Hemodynamic Variables and Tissue Energetics during Resuscitation of Porcine Hemorrhagic Shock with Hextend® or Lactated Ringer's Solution

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SUMMARY

There has been recent interest in resuscitation of hemorrhagic shock using new colloid solutions such as Hextend® (6% hydroxyethyl starch in balanced salt solution). We examined the effects of resuscitation of porcine hemorrhagic shock with Hextend® or lactated Ringer's (LR) solution on hemodynamic parameters and tissue energetics. Anesthetized, instrumented pigs underwent hemorrhagic shock (35% total blood volume, 90 minutes) and were randomized to resuscitation with Hextend® 10 cc/kg (n=5), Hextend® 20 cc/kg (n=8), or LR 20 cc/kg (n=10) per step in four steps. Endpoints measured included invasive hemodynamics, near infrared (NIR) spectroscopic measures of tissue hemoglobin saturation (StO_2) in stomach, liver, and hind limb, and in vivo nuclear magnetic resonance (NMR) measures of tissue phosphoenergetics of liver and hind

limb. Both groups receiving Hextend® resuscitation demonstrated increased cardiac output and oxygen delivery (DO_2) compared to animals resuscitated with LR. Phosphoenergetics (hind limb phosphocreatine) improved more rapidly in Hextend® groups compared to animals receiving LR. There were no significant differences between groups with respect to StO_2 in hind limb or stomach. Hextend® resuscitation resulted in improved hemodynamics and tissue energetics secondary to improved filling pressures in this porcine model of controlled hemorrhagic shock. Equivalent hemodynamic improvement was achieved with Hextend® at 1/3 the volume of LR. This product may have significant application in austere environments where the volume of resuscitative fluid is limited due to mission constraints.

1.0 INTRODUCTION

The ideal fluid for volume expansion in both civilian and military trauma has been the topic of debate for several decades. In terms of outcome, the ideal fluid has not yet been identified. A number of recent expert panels have suggested various fluids in the setting of a paucity of randomized, prospective trials.[1,2,3] Without an obvious optimal fluid, lactated Ringer's (LR) has been the suggested resuscitation fluid for acute treatment of victims of trauma.[4] However, this strategy has the potential disadvantages of immunosuppression[5] and the need for administration of at least three times the volume of blood lost to provide intravascular volume expansion. For the military in a combat/field situation, the "cube" (or weight and volume) of the fluid is a significant consideration. For this reason, recent military planners have included colloid (Hextend®) as the resuscitation fluid of choice in far forward units.[6] Hextend® is a preparation of hydroxyethylstarch in a physiologically balanced electrolyte solution. However, its effects on coagulation and effectiveness in restoration of intravascular volume are still controversial.[7] Additionally, recent research into low-volume resuscitation has raised questions regarding the appropriate volume of fluid and whether vigorous restoration of intravascular volume actually risks furthering hemorrhage.[8,9,10] As a result, low-volume fluid resuscitation has more recently been considered to avoid "popping the clot."

Arguably, an endpoint for resuscitation after hemorrhage is restoration and normalization of tissue energetic levels, as prompt restoration of these levels would be optimal for survival of cells and tissues. Although direct measurement of mitochondrial energy production is not clinically available, measurements of tissue energetics can be assessed in the experimental setting. With use of nuclear magnetic resonance (NMR) spectroscopy, tissue energetics in the form of high-energy phosphates can be evaluated in real-time during experimental shock for a dynamic perspective during shock and resuscitation.[11-13] It has been demonstrated that energy levels are highly conserved during ischemia, likely through use of alternate ATP-producing pathways and down-regulation of non-essential processes.[14-15] Animal studies have suggested that organ failure from shock is coincident with energetic failure.[16] However, a direct comparison of energetic levels *in vivo* between colloid and crystalloid resuscitation after hemorrhagic shock has not previously been reported.

This study was performed to evaluate tissue energetics during resuscitation from hemorrhagic shock with use of Hextend® as compared to LR *in vivo* and in real time with use of nuclear magnetic resonance (NMR) and near-infrared (NIR) spectroscopy. We hypothesized that the two fluids would be similar in their ability to restore post-shock tissue energetics.

## 2.0 METHODS

2.1 Animal Protocol:
This experimental protocol was approved by the University of Minnesota Animal Use Committee and was conducted in accordance with established guidelines of the treatment of laboratory animals. Thirty-two male Yorkshire-Landrace pigs (Fanning Farms, Howe, Indiana) weighing 13-20 kg were used for experimentation.

Each animal was maintained without food and with free access to water for 12 hours prior to the experiment. Animals were anesthetized with althesin and inhaled nitrous oxide as previously described.[12-13]

The following devices were placed: Pulmonary artery catheter via the right internal jugular vein, 12 Fr venous bypass catheter in the inferior vena cava (IVC), cystostomy catheter in the bladder, and an arterial catheter in the right carotid artery. Near-infrared spectroscopy probes (Hutchinson Technology, Inc, Hutchinson, Minnesota) were placed directly on the liver at laparotomy, on the surface of the hind limb, and into the stomach via a modified nasogastric tube. Specially-constructed NMR surface coils were placed on the liver surface as well as on the hind limb.

Splenectomized and instrumented animals were randomized to receive either Hextend® at 10 cc/kg, Hextend® at 20cc/kg, or LR at 20 cc/kg for resuscitation. The shock/resuscitation protocol is illustrated in Figure 1. Hemorrhagic shock was induced by a 35% bleed (estimated by weight) into a heparinized blood collection bag via IVC cannula. The animals remained in shock for 90 minutes at which time, following measurements, they received resuscitation with either Hextend® or LR. Resuscitation was divided into four boluses using either Hextend® at 10 cc/kg/bolus, Hextend® at 20 cc/kg/bolus, or LR at 20 cc/kg/bolus, depending on pre-shock randomization. Hemodynamic, NMR, and NIR measurements were taken at baseline, during shock (every 30 minutes for 90 minutes), and after each fluid bolus. Surviving animals were euthanized at the end of the fourth resuscitative measurement.

Figure 1: Shock and resuscitation protocol
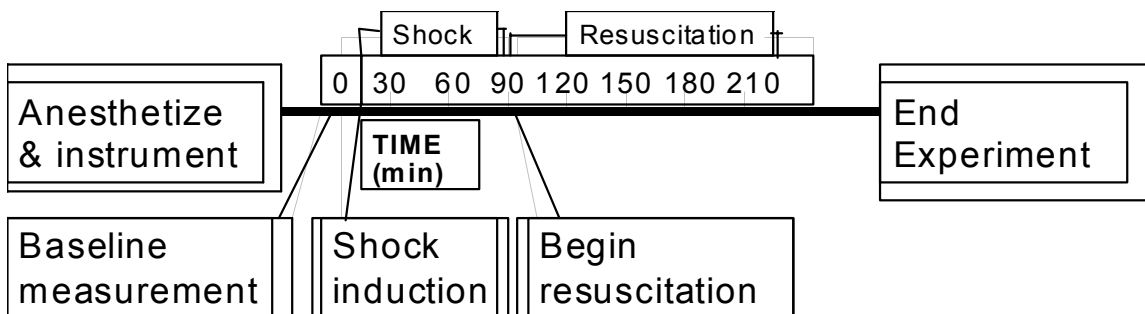
2.2 Measurements:

An NIR reflectance probe was placed in the stomach, liver, and hind limb. Percent StO_2 is a measure of hemoglobin oxygen saturation of blood contained in the volume of tissue illuminated by near-infrared light. For each StO_2 measurement the multiple optical absorbance values were processed as previously described[17]. In vivo ^{31}P NMR spectra were recorded at 25.80 MHz in a 1.5T whole-body superconducting magnet (Magnex Scientific, Abingdon, UK) interfaced to an Apollo spectrometer (Tecmag Inc., Houston, USA) as described.[12-13] Pulmonary artery catheter measurements of cardiac output (CO) were made via thermodilution and obtained at baseline and in synchrony with NMR measurements. These measurements were used to calculate oxygen delivery (DO_2) and oxygen consumption (VO_2) and indexed by animal weight in kilograms. Arterial and mixed venous blood gases as well as lactate, hemoglobin (Instrument Laboratories, Lexington, MA) and hemodynamic parameters were measured at baseline and in synchrony with the NMR measurements.

2.3 Analysis of NIR/NMR data:

NIRS measurements were expressed as percent oxyhemoglobin saturation (StO_2). NMR spectra were imported into ACD/Spec Manager Software (Advanced Chemistry Development Inc., Toronto, Ontario, Canada) to determine peak height and area. Tissue pH (pHi) was determined using the chemical shift of the inorganic phosphate (Pi) peak relative to phosphocreatine (PCr) peak as described.[18] NMR measurements were expressed as area under the curve relative to total phosphorus and were normalized to baseline measurements.

2.4 Analysis of data:

Animals that survived the hemorrhagic shock protocol to receive resuscitation were used for analysis. No intent-to-treat analysis was performed. Three groups were compared: 1) animals resuscitated with LR at 20cc/kg/bolus 2) animals resuscitated with Hextend® at 10cc/kg/bolus (Hextend-10) 3) animals resuscitated with Hextend® at 20cc/kg/bolus (Hextend-20). Comparisons of hemodynamics, NIRS, and NMRS measurements were made between groups at baseline, after 30, 60, and 90 minutes of shock, and after each of four resuscitative steps. A one-way analysis of variance (ANOVA) with least squared post-hoc testing was performed to determine statistically significant differences between groups at each time point. A p-value of <0.05 defined significance.

3.0 RESULTS

Thirty-two animals were randomized to one of three resuscitative strategies (Figure 2).
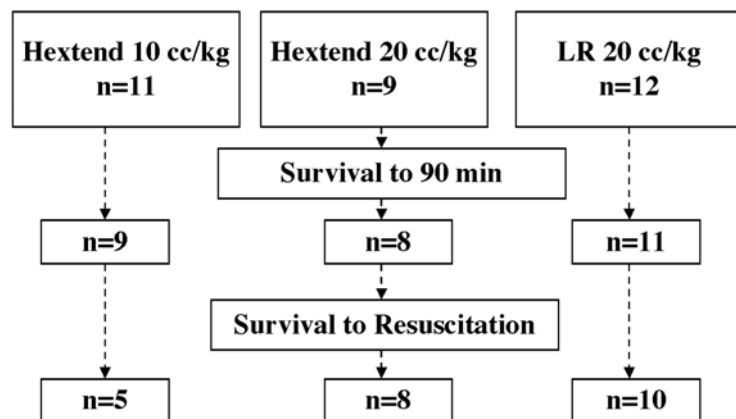

Figure 2: Total animals enrolled in protocol, surviving shock, and surviving to resuscitation

Animals in the three groups were similar with respect to body weight, hemorrhage volume, baseline mean arterial pressure (MAP) and other hemodynamic parameters (Table 1a and b).

Table 1a: Baseline weights (in kilograms) and hemorrhage volumes (in cc/kg) for each group. Mean ± standard deviation. LR=lactated Ringer's group; H-10=Hextend-10 group; H-20=Hextend-20 group.

Weight in kilograms			Volume hemorrhaged		
LR	H-10	H-20	LR	H-10	H-20
15.8 (2.3)	17.1 (2.0)	17.8 (1.7)	28.0 (5.1)	29.6 (2.8)	28.7 (6.6)

Table 1b: Hemodynamic variables between the three resuscitation groups. Mean ± standard deviation. (LR=lactated Ringer's group resuscitated at 20 cc/kg; H-10=Hextend-10 group resuscitated at 10 cc/kg; H-20=Hextend-20 group resuscitated at 20 cc/kg; MAP = mean arterial pressure in mmHg; Hgb = hemoglobin in mg/dL; PCWP = pulmonary capillary wedge pressure in mmHg; DO_2 = oxygen delivery in cc/kg/minute; VO_2 = oxygen consumption in cc/kg/minute; lactate expressed in mmol/L).

	MAP			Hgb			PCWP		
	LR	H-10	H-20	LR	H-10	H-20	LR	H-10	H-20
Baseline	82.2±13.2	92.7±9.7	81.8±15.2	10.8±1.7	11.3±1.0	10.0±1.2	9±2	6±5	9±4
Shock-90	62.5±12.6	36.2±4.5	54.8±14.4	9.1±0.9	8.7±1.1	8.8±0.6	7±3	8±3	5±3
Resus-1	68.0±12.7	48.1±13.7	75.9±17.7	8.0±0.9	7.3±1.5	5.8±0.3	8±3	9±3	8±3
Resus-4	78.9±16.7	82.7±19.8	89.3±25.4	6.8±1.2	5.8±1.5	6.5±2.1	9±4	11±4	15±4

	DO_2			VO_2			lactate		
	LR	LR	H-10	H-20	LR	H-10	H-20	H-10	H-20
Baseline	24.6±6.5	6.2±2.4	5.9±0.9	5.7±1.7	3.2±2.8	2.7±1.6	2.3±1.1	25.6±5.6	22.5±3.5
Shock-90	11.8±1.6	8.1±2.4	5.7±1.2	7.4±1.9	5.5±1.9	9.9±3.0	4.6±2.1	8.2±2.5	10.7±3.3
Resus-1	14.4±2.0	6.7±2.8	6.3±1.3	6.8±2.2	7.2±3.2	12.2±5.1	4.8±2.9	13.2±3.2	16.1±5.4
Resus-4	15.8±3.1	6.0±1.5	7.6±1.0	9.4±3.2	5.5±3.7	10.4±5.5	2.7±1.5	22.5±6.0	32.0±13.0

With hemorrhage, the cardiac output of all animals predictably dropped, and the Hextend-10 group dropped significantly lower than the other two groups at 60 minutes of shock (Figure 3). This drop was also reflected in a significantly lower hind limb tissue pH (pHi) for the Hextend-10 group at 60 minutes of shock (Figure 4). With resuscitation, significant differences in cardiac output developed between groups. After the first and second resuscitative steps, the Hextend-20 group had a significantly greater cardiac output than either the LR or the Hextend-10 group. However, by the fourth resuscitative step, both Hextend® groups averaged a significantly greater cardiac output than the LR group, with no significant difference between Hextend® groups (Figure 3).
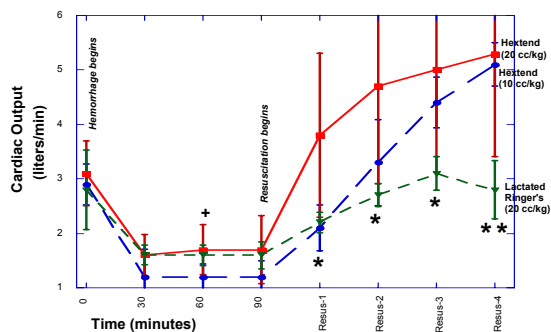

Figure 3: Cardiac output during shock and resuscitation. Hextend-10 group significantly lower than either LR or Hextend-20 group at 60 minutes of shock ([+]p=0.044). Hextend-20 group significantly greater than LR or Hextend-10 groups at Resus-1 and Resus-2 (*p<0.012). At Resus-3, Hextend-20 group significantly greater than LR group alone (*p<0.012). At Resus-4, LR group significantly lower than either Hextend group (**p=0.001).
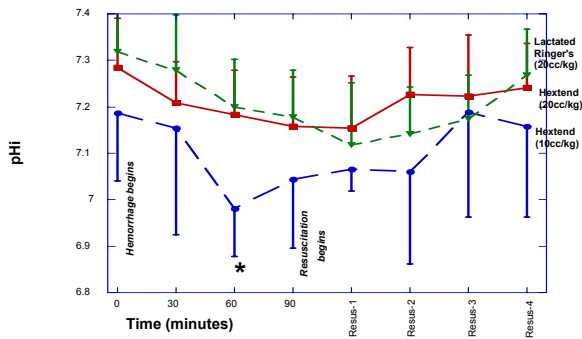
Figure 4: Hind limb tissue pH (pHi)　as determined by chemical shift of *in vivo* NMR spectroscopy during shock and resuscitation. Hextend-10 group significantly lower than Hextend-20 or LR group (*p<0.05).

The amount of change in cardiac output between the end of shock (Shock-90) to the end of resuscitation (Resus-4) was significantly greater for the Hextend® groups than the LR group. These differences were reflected in oxygen delivery (DO_2) values which were significantly higher for both Hextend® groups compared to the LR group by Resus-4 (Table 1b; p<0.05). There was no statistical difference in DO_2 between Hextend® groups (Table 1b; p=0.157) at Resus-4. With respect to increased cardiac output and DO_2 with resuscitative measures, LR given at 60 cc/kg in 2 doses was equivalent to Hextend® at 20 cc/kg given as either a single dose or two doses (Figure 5).
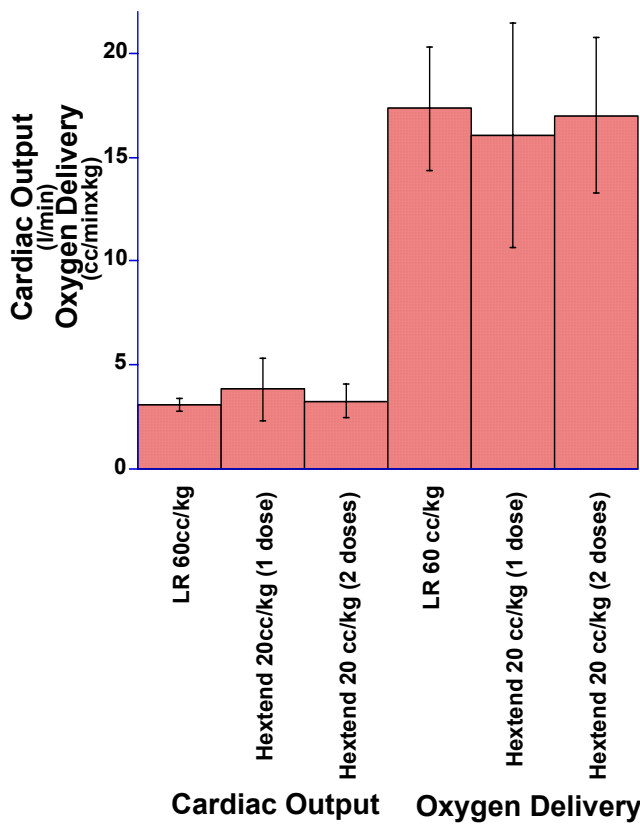

Figure 5: Cardiac output and oxygen delivery after resuscitation with 60 cc/kg (3 boluses) of LR, 20 cc/kg Hextend in one bolus (1 dose), or 20 cc/kg Hextend in two boluses (2 doses).

Changes in tissue phosphoenergetics were also observed between the three groups during resuscitation. After the second and third resuscitative steps, hind limb (skeletal muscle) levels of phosphocreatine (PCr) were greater in the Hextend-10 group than the Hextend-20 or LR groups. However, by the end of resuscitation (Resus-4), both Hextend® groups were significantly greater than the LR group, and there was no difference between Hextend® groups (Figure 6a). No significant differences between tissue ATP levels in the hind limb (Figure 6b) or liver (not shown) were observed between groups during resuscitation, except exclusively after the third resuscitative step. As previously reported, hind limb phosphomonoester (PME) levels uniformly increased during shock in all groups and then decreased with resuscitation.[12] Consistent with previous observations, the animals that were more severely affected by the shock protocol had a significant elevation in PME levels during shock (Hextend-10 group) (Figure 6c). [12,13]
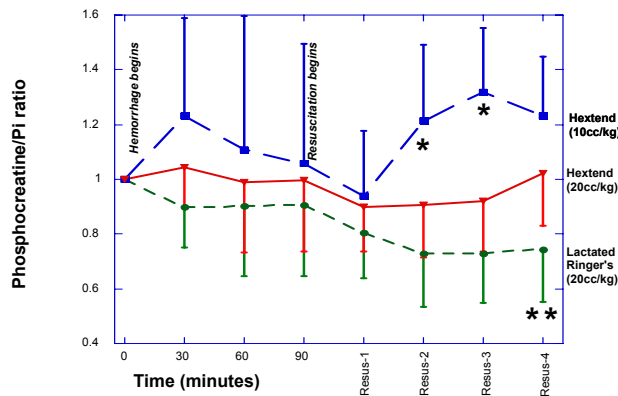

Figure 6a: Hindlimb phosphocreatine/inorganic phosphate (Pi) ratio *in vivo* during hemorrhagic shock and resuscitation. *p<0.05 when Hextend-10 group compared to other groups. **p<0.01 when LR group compared to both Hextend® groups.

Figure 6b: Adenosine triphosphate (ATP)/ inorganic phosphate (Pi) ratio in hind limb (skeletal muscle) during shock and resuscitation as determined by *in vivo* NMR spectroscopy. *p<0.02 when compared to Hextend-10 group.
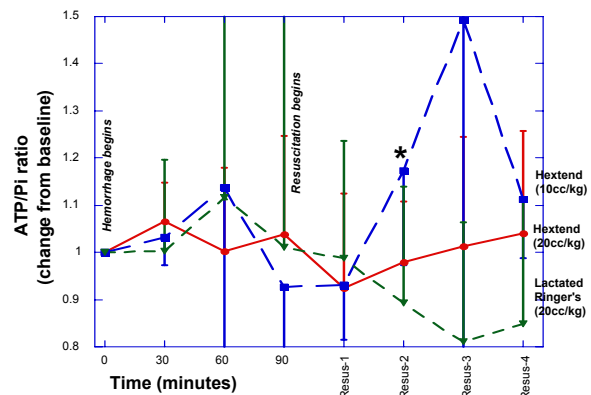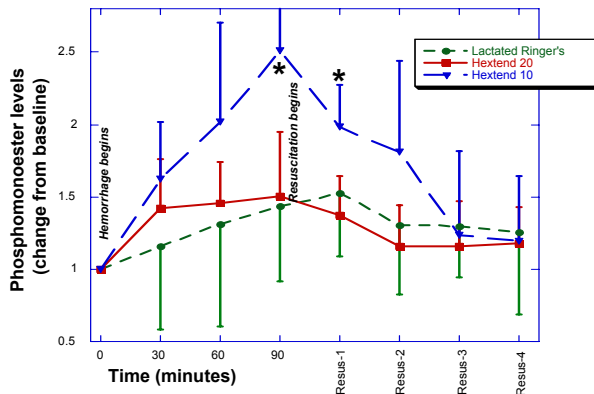
Figure 6c: Hind limb (skeletal muscle) phosphomonoesters as determined by in vivo NMR spectroscopy during shock and resuscitation. *p<0.01 when compared to Hextend-10 group.

Predictably, StO_2 of the liver, stomach and hind limb dropped in all groups with shock and returned towards baseline levels with resuscitation. Interestingly, we noted a significant decrease in liver StO_2 during the later phases of resuscitation in the animals receiving 20 cc/kg/step of Hextend® as compared to animals resuscitated with LR (Resus-3, p=0.03). There were no other significant differences in StO_2 between groups.

4.0 DISCUSSION

Hextend® resuscitation resulted in increased systemic oxygen delivery (DO_2) and improved tissue energetics as measured by NMR spectroscopy. We believe that this increase is secondary to the early increased filling pressure in the heart from colloid administration as demonstrated by increased pulmonary capillary wedge pressure (PCWP) in animals receiving Hextend®. This increase contributed to an earlier improvement in cardiac output and the resulting improvement in DO_2 and phosphoenergetics. Interestingly, this increase was not reflected in an increase in StO_2 in any of the tissue beds monitored. The volume expansion achieved with Hextend® was similar to that classically described,[4,19] with a similar increase in global hemodynamic parameters achieved with 1/3 the volume of crystalloid resuscitative fluid.

One issue not addressed in our controlled hemorrhage model was the level of blood pressure safe to prevent "popping the clot", resulting in increased hemorrhage. Human studies have demonstrated the safety and efficacy of hypotensive resuscitation in short term care of trauma patients. [8] Animal studies have placed the "safe" pressure limit at approximately 60 mm Hg. [20] The US military has adapted the previous ATLS protocol for care of injured patients in the field to limit intravenous fluids to those patients with abnormal pulses and altered mental status.[21] Hextend®, with its more effective volume expansion, may be more apt to cause loss of the primary protective clot due to its more rapid restoration of cardiac output.

One of the weaknesses of this study was the randomization scheme, which randomized animals prior to shock. This resulted in fewer animals available for analysis in the Hextend-10 group than the other two groups due to death of several more animals during the shock period. Additionally, it appears from the hemodynamic profile and elevated lactate levels of the analyzed animals in this group that these animals were more severely stressed by the shock protocol than animals in the other groups. Interestingly, despite this issue, animals receiving Hextend® appeared to improve more rapidly with resuscitation compared to animals treated with LR as demonstrated by hemodynamic parameters and tissue energetic indices (Figure 3). NMR data from the

liver, which would have aided in interpretation of changes in StO$_2$ signal in this organ, was not interpretable during major portions of this protocol due to poor signal/noise ratio. Finally, one must be careful in application of findings derived from a controlled animal shock model to a clinical scenario.

In conclusion, we have demonstrated that Hextend® resuscitation results in improved hemodynamics and tissue energetics secondary to improved filling pressures in a porcine model of controlled hemorrhagic shock. This product may have significant application in austere environments where the volume of resuscitative fluid is limited due to mission constraints. Further study of this product is needed in clinical settings to validate these results.

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