH groups ($P<0.001$). Platelets increased in all groups on Day 7 and 14 ($P<0.001$). Fibrinogen levels were elevated in all groups on Day 1 and Day 3 after hemorrhage and PH which corresponded with an increase in PT on Day 1 ($P<0.001$). Exposure to hemorrhage and PH did not affect PTT as there were no significant differences from baseline in any groups ($P>0.07$).

PH Duration Effects on End Organ Injury

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) blood levels were monitored as indices of liver dysfunction (Figure 3A and 3B). Creatine phosphokinase (CPK) and lactic acid dehydrogenase (LDH) levels in blood were monitored as indices of muscle dysfunction (Figure 4A and 4B). Kidney function was assessed by blood urea

nitrogen (BUN) and blood creatinine (Figure 5A and 5B). These indices were monitored at baseline, immediately after transfusion (Recovery), and 1, 3, 7, 14, and 21 days after the experiment. ALT levels (Figure 3A) were significantly elevated ($P<0.001$) on Day 1 and Day 3 in the animals subjected to hypotension for 1, 2 and 3 hours, and these levels were significantly higher ($P<0.001$) in the 2hr and 3hr PH groups compared with controls (0hr PH). On Day 1 after prolonged hypotension, AST levels (Figure 3B) were increased ($P<0.001$) in the animals subjected to hypotension for 1, 2 and 3 hours which were significantly higher ($P<0.001$) in the 2hr and 3hr PH groups compared to 0hr controls. CPK and LDH (Figure 4A and 4B) also increased significantly ($P<0.001$) in the 2hr and 3hr PH groups compared to baseline and to the 0 hr PH group on Day 1 after prolonged hypotension. The elevations in ALT, AST, CPK, and LDH were transient and returned to baseline values within 7 days after hypotension. In contrast, BUN and creatinine (Figure 5A and 5B) did not change ($P>0.10$) from baseline in any group during the 3 weeks after hypotension, and remained at normal values throughout the study.

Discussion

The results of the study revealed that hemorrhage of 30% blood volume followed by hypotension for 1, 2 and 3 hours was associated with elevations in biomarkers of liver and muscle dysfunction 1 and 3 days after recovery, while no evidence of renal dysfunction was observed. These findings support our hypothesis that longer duration of hypotension after hemorrhage resulted in greater organ dysfunction, but this effect was observed only in muscle and liver, and not in kidney. Previous studies have shown that severe hemorrhage requires resuscitation to support adequate perfusion of vital organs²⁰. Numerous studies have been conducted to determine the acute effects of hemorrhage and resuscitation maneuvers on hemodynamic stability and survivability^{9, 11, 21, 22}. These studies were critical in determining the military guidelines for dysfunction control resuscitation which support the use of whole blood or blood products as resuscitation fluids to be given until detection of a radial pulse or a hypotensive systolic blood pressure of 80–90 mmHg are attained¹. However, no studies have investigated the long-term consequences of hemorrhage and hypotension after surviving such an insult. The current study is the first to address these consequences. While swine are commonly used as the animal model for hemorrhage and resuscitation studies, this study was conducted in baboons because a critical component of the study was 100% survival of the animals. Nonhuman primates provide a better model than swine when survival and recovery from hemorrhage procedures are required because swine have a robust splenic contraction which adds a variable not seen in nonhuman primates or humans. As a result, swine often must undergo splenectomy prior to hemorrhage studies while nonhuman primates do not require this surgical procedure. Thus, the non-human primates used in this study were not surgically altered, survived the experiment, and were returned to their outdoor enclosures at the end of the study. Nonhuman primates are physiologically, immunologically, and genetically, similar to humans making them ideal animal models for translational research^{23–25}. In addition, previous studies in our group demonstrated the similarity between baboons and humans in their hemodynamic response to hemorrhage¹⁹.

Experimental Condition vs. Prolonged Field Care Situation

In order to study the effects after survival from hemorrhage and hypotension, it was important to create an experimental condition that was 100% survivable. We used a mild controlled hemorrhage of 30% blood volume without resuscitation except when SAP was below 80 mm Hg after hemorrhage. Under these conditions, a small amount of shed blood was returned to the animal to raise SAP to 80 mmHg. Only 5 of the 15 animals in the 1, 2, and 3hr PH groups required this maneuver. The hemodynamic responses to the hemorrhage were variable among the baboon (similar to humans), and resulted in differences in SAP at the end of hemorrhage between the groups (Figure 1A). Additional blood could not be removed to equalize blood pressure between groups because hemorrhage was limited to 30% of total blood volume. Within 30–45 minutes after hemorrhage, the animals auto resuscitated to blood pressures that were higher than the target SAP of 90 mmHg. As the maximum allowed hemorrhage volume was 30% of total blood volume, no additional blood could be removed to lower the blood pressure. After hemorrhage, blood pressure remained 25–35 mmHg below baseline. This relative hypotension after hemorrhage was held for 0, 1, 2 or 3 hours at an average SAP of less than 110 mmHg (Figure 1A).

The level of sedation was also a critical factor in conducting the experiment because it was important to maintain cardiovascular reflex function to compensate for blood loss. Under experimental conditions while the animal was sedated, HR increased during hemorrhage which confirmed functional baroreflexes to compensate for blood loss (Figure 1C). A side effect of sedation was a condition of mild respiratory acidosis as represented by a low blood pH and a compensatory increase in HCO_3^- (Figure 2A and 2B). However, hemorrhage and subsequent duration of hypotension did not affect pH or HCO_3^- levels, and these parameters returned to normal after the animal recovered from sedation (data not shown). Blood lactate was measured during hemorrhage and PH as an indicator of tissue hypoxia. The increases in blood lactate levels were within the normal physiological range, and confirmed that the level of hemorrhage produced only mild tissue hypoxia. This experimental approach resulted in 100% survival of the animals in the study.

Clearly, this experimental protocol does not replicate the environment in a prolonged field care situation as the animals are sedated, receiving oxygen, hemorrhaged, and not exposed to additional traumatic injury. In addition, volume controlled hemorrhage has many confounding factors which are not consistent with uncontrolled hemorrhage²⁶. However, this approach provided a controlled experimental environment in which to investigate the long-term consequences of delaying resuscitation for up to 3 hours after a mild hemorrhage. This study is the first to attempt this approach in understanding the long-term consequences of hemorrhage and hypotension after surviving such an insult.

PH Duration Effects on Blood Cell Counts and Coagulation Enzymes

Blood cell counts and fibrinogen were significantly affected by hemorrhage and hypotension (Table 1), but these effects were mild as the observed levels were all within normal physiological ranges and not different between groups ($P>0.20$). Hemorrhage and hypotension had minimal effects on PT and PTT. Previous studies on the effect of hemorrhage on blood cell counts and coagulation factors have clearly shown that

hemorrhage can result in anemia and coagulopathy^{27, 28}. These studies have focused on the acute response within hours of hemorrhage, but information regarding the long-term (21 days) effects on blood cells and coagulation properties in response to different durations of hypotension after hemorrhage has not been available prior to this study.

PH Duration Effects on End Organ Injury

Previous studies have shown that hemorrhage followed by resuscitation results in oxidative stress, coagulopathy, inflammatory responses, and mitochondrial dysfunction^{21, 29–31} which can lead to acute injury of the liver, muscle, and kidneys^{32–35}. In the current study, we investigated the long-term effect of prolonged hypotension on liver, muscle and kidney dysfunction. Liver dysfunction was assessed based on blood levels of ALT and AST as both enzymes are released into the blood in the presence of liver dysfunction. The results of this study showed that PH caused increases in ALT and AST, and these increases were significantly greater in 2hr PH and 3hr PH groups than in controls (0hr PH). While both ALT and AST are biomarkers for liver dysfunction, high circulating levels of ALT are more specific to liver dysfunction³⁶. Interestingly, the observed effect of PH on ALT levels was more robust than the effect on AST levels. These findings clearly indicate that the duration of PH had a transient impact on liver function such that the longer duration of hypotension was associated with higher ALT and AST levels.

Muscle dysfunction was assessed by blood levels of CPK and LDH, which were significantly elevated in the 1hr, 2hr, and 3hr PH groups on Day 1, and were significantly greater in 2hr PH and 3hr PH groups compared to controls (0hr PH) (Figure 4A, 4B). Interestingly, the circulating levels of CPK and LDH observed in the current study were 30–75 times (for CPK), and 5–13 times (for LDH) greater than the upper limit of normal ranges for these enzymes. These values greatly exceeded the range for diagnosis of severe rhabdomyolysis. Fortunately, these extremely high levels of CPK and LDH were observed only transiently, and returned to normal levels within 7 days of the experiment. Serum levels of CPK arise largely from skeletal muscle, with smaller amounts from cardiac muscle³⁷. Similarly, isoforms of LDH are specific to tissues of origin, and can be produced by multiple organs including skeletal and cardiac muscle. We did not differentiate the isoforms of CPK or LDH in our study, but we speculate that skeletal muscle produced the majority of CPK and LDH in the current study due to skeletal muscle ischemia during the experiment as a result of the hemorrhage³⁸.

Kidney dysfunction was not detected after hemorrhage and hypotension. Blood levels of creatinine and BUN were normal in all groups throughout the study. The animals received fluids while recovering from sedation equivalent to a daily maintenance need, and this extra fluid could have contributed to normal creatinine and BUN. However, it is unlikely that the extra fluid was enough to prevent myoglobinuric renal injury, which typically is seen a few days after muscle dysfunction occurs. Thus, these results indicate that the kidney was protected from the impact of prolonged hypotension up to 3 hours. These findings are consistent with those of Kovlov et al. which showed that hemorrhage and hypotension for 2 hours in rats affected mitochondrial function and produced endoplasmic reticulum stress

marker in the liver, but not the kidney³⁰. Thus, evidence in rats and non-human primates suggests that the kidney is less susceptible to PH than the liver or skeletal muscle.

Study Limitations

While the current study provides valuable information regarding the consequences of surviving a prolonged field care scenario, the experimental design is not without limitations. As mentioned previously, the level of hemorrhage (30% total blood volume) and the ensuing systemic tissue hypoxia, as indicated by blood lactate levels, were mild. In addition, SAP was maintained below 110 mm Hg during the hypotension which is higher than the recommended target SAP of 80–90 mmHg for hypotensive resuscitation. This experimental model was used to ensure survival of the animal, and to attain the goal of our study of monitoring the long-term (21 days) effects of hemorrhage and hypotension. As a result, this study did not replicate the hemodynamic pattern of the classic model of Class III hemorrhagic shock (SAP = 50 mmHg) followed by hypotensive resuscitation to SAP 80–90 mm Hg^{22, 39, 40}. Additionally, the experiment did not include tissue trauma other than that produced by the percutaneous insertion of catheters, and thus results reflect the effects of hemorrhage and PH alone. Despite these shortcomings in the severity of the experimental model, the results of the study showed significant effects on blood cell counts, coagulation, and organ dysfunction in liver and muscle within 1–3 days after PH.

In summary, this study is the first to evaluate the effects of surviving multiple durations of PH after hemorrhage for up to 21 days after recovery. The results provide evidence that prolonged hypotension for up to 3 hours did not result in clinically significant renal insult, rhabdomyolysis or liver injury in a non-human primate model, further supporting the validity of permissive, and even prolonged, hypotension as a resuscitative strategy for those in hemorrhagic shock. However, several factors could exacerbate the responses observed in the current study such as a more severe hemorrhage to produce Class III hemorrhagic shock, the presence of tissue trauma representing battlefield wounds, and longer periods of PH. Therefore, further investigations are needed to assess how these factors could result in permanent organ dysfunction in soldiers who survive prolonged field care.

Acknowledgements

We thank Martha Avila, Manuel Aguilar, Martin Carias, Lateya Smith, and Cathy Perez for their excellent technical assistance. This study was funded by the United States Army, Medical Research and Materiel Command.

References

1. Butler FK, Holcomb JB, Schreiber MA, Kotwal RS, Jenkins DA, Champion HR, Bowling F, Cap AP, Dubose JJ, Dorlac WC, et al. Fluid resuscitation for hemorrhagic shock in tactical combat casualty care. TCCC guideline change 14–01-2 June 2014. *J Spec Oper Med.* 2014;14(1):13–14.
2. Cannon WB, Fraser J, Cowell EM. The preventative treatment of wound shock. *JAMA.* 1918;709:618–21.
3. Holcomb JB, Wade CE, Michalek JE, Chisholm GB, Zarzabal LA, Schreiber MA, Gonzalez EA, Pomper GJ, Perkins JG, Spinella PC, et al. Increased plasma and platelet to red blood cell ratios improves outcome in 466 massively transfused civilian trauma patients. *Ann Surg.* 2008;248:447–58. [PubMed: 18791365]

4. Champion HR. Combat fluid resuscitation: introduction and overview of conferences. *J Trauma*. 2003;54(5 Suppl):S7–12. [PubMed: 12768095]
5. Dula DJ, Wood GC, Reimer AR, Starr M, Leicht M. Use of prehospital fluids in hypotensive blunt trauma patients. *Prehosp Emerg Care*. 2002;6(4):417–20. [PubMed: 12385609]
6. Kaweski SM, Sise MJ, Virgilio RW. The effect of prehospital fluids on survival in trauma patients. *J Trauma Acute Care Surg*. 1990;30(10):1215–18.
7. Rafie AD, Rath PA, Michell MW, Kirschner RA, Deyo DJ, Progh DS, Grady JJ, Kramer GC. Hypotensive resuscitation of multiple hemorrhages using crystalloid and colloids. *Shock*. 2004;22(3):262–69. [PubMed: 15316397]
8. Skarda DE, Mulier KE, George ME, Beilman GJ. Eight hours of hypotensive versus normotensive resuscitation in a porcine model of controlled hemorrhagic shock. *Acad Emerg Med*. 2008;15(9):845–52. [PubMed: 19244635]
9. Cotton BA, Guy JM, Morris JA Jr, Abumrad NN. The cellular, metabolic, and systemic consequences of aggressive fluid resuscitation strategies. *Shock*. 2006;26(2):115–21. [PubMed: 16878017]
10. Hahn RG. Fluid therapy in uncontrolled hemorrhage--what experimental models have taught us. *Acta Anaesthesiol Scand*. 2013;57(1):16–28. [PubMed: 22946747]
11. Sondeen JL, Coppes VG, Holcomb JB. Blood pressure at which rebleeding occurs after resuscitation in swine with aortic injury. *J Trauma*. 2003;54(5 Suppl):S110–7. [PubMed: 12768112]
12. Morrison CA, Carrick MM, Norman MA, Scott BG, Welsh FJ, Tsai P, Liscum KR, Wall MJ Jr, Mattox KL. Hypotensive resuscitation strategy reduces transfusion requirements and severe postoperative coagulopathy in trauma patients with hemorrhagic shock: Preliminary results of a randomized clinical trial. *J Trauma*. 2011;70(3):652–63. [PubMed: 21610356]
13. Bickell WH, Wall MJ, Pepe PE, Martin RR, Ginger VF, Allen MK, Mattox KL. Immediate versus delayed fluid resuscitation for hypotensive patients with penetrating torso injuries. *N Engl J Med*. 1994;331:1105–09. [PubMed: 7935634]
14. Dutton RP, Mackenzie CF, Scalea TM. Hypotensive resuscitation during active Hemorrhage: Impact on in-hospital mortality. *J Trauma Acute Care Surg*. 2002;52:1141–46.
15. Kotwal RS, Howard JT, Orman JA, Tarpey BW, Bailey JA, Champion HR, Mabry RL, Holcomb JB, Gross KR. The effect of a Golden Hour Policy on the morbidity and mortality of combat casualties. *JAMA Surg*. 2016;151(1):15–24. [PubMed: 26422778]
16. Farr WD. The death of the golden hour and the return of the future guerrilla hospital. In: University JSO, editor. MacDill Air Force Base, Florida: The JSOU Press; 2017.
17. Keenan S, Riesberg JC. Prolonged field care: Beyond the “Golden Hour”. *Wilderness Environ Med*. 2017;28:S135–S39. [PubMed: 28601206]
18. Cotton BA, Reddy N, Hatch QM, LeFebvre E, Wade CE, Kozar RA, Gill BS, Albarado R, McNut tMK, Holcomb JB. Damage control resuscitation is associated with a reduction in resuscitation volumes and improvement in survival in 390 damage control laparotomy patients. *Ann Surg*. 2011;254(4):598–605. [PubMed: 21918426]
19. Hinojosa-Laborde C, Shade RE, Muniz GW, Bauer C, Goei KA, Pidcoke HF, Chung KK, Cap AP, Convertino VA. Validation of lower body negative pressure as an experimental model of hemorrhage. *J Appl Physiol*. 2014;116:406–15. [PubMed: 24356525]
20. Shoemaker WC, Peitzman AB, Bellamy R, Bellomo R, Bruttig SP, Capone A, Dubick MA, Kramer GC, McKenzie JE, Pepe PE, et al. Resuscitation from severe hemorrhage. *Crit Care Med*. 1996;24(2 Suppl):S12–S23. [PubMed: 8608703]
21. Doran CM, Doran CA, Woolley T, Carter A, Male K, Midwinter MJ, Mahoney PF, Watts S, Kirkman E. Targeted resuscitation improves coagulation and outcome. *J Trauma*. 2012;72(4):835–43.
22. Kheirabadi BS, Valdez-Delgado KK, Terrazas IB, Miranda N, Dubick MA. Is limited prehospital resuscitation with plasma more beneficial than using a synthetic colloid? An experimental study in rabbits with parenchymal bleeding. *J Trauma Acute Care Surg*. 2015;78(4):752–59. [PubMed: 25807404]

23. Hauser CJ. Preclinical models of traumatic hemorrhagic shock. *Shock*. 2005;Suppl 1(24–32). [PubMed: 16374369]
24. Redl H, Bahrami S. Large animal models: baboons for trauma, shock, and sepsis studies *Shock*. 2005;Suppl 1:88–93.
25. Schaub LJ, Moore HB, Cap AP, Glaser JJ, Moore EE, Sheppard FR. Nonhuman primate model of polytraumatic hemorrhagic shock recapitulates early platelet dysfunction observed following severe injury in humans. *J Trauma Acute Care Surg*. 2017;82(3):461–69.
26. Sondeen JL, Dubick MA, Holcomb JB, Wade CE. Uncontrolled hemorrhage differs from volume- or pressure-matched controlled hemorrhage in swine. *Shock*. 2007;28(4):426–33. [PubMed: 17558347]
27. Holcomb JB, Tilley BC, Baraniuk S, Fox EE, Wade CE, Podbielski JM, del Junco DJ, Brasel KJ, Bulger EM, Callcut RA, et al. Transfusion of plasma, platelets, and red blood cells in a 1:1:1 vs a 1:1:2 ratio and mortality in patients with severe trauma The PROPPR Randomized Clinical Trial. *JAMA*. 2015;313(5):471–82. [PubMed: 25647203]
28. Hess JR, Brohi K, Dutton RP, Hauser CJ, Holcomb JB, Kluger Y, Mackway-Jones K, Parr MJ, Rizoli SB, Yukioka T, et al. The coagulopathy of trauma: a review of mechanisms. *J Trauma*. 2008;65(4):48–54.
29. Mapstone J, Roberts I, Evans P. Fluid resuscitation strategies: A systematic review of animal trials. *J Trauma*. 2003;55(3):571–89. [PubMed: 14501908]
30. Kozlov AV, Duvigneau JC, Hyatt TC, Raju R, Behling T, Hartl RT, Staniek K, Miller I, Gragor W, Redl H, et al. Effect of estrogen on mitochondrial function and intracellular stress markers in rat liver and kidney following trauma-hemorrhagic shock and prolonged hypotension. *Mol Med*. 2010;16(7–8):254–61. [PubMed: 20379612]
31. Cai B, Dong W, Sharpe S, Deitch EA, Ulloa L. Survival and inflammatory responses in experimental models of hemorrhage. *J Surg Res*. 2011;169:257–66. [PubMed: 20189589]
32. Lefkowitz JH, Mendez L. Morphologic features of hepatic injury in cardiac disease and shock. *J Hepatol*. 1986;2:313–27. [PubMed: 3722787]
33. Moran A, Thacker SA, Arikian AA, Mastrangelo MA, Wu Y, Yu B, Tweardy DJ. IL-6-mediated activation of Stat2alpha prevents trauma/hemorrhage shock-induced liver inflammation. *PLOS ONE*. 2011;6(6):e21449. [PubMed: 21738667]
34. Mayeur N, Nimville V, Jaafar A, Allard J, al Saati T, Guilbeau-Frugier C, Fourcade O, Girolami JJ, Schaak S, Ivan T. Morphologic and functional renal impact of acute kidney injury after prolonged hemorrhagic shock in mice. *Crit Care Med*. 2011;39(9):2131–38. [PubMed: 21572325]
35. Anaya-Prado R, Toledo-Pereyra LH, Collins JT, Smejkal R, McClaren J, Crouch LD, Ward PA. Dual blockade of P-selectin and beta-integrin in the liver inflammatory response after uncontrolled hemorrhagic shock. *J Am Coll Surg*. 1998;187(1):22–31. [PubMed: 9660021]
36. Sookoian S, Pirola CJ. Alanine and aspartate aminotransferase and glutamine-cycling pathway: their roles in pathogenesis of metabolic syndrome. *World J Gastroenterol*. 2012;18(29):3775–81. [PubMed: 22876026]
37. Wu AH. Creatine kinase isoforms in ischemic heart disease. *Clin Chem*. 1989;35(1):7–13. [PubMed: 2642764]
38. Jacobs DS, Robinson RA, Clark GM, Tucker JM. Clinical significance of the isomeric pattern of the isoenzymes of serum lactate dehydrogenase. *Annals of Clinical and Laboratory Science*. 1977;7(5):411–20. [PubMed: 900865]
39. Kheirabadi BS, Miranda N, Terrazas IB, Voelker AN, Grimm RC, Dubick MA. Influences of limited resuscitation with plasma or plasma protein solutions on hemostasis and survival of rabbits with noncompressible hemorrhage. *J Trauma Acute Care Surg*. 2016;81(1):42–49. [PubMed: 27120325]
40. Kheirabadi BS, Miranda N, Terrazas IB, Gonzales MD, Grimm RC, Dubick MA. Does small-volume resuscitation with crystalloids or colloids influence hemostasis and survival of rabbits subjected to lethal uncontrolled hemorrhage. *J Trauma Acute Care Surg*. 2017;82(1):156–64.

Figure 1:

Systolic arterial pressure (SAP, A), pulse pressure (PP, B) and heart rate (HR, C) are shown during the hemorrhage and hypotensive experiment. The experimental phases represented are Baseline, Hemorrhage/Hold, Hypotension, Transfusion, and Recovery. Animal groups are no prolonged hypotension (PH) (0hr PH, n=3, black circles), 1hr PH (n=5, green diamonds), 2hr PH (n=5, blue triangles), and 3hr PH (n=5, red squares). Data are expressed as mean $\pm$ standard deviation.

Figure 2:

Blood values of pH (A), bicarbonate (HCO_3^- , B) and lactate (C) are shown during the hemorrhage and hypotensive experiment. Normal ranges are indicated as a yellow band. Blood sample were collected during Baseline, End Hemorrhage, Hypotension, and Recovery. Animal groups are no prolonged hypotension (PH) (0hr PH, n=3, black circles), 1hr PH (n=5, green diamonds), 2hr PH (n=5, blue triangles), and 3hr PH (n=5, red squares). Data are expressed as mean $\pm$ standard deviation. Significant differences from baseline are denoted by + ($P < 0.05$)

Figure 3:

Blood values of alanine aminotransferase (ALT, A), and aspartate aminotransferase (AST, B). Normal ranges are indicated as a yellow band. Blood samples were collected at baseline, immediately after the experiment (Recovery), and 1, 3, 7, 14, and 21 days after the experiment. Animal groups are no prolonged hypotension (0hr PH, n=3, black circles), 1hr PH (n=5, green diamonds), 2hr PH (n=5, blue triangles), and 3hr PH (n=5, red squares). Data are expressed as mean $\pm$ standard deviation. Significant differences from baseline are denoted by * ($P < 0.001$), or + ($P < 0.05$). Significant differences from the control group, 0hr PH, are denoted by # ($P < 0.001$).

Figure 4:

Blood values of creatine phosphokinase (CPK, A), and lactic acid dehydrogenase (LDH, B). Normal ranges are indicated as a yellow band. Blood samples were collected at baseline, immediately after the experiment (Recovery), and 1, 3, 7, 14, and 21 days after the experiment. Animal groups are no prolonged hypotension (0hr PH, n=3, black circles), 1hr PH (n=5, green diamonds), 2hr PH (n=5, blue triangles), and 3hr PH (n=5, red squares). Data are expressed as mean $\pm$ standard deviation. Significant differences from baseline are denoted by * ($P < 0.001$), or + ($P < 0.05$). Significant differences from the control group, 0hr PH, are denoted by # ($P < 0.001$).

Figure 5:

Blood values of blood urea nitrogen (BUN, A), and creatinine (B). Normal ranges are indicated as a yellow band. Blood samples were collected at baseline, immediately after the experiment (Recovery), and 1, 3, 7, 14, and 21 days after the experiment. Animal groups are no prolonged hypotension (0hr PH, n=3, black circles), 1hr PH (n=5, green diamonds), 2hr PH (n=5, blue triangles), and 3hr PH (n=5, red squares). Data are expressed as mean $\pm$ standard deviation.

Table 1.

Effect of prolonged hypotension on blood cell counts and coagulation

	PH	Baseline	Day 1	Day 3	Day 7	Day 14	Day 21
Hematocrit	0 hr	42.6 ± 1.2	35.4 ± 6.5 *	37.7 ± 6.0	38.7 ± 4.3	40.9 ± 3.1	40.9 ± 1.4
%	1 hr	40.5 ± 0.8	34.4 ± 2.7 *	34.4 ± 4.6 *	36.9 ± 2.6	38.1 ± 1.1	40.1 ± 1.4
(35.2–44.8)	2 hr	43.4 ± 1.5	36.8 ± 1.9 *	36.5 ± 3.5 *	39.4 ± 2.9	40.6 ± 2.8	41.5 ± 2.2
	3 hr	42.8 ± 4.8	34.5 ± 1.1 *	35.2 ± 2.1 *	39.3 ± 2.2	38.9 ± 2.7	41.2 ± 3.3
Hemoglobin	0 hr	13.9 ± 0.3	11.5 ± 2.1 *	12.4 ± 2.0	12.6 ± 1.4	13.3 ± 0.9	13.4 ± 0.4
g/dL	1 hr	13.5 ± 0.4	11.4 ± 0.9 *	11.4 ± 1.4 *	12.1 ± 0.8	12.6 ± 0.4	13.2 ± 0.4
(11.6–15.3)	2 hr	14.4 ± 0.4	12.0 ± 0.7 *	12.1 ± 1.1 *	13.0 ± 0.9	13.3 ± 0.8	13.4 ± 1.0
	3 hr	14.1 ± 1.5	11.3 ± 0.4 *	11.7 ± 0.7 *	12.8 ± 0.7	12.7 ± 0.8	13.4 ± 1.0
WBC	0 hr	6.3 ± 0.8	13.3 ± 1.3	11.6 ± 0.9	12.6 ± 0.5	9.4 ± 1.2	7.6 ± 1.5
10 ³ /mm ³	1 hr	6.1 ± 0.9	14.9 ± 3.9 *	11.4 ± 3.1	9.3 ± 1.4	8.0 ± 2.2	7.6 ± 1.9
(5.4–15.0)	2 hr	6.4 ± 1.2	16.8 ± 4.4 *	8.6 ± 1.9	10.1 ± 2.6	9.0 ± 2.5	10.3 ± 5.4
	3 hr	6.6 ± 1.0	14.9 ± 3.4 *	11.0 ± 3.8	8.5 ± 1.2	10.2 ± 1.9	9.5 ± 0.9
RBC	0 hr	5.4 ± 0.1	4.5 ± 0.8 *	4.8 ± 0.8 *	4.9 ± 0.6	5.2 ± 0.3	5.2 ± 0.2
10 ⁶ /mm ³	1 hr	5.1 ± 0.1	4.4 ± 0.4 *	4.4 ± 0.6 *	4.7 ± 0.4	4.8 ± 0.2	5.0 ± 0.3
(4.4–5.7)	2 hr	5.5 ± 0.2	4.7 ± 0.3 *	4.7 ± 0.5 *	5.0 ± 0.4	5.2 ± 0.4	5.3 ± 0.4
	3 hr	5.5 ± 0.7	4.4 ± 0.3 *	4.5 ± 0.3 *	5.0 ± 0.4	5.0 ± 0.4	5.3 ± 0.5
Platelets	0 hr	205 ± 63	178 ± 62	248 ± 72	359 ± 114 *	375 ± 101 *	255 ± 44
10 ³ /mm ³	1 hr	174 ± 21	163 ± 58	187 ± 60	328 ± 144 *	279 ± 107 *	213 ± 63
(130–430)	2 hr	235 ± 80	194 ± 68	238 ± 78	352 ± 122 *	328 ± 109 *	273 ± 114
	3 hr	264 ± 71	170 ± 94	234 ± 58	356 ± 86 *	376 ± 58 *	298 ± 90
Fibrinogen	0 hr	254 ± 2	455 ± 192 *	591 ± 174 *	436 ± 102 *	300 ± 30	273 ± 31
mg/dL	1 hr	269 ± 56	398 ± 57 *	475 ± 155 *	381 ± 88	292 ± 53	274 ± 39
(175–492)	2 hr	266 ± 35	420 ± 75 *	489 ± 80 *	366 ± 35	269 ± 37	285 ± 45
	3 hr	279 ± 37	465 ± 25 *	588 ± 64 *	429 ± 38 *	325 ± 24	308 ± 24
PT	0 hr	11.4 ± 0.7	12.8 ± 0.9	11.0 ± 0.2	11.0 ± 0.3	11.3 ± 0.4	10.9 ± 0.5
seconds	1 hr	12.2 ± 0.9	13.7 ± 0.6 *	11.5 ± 0.6	11.4 ± 0.3	11.9 ± 0.7	12.2 ± 0.6
(10.2–14.0)	2 hr	12.4 ± 0.9	13.7 ± 1.1 *	11.5 ± 0.8	11.4 ± 0.6	12.0 ± 1.1	12.3 ± 0.8
	3 hr	11.2 ± 0.9	13.8 ± 1.7 *	11.5 ± 0.8	11.1 ± 0.4	11.5 ± 0.6	11.8 ± 0.9
PTT	0 hr	29.9 ± 0.8	33.0 ± 1.6	31.7 ± 2.0	29.1 ± 2.2	29.7 ± 1.0	29.7 ± 0.2

	PH	Baseline	Day 1	Day 3	Day 7	Day 14	Day 21
seconds	1 hr	29.5 ± 2.2	31.1 ± 1.8	28.6 ± 3.2	28.8 ± 1.7	27.6 ± 0.9	28.7 ± 1.2
(27.2–36.8)	2 hr	29.8 ± 4.1	30.1 ± 4.1	30.6 ± 3.5	29.0 ± 2.6	29.9 ± 0.8	29.8 ± 2.9
	3 hr	27.3 ± 2.2	30.0 ± 2.7	28.9 ± 1.7	28.5 ± 1.1	29.0 ± 1.2	28.5 ± 1.4

Values for hematocrit, hemoglobin, white blood cells (WBC), red blood cells (RBC), platelets, fibrinogen, prothrombin time (PT), and partial thromboplastin time (PTT) are shown as mean ± standard deviation. Normal ranges are indicated in parenthesis.

* denotes significant difference from Baseline, $P < 0.001$.