Albumin Saturated with Fatty Acids Prevents Decompensation in a Rat Hemorrhagic Shock Trauma Model with Tourniquet and Hypotensive Resuscitation

Alexander H. Penn[1],

Michael A. Dubick[2],

Ivo P. Torres Filho[2]

[1] TriService Research Laboratory, JBSA Fort Sam Houston, San Antonio, Texas

[2] US Army Institute of Surgical Research, JBSA Fort Sam Houston, San Antonio, Texas

Corresponding Author (and reprint address):

Alexander H. Penn, PhD

4141 Petroleum Rd.

JBSA Fort Sam Houston, TX 78234-2644

alexander.penn.ctr@us.af.mil

phone: 210-539-8080

Conflicts of Interest and Sources of Funding: No authors report a conflict of interest. Funding for this study was provided by the US Army Medical Research & Development Command.

The views expressed in this article are those of the author(s) and do not reflect the official policy or position of the U.S. Army Medical Department, Department of the Army, DoD, or the U.S. Government.

Abstract (WORD COUNT 247)

Decompensation is a major pre-hospital threat to survival from trauma/hemorrhage shock (T/HS) after controlling bleeding. We recently showed higher than expected mortality from a combat-relevant rat model of T/HS (27 ml/kg hemorrhage) with tourniquet (TQ) and permissive hypotensive resuscitation (PHR) with Plasmalyte. Mortality and fluid requirements were reduced by resuscitation with 25% albumin pre-saturated with oleic acid (OA-sat) compared to fatty-acid (FA)-free albumin or Plasmalyte. The objective of this follow-up study was to analyze the role of decompensation in those outcomes. We observed two forms of decompensation: slow (accelerating fluid volumes needed to maintain blood pressure) and acute (continuous fluid administration unable to prevent pressure drop). Combined incidence of decompensation was 71%. Acute decompensations caused 21 of 22 total deaths observed by 3 h and began as slow decompensations. Prior to transition from slow to acute, acute decompensators were distinguishable from stable animals by their diastolic pressure response to fluids. Decompensation was not due to loss of cardiac performance. Acute decompensators had significantly lower heart rate and diastolic blood pressure variability and high-frequency spectral power. Colloid administration (FA-free albumin) to increase oncotic pressure added to vascular volume but only delayed the need for high fluid volumes to maintain pressure. In contrast, OA-sat albumin rats maintained pressure with less fluid, in line with decreased decompensation. Our findings suggest acute decompensation may be common after trauma and severe hemorrhage treated with TQ and PHR and that OA-sat albumin may benefit early survival and reduce transfusion volume by preventing decompensation.

Key words: fatty acids; resuscitation; colloid; hemolysis; acute decompensation

Introduction

Most military deaths in recent US conflicts occurred during the pre-hospital phase, and 91% of the deaths from potentially survivable injuries occurred as result of hemorrhage, of which 24% were deemed potentially survivable (1). Investigation into the causes of these potentially survivable deaths is vital to the development of new treatments and technologies to prevent them. The most immediate threat to survival in the pre-hospital setting is uncontrolled bleeding and we have made considerable strides in hemorrhage control through improved use of tourniquets (TQ), hemostatic dressings, and damage control resuscitation strategies including permissive hypotensive resuscitation (PHR) (2-4). However, casualty transport times to medical treatment facilities are projected to increase in future conflicts associated with multi-domain operations. Despite the improvements in temporary hemorrhage control, these scenarios may increase the fatalities resulting instead from decompensation after hemorrhage.

Decompensation is the failure of the body's compensatory mechanisms for handling blood loss. These mechanisms include: 1) increasing cardiac heart rate and/or contraction strength to improve cardiac output and oxygen delivery, 2) adjusting arterial constriction to shunt more of the remaining blood to heart and brain, 3) increasing venous constriction to mobilize blood reserves (5), and 4) autotransfusion to shift extravascular fluid into the intravascular space. Except for autotransfusion, these mechanisms are active compensatory responses governed by the sympathetic nervous system (SNS) (6). Death in the first few hours of shock from controlled hemorrhage or gut ischemia appears to be more closely related to cardiovascular failure (i.e. decompensation) than failure of other organs (7, 8). Despite this, few studies attempt to parse out the effects of treatments on different compensatory mechanisms, even when early death is a primary outcome of the study.

Recently, we created a rat model of trauma plus hemorrhagic shock (T/HS) with TQ at time of injury and PHR with Plasmalyte, to model injury and early treatments in pre-hospital combat casualty care (8). We tested restoration of the albumin lost in hemorrhage with a 25% albumin bolus given prior to PHR,. Since albumin is the primary source of oncotic pressure in plasma, hyper-oncotic albumin should have increased fluid auto-transfusion from extravascular compartments, reducing the overall fluid requirements needed to maintain the hypotensive pressure goal (9). This would reduce fluid overload (10, 11) and the weight in medics' packs (2). We also investigated the effect of non-esterified fatty acids (NEFAs) attached to albumin by comparing NEFA-free to NEFA-saturated albumin.

The findings were somewhat unexpected. First, the mortality was unusually high and early for the degree of hemorrhage (27 ml/kg; 45% of blood volume) and experiment duration (3 h) (8). 15% of the animals died before receiving treatment and an additional 47% of the animals that received vehicle bolus died before the end of experiment. Second, the rate of decompensation was unusually high. A large proportion of animals began losing blood pressure and dying despite *continuous* fluid resuscitation. Of 22 deaths, 21 were due to this *acute decompensation*. Third, NEFA-saturated albumin was overwhelmingly beneficial since death and fluid requirements were significantly reduced. In contrast, oncotically-equivalent NEFA-free albumin provided only a short-term (1^{st} hour) benefit to fluid requirements, with no benefit to total fluid requirements.

Clearly, high and early death was attributable to acute decompensations. Fluid requirements are also directly tied to decompensation, because PHR uses pressure drop as a trigger for fluid administration. Unfortunately, acute decompensations are poorly studied. This may be due to the difficulty of collecting pre-hospital data, especially on the battlefield. There may also be a tendency to assume, regardless of evidence, that any pre-hospital drop in blood pressure must be due to further blood loss, either from a trauma that was "missed" in initial evaluation or from re-bleeding triggered

by resuscitation. It may also be due to the tendency in animal studies to either exclude subjects that acutely decompensate or from failure to recognize them as distinct events, lumping them with deaths caused by other mechanisms.

The goals of this follow-up analysis were to 1) characterize the acute decompensators, determining what traits, if any, set them apart from the slow decompensators and non-decompensating subjects and 2) determine the relative contribution of different compensatory mechanisms to the initial compensation and decompensation, and how these mechanisms were affected by the treatment groups.

Methods

T/HS + TQ + PHR Procedure

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's Institutional Animal Care and Use Committee approved all research conducted in this study. The facility where this research was conducted is fully accredited by AAALAC. The surgical procedure has been described in-depth previously (8). In brief, isoflurane anesthetized rats (male; Sprague-Dawley; 275-350 g body weight, BW) were cannulated in the carotid artery for continuous blood pressure recording, femoral artery for controlled hemorrhage, and the femoral vein for blood sampling and resuscitation fluids. Rats received a trauma (transverse laparotomy) immediately before hemorrhage (2 ml/min/kg). Hemorrhage continued at this rate until mean arterial pressure (MAP) dropped to 35 mmHg (designated as time t = 0). At this time, corresponding to "first aid", a pneumatic TQ was inflated around the right hind limb, and hemorrhage rate was reduced to 0.6 ml/min/kg. Rate was adjusted to maintain MAP = 35 ± 3 mmHg until 45% of the estimated blood volume (6% of BW) had been removed. The time from t = 0 – 1 h is referred to as

the **Ischemic** phase. If the MAP dropped below 34 mmHg after completion of hemorrhage but before the end of the Ischemic phase, we administered Plasmalyte A (Baxter Healthcare Corporation, Deerfield, IL) at 0.5 ml/min until MAP reached 37 mmHg.

At the start of the **Pre-Hospital** care phase (t = 1 h), rats were resuscitated with 2.69 ml/kg BW of 250 mg/ml bovine serum albumin (BSA) or vehicle ("Plasmalyte", N=17),corresponding to the estimated albumin lost in hemorrhage (assuming 42% hematocrit and 43 mg/ml albumin in plasma). BSA was either NEFA-free ("FA-free BSA", N=15) or was saturated ("OA-sat BSA", N=13) with oleic acid as previously described (8, 12). Following bolus treatment, PHR was initiated with MAP < 50 mmHg as the trigger for Plasmalyte administration (1 ml/min; ceasing at MAP > 53 mmHg).

At start of the **In-Hospital** phase (t = 2 h), the TQ was removed and the threshold for Plasmalyte administration was increased to 60 mmHg (ceasing at MAP > 63 mmHg). Total Plasmalyte infusion in all phases was limited to 4 times the HV. At t = 3 h, surviving animals were euthanized by intravenous administration of 0.2 ml of Fatal Plus (390 mg/ml sodium pentobarbital; Vortech Pharmaceuticals, Dearborn, MI), while under anesthesia. Venous blood samples in heparin (0.5 ml each) were collected at baseline and at the end of each phase.

Blood analysis

Hematocrit (Hct) was measured by capillary tube centrifugation before spinning the remaining blood to obtain plasma (stored -80 ºC until analysis). Vascular volume was calculated from Hct assuming that the only change in blood volume occupied by red blood cells was due to hemorrhage and blood sampling. Plasma samples were assayed for albumin concentration by bromocresol purple assay, performed with a kit (Sigma-Aldrich) using reconstituted rat serum albumin (Sigma-Aldrich) for calibration. Additional assays (blood gases, electrolytes, NEFA concentration and binding capacity,

Syndecan-1 concentration, and alanine transaminase activity) using blood and plasma were performed and reported previously (8).

Decompensation Analysis

Animals were divided post-hoc into 3 categories based on their responses to fluid therapy. In animals defined as *acute decompensators*, the blood pressure dropped resulting in death despite continuous fluid administration to achieve the target MAP. The remaining animals were grouped based on the final fluid administration rate. *Stable* animals needed less than 50% of their HV (13.5 ml/kg) in a 20 min period, while animals needing 50% HV or more were classed as *slow decompensators*. To better understand events leading to acute decompensation, we analyzed the 20 min period prior to the point those animals ceased responding positively to fluids and compared it to the final 20 min that animals could receive fluid (i.e. prior to reaching the maximum allowed or to t = 3 h) for slow decompensators and stable animals. We examined MAP, diastolic arterial pressure (DAP), systolic arterial pressure (SAP), and heart rate (HR) during that entire period and while the animals were receiving fluid (i.e. response to fluid administration).

Heart Rate and Blood Pressure Variability

To approximate the degree of autonomic changes that may be involved in decompensation, we analyzed heart rate variability (HRV) and arterial pressure variability (APV) in time and frequency domains. Pressures (mean, diastolic, and pulse) and peak-to-peak intervals (i.e. heart period) were extracted and processed for 2-min intervals at indicated time points from arterial pressure data using commercial software (Acqknowledge, Biopac, Goleta, CA; Excel, Microsoft Corp, Redmond, WA). Missed beats and double-counted beats were interpolated. Time domain variability is described by the

standard deviation of the signal in the 2-min interval. For frequency domain measures, time domain data was first re-sampled at 5 Hz (suitable for catching the entire spectrum in rats) and the mean value of each series was subtracted from each interval measure to remove trend (13). Power spectrum was determined by fast Fourier transform (Matlab, MathWorks; Natick, MA). High frequency spectral power is synchronous with respiration and may quantitatively evaluate respiratory arrhythmias, therefore serving as index of vagal (i.e. parasympathetic) activity (14). The baseline respiratory rate in this study was approximately 60-70 breaths/min (1.0 – 1.2 Hz). Therefore, we defined high frequency (HF) power as power > 0.75 Hz up to the Nyquist frequency of 2.5 Hz (13). Mayer waves corresponding to SNS efferent activity can be detected at ~0.4 Hz in APV (but not HRV) in conscious rats (15), however, similar to sinoaortic baroreceptor denervated rats (15), we found no evidence for a peak corresponding to Mayer waves at any time point. Possibly, Mayer waves were greatly diminished and/or shifted by the anesthesia into the very low-frequency range (15) where they would be concealed within the fast Fourier transform artifact (**Supplemental Figure 1**). As a consequence, we only report HF spectral power findings.

Statistics

Results are described as mean ± standard deviation or as median (interquartile range). Continuous measures were analyzed by ANOVA (repeated measures ANOVA in cases where both time and group comparisons were made) followed by post-hoc Student's t-test or, for non-normally distributed data, by Kruskal-Wallis test followed by post-hoc ranked Tukey or Dunn's multiple comparison test. Categorical data were analyzed by Chi-square analysis. Significance was set to $p < 0.05$ for the non-parametric tests. To correct for multiple comparisons in the t-tests and Chi-square analysis, significance was set to $p < 0.025$.

Results

Compensatory Mechanisms

Previously (8), we reported data related to cardiac and arterial compensatory mechanisms. For this analysis, we extracted information on the other two compensatory mechanisms, venous constriction and autotransfusion, using estimates of vascular volume (venous capacity being the largest contributor) and albumin concentration (a surrogate for oncotic pressure).

Plasma albumin concentration dropped by the end of the ischemic phase compared to baseline in all groups ($p < 0.0001$, **Figure 1A**). As expected, groups that received albumin treatment maintained or increased albumin concentrations between 1 and 2 h, and their concentrations at 2 h were greater than the Plasmalyte group, which continued to decrease. By the final time point (3 h), however, Plasmalyte and FA-free BSA groups' survivors appear to have equilibrated to the same albumin concentration, which was lower than baseline and the final concentration in the OA-sat BSA group **(Figure 1A)**. These results suggest that the OA-sat BSA group was able to maintain a higher plasma oncotic pressure.

The vascular volume was estimated using hematocrit, hemorrhaged volume, blood sample volume, and the estimated starting blood volume. Hct for all groups was ~43% at baseline and dropped to the same levels at 1 and 2 h (**Figure 1B**). By 3 h, Hct stabilized in Plasmalyte and OA-sat BSA groups but continued to drop in the FA-free BSA group to 25%, on average. Total blood volume decreased after hemorrhage (**Figure 1C**), though by 1 h it was at 63% of starting volume, compared to the 55% we assume was left immediately after 45% hemorrhage. This difference corresponds to a 4.8-ml/kg autotransfusion of fluid, which almost exactly accounts for the observed drop in albumin concentration (calculated plasma albumin concentration at T = 1 h is 39.2 mg/ml versus the measured

value of 39.4 mg/ml; see **Supplement** for details). Survivors continued to increase their blood volume to the same extent during the Pre-Hospital phase. The Plasmalyte group showed no further change at the final time point, suggesting that all fluid given in the In-hospital phase (see (8)) leaked out again. The FA-free BSA group continued to expand its vascular volume until it was nearly equivalent to pre-hemorrhage (Baseline) levels, however the volume increase was still less than the fluid volume given, suggesting the rest also leaked out. The OA-sat BSA group, like Plasmalyte, showed no further change at the final time point, yet most of these animals needed no fluid.

Total albumin in the vasculature was calculated by multiplying albumin concentration by the estimated plasma volume (**Figure 1D**). The OA-sat BSA group had a slightly greater amount of albumin than other groups before resuscitation (1 h). The FA-free BSA and OA-sat BSA groups increased their total vascular albumin after albumin bolus, but did not return to baseline. This is due in part to the difference between baseline starting concentrations (48.6 ± 6.5 mg/ml) and the literature value of 43 mg/ml, used for our dosage calculation. The total vascular albumin in Plasmalyte and FA-free BSA groups remained constant between 2 and 3 h, suggesting that the vasculature was not more permeable than normal to large molecules (or at least that the rate of albumin exiting the vasculature was equal to albumin return via lymph). In contrast, total vascular albumin fell in the OA-sat BSA group in that period ($p = 0.006$), though it remained significantly elevated compared to the Plasmalyte group (**Figure 1D**), suggesting that vascular permeability to large molecules may have been affected.

Decompensation

In models where fluid is used to maintain target blood pressures, decompensation must be tracked by measuring fluid requirements rather than pressure. Once our animals' MAPs dropped to the point that fluids were required, we observed three patterns of fluid administration corresponding to

decompensation outcome types (acute decompensation, slow decompensation, and stable) described in Methods (**Figure 2**). Decompensating animals typically showed an accelerating need for fluid compared to stable animals. This acceleration is especially apparent in acute decompensators, prior to the point they become unresponsive (indicated by the final, long, uninterrupted period of fluid administration). Prior to unresponsiveness, *every animal that acutely decompensated had already met or exceeded the rate of fluid administration that defines slow decompensation*. After unresponsiveness, the drop in pressure was rapid (time between unresponsiveness and death: 6.1 ± 6.5 min, N = 21). Of the 53 animals, 21 were acute decompensators, 9 were slow decompensators, and 23 were stable (**Table 1**). Eight animals acutely decompensated prior to treatment bolus (15% decompensation rate prior to start resuscitation). Table 1 shows the distribution of decompensation outcomes in each treatment group. Significantly fewer animals decompensated after OA-sat BSA (15%) than following FA-free BSA (60%) or Plasmalyte (65%) ($p<0.02$).

Fluid Requirements

Total fluid requirements were 26% (IQR 10-77%) of HV (7 ml/kg) for stable animals, 275% (193-405%) of HV (74 ml/kg) for slow decompensating animals, and 135% (60-147%) of HV (36 ml/kg) for acutely decompensating animals (stable animals significantly different from slow and acutely decompensating animals; N = 23 stable, 9 slow, and 21 acute). Total fluid requirements for acute decompensators are skewed downwards by their early death, however. Examination of fluid requirements in each phase shows a trend for *more* fluids needed by acute than slow decompensators in every phase (**Figure 3**). Stable animals required significantly less fluid than acute decompensators in every phase and less fluid than slow decompensators in the In-Hospital phase. The median stable animal needed no fluid other than the treatment bolus.

Pressure Response to Fluids

Figure 4 shows a representative example of the arterial blood pressure trace for each type of fluid response. Prior to becoming unresponsive to fluid, a common feature of acute decompensators (and some slow decompensators) was decreased DAP with increased pulse pressure (PP) as fluids were added, suggesting early differential response to fluids (**Figure 4B**). To test this, we determined the change in DAP and PP for each outcome type while they were receiving fluids. Fluids increased PP but had almost no effect on DAP in acute decompensators, compared to stable animals that increased PP and DAP in response to fluid (**Figure 5**). Slow decompensators had an intermediate pressure response to fluid.

Vascular versus Cardiac Decompensation

Typically, PP (closely related to stroke volume (16)) and HR are associated with cardiac function, while in relation DAP (i.e. the residual pressure when the heart is *not* shifting blood into the arterial vasculature) is associated more with vascular function. To help determine whether the decompensation was more likely due to cardiac or vascular decompensation, we compared DAP, PP, and HR. At t = 3 h, slow decompensators had significantly higher HR and PP, and lower DAP, compared to stable animals (**Figure 6**). Acute decompensators were all dead by t = 3 h, but in the 20 min prior to becoming unresponsive, DAP decreased (32±11 to 27±5 mmHg, N=20, p=0.025) and PP tended to increase (43±19 to 51±18 mmHg, p=0.036), with essentially no change in heart rate (443±55 to 433±47 BPM, p=0.35). In contrast, stable animals in the final 20 min showed no trend for change in DAP (47±8 to 45±7 mmHg, N=22, p=0.2), PP (52±11 to 50±12 mmHg, p=0.5), or heart rate (423±57

to 422±60 BPM, p=0.77). These results suggest that the failure to compensate is vascular rather than cardiac in origin for both acute and slow decompensators.

Heart Rate and Blood Pressure Variability Analysis

Variability in the time domain of the heart period (HP) dropped during the hemorrhage, hitting the low end of its range just prior to resuscitation (**Figure 7A**). Resuscitation improved HP variability in stable animals significantly more than animals that would later decompensate, on whom it had little effect. DAP variability decreased in all groups with hemorrhage, but recovered and was elevated by the end of the ischemic phase in stable animals compared to animals that would later decompensate (**Figure 7B**). Slow decompensators recovered DAP variability by the end of the Pre-Hospital period, but acute decompensators did not recover. Pulse pressure variability roughly paralleled PP itself (Figure 6), all groups recovering some variability during the ischemic period and slow decompensators eventually achieving higher variability by T = 3 h (**Figure 7D**). Unsurprisingly, MAP variability behaved like a blend of DAP and PP variability (**Figure 7C**). HF spectral power was qualitatively similar to the variability in the time domain (**Figure 8**). Particularly, we observed greater HP and DAP power in stable animals compared to acute decompensators and saw that slow decompensators had large increases in PP power (not significant) as resuscitation continued. MAP power behaved, again, like a blend of DAP and PP power.

Discussion

We previously reported a rat model of T/HS + TQ + PHR with greater mortality than similar models without either TQ or TQ + PHR (8). We eliminated organ damage, continued bleeding, and potassium as causes of mortality, and deduced that mortality was likely due to decompensation.

Treatment with OA-sat BSA reduced mortality and fluid requirements (8). For this report, we investigated the connections among decompensation, mortality, fluid requirements, fluid response, HRV, APV, and treatment. Though the experiment was not originally designed to study compensation, we will demonstrate how the compensatory mechanisms can still be qualitatively accessed using measurements of hemodynamics, hematocrit, and albumin concentration, and how this can lead to a more complete interpretation of findings.

Compensatory Mechanisms

Cardiac compensation can be assessed via HR and PP, which correlates to stroke volume (16). We had shown previously that HR increased similarly for all groups (8). Grouping animals by decompensatory outcome revealed that cardiac compensations as approximated by HR and PP were *stronger* in animals that were decompensating. This supports the argument that decompensation is not cardiac in this model. It also argues that arterial compensation has not failed, since the purpose of arterial compensation is to shunt blood towards the heart and brain, and clearly the heart was functioning well. We previously reported that DAP was significantly improved by OA-sat BSA treatment (8), however we cannot say that this is due to increased arterial constriction, because arterial pressure is affected by both arterial constriction and venous constriction (through the latter's effect on cardiac output, see below). OA-BSA treatment did not increase lactate or base deficit relative to the other treatments (8), which suggests equivalent tissue perfusion and supports the argument that it is venous constriction, not arterial constriction, that is improving DAP.

Autotransfusion can be assessed by serial measurement of vascular volume, which for controlled hemorrhage can be calculated using hematocrit and the assumptions that red blood cells neither enter nor leave the vasculature and stay the same size (a limitation of this study is that we did

not measure mean corpuscular volume with a complete blood cell count). In this study, autotransfusion only restored 8% of the starting blood volume, prior to treatment. In the hour after treatment, all groups recovered additional vascular volume in excess of the 4% of starting volume provided by the bolus. The Plasmalyte group received more than enough fluid to account for the volume increase (8) (most of what was given leaked out again), but since the albumin groups did not need exogenous crystalloid in that hour, the extra volume must have come from additional autotransfusion.

To understand why adding albumin increases autotransfusion, to explain the group effects on volume in the final hour, and to qualitatively assess systemic venous constriction, we make use of the Starling principle. In its simplest form, the Starling principle describes the flow of fluid across the capillary wall using the equation $J = k * (\Delta P - \Delta \Pi)$, where J = flow of fluid from the vasculature, k = permeability, which is positive and non-zero for most capillaries, ΔP = the hydrostatic transmural pressure gradient, and $\Delta \Pi$ = the oncotic pressure gradient. When there is sufficient hemorrhage to drop ΔP below $\Delta \Pi$, the normal outward leak of fluid ceases and fluid instead enters from the extravascular space and continues entering from the lymph (i.e. autotransfusion) (9). Because the incoming fluid has lower concentrations of albumin and other plasma proteins than plasma (17), the plasma oncotic pressure will drop. Autotransfusion will end when oncotic pressure drops to meet hydrostatic pressure. Therefore, *autotransfusion is limited by the plasma protein concentration*, of which albumin is the prime component (18). Adding albumin therefore increases autotransfusion. In our study, the albumin concentration calculated for T = 1 h, based solely on dilution from autotransfusion, matched the measured concentration, suggesting that prior to resuscitation there is no significant movement of albumin into or out of the vasculature. Low albumin concentration on admission has previously been associated with poor outcome after trauma (19, 20), but the reasons were not well understood. Our

results suggest that equilibrium of fluid flux would have been fast enough to attribute the low albumin directly to low capillary hydrostatic pressure.

Because venous resistance is small compared to the resistance in arterial vessels and capillaries, changes in capillary hydrostatic pressure are more dependent on changes to venous pressure than changes to arterial pressure. Venous pressure, in turn, is determined by the "stressed volume". Stressed volume refers to the blood volume in excess of the volume needed to fill the vasculature without stretching it (i.e. the "unstressed volume"). The elastic recoil of the vessels, particularly the veins where the majority of blood volume resides (21), provides the venous pressure that is then converted by the heart into venous return/cardiac output (5). This pressure therefore determines the upper limit of cardiac output. The body can convert unstressed volume into stressed volume by increasing venous constriction, essentially redefining the volume at which the vasculature is full (21). As a result, there is a surprisingly direct qualitative relation between venous constriction and albumin concentration (Figure 9).

We can use these relations to interpret our findings. In the Plasmalyte-only group, albumin concentration dropped even further after resuscitation, suggesting venous constriction was dropping. Though vascular volume expanded with the incoming fluid, the drop in venous constriction suggests the fluid increased unstressed volume, but not the hemodynamically critical stressed volume. In the surviving animals, a steady state was eventually reached (no further drop in albumin). After that point, when fluid was added to try to maintain PHR, it no longer increased vascular volume, suggesting albumin was diluted to the point that oncotic pressure was dropping below capillary hydrostatic pressure, forcing the fluid to extravasate. Essentially 100% of that fluid went to edema. This is the main reason crystalloids are only useful as short-term solutions for hypovolemia. In the FA-free BSA group, we replaced the majority of the albumin lost to hemorrhage. This increased autotransfusion as

discussed above, and eventually restored the vascular volume in survivors (actually more than expected, considering the volume of hemorrhaged red blood cells was also replaced). However, with that volume came a drop in albumin, suggesting that, like the Plasmalyte group, the fluid became unstressed volume. This is consistent with the observation that decompensation was not better with FA-free BSA than it was with Plasmalyte alone. At some point in the final hour, albumin concentration dropped to the same point as the Plasmalyte group, explaining why the fluid requirement also increased to match the Plasmalyte group in that hour (8). In contrast, after the OA-sat group received its albumin bolus, it increased volume to the same degree as the Plasmalyte group, but needed no exogenous volume, and its albumin concentration remained high. This means that hydrostatic pressure was high and only a small amount of autotransfusion was needed to bring oncotic pressure down to it. These findings indicate that venous constriction remained high in the OA-sat BSA group. Since this was the group that showed significantly higher survival, it supports the argument that, at least in this model, venous compensation is the key mechanism. While cardiac compensation can increase oxygen delivery and arterial compensation makes sure the heart and brain get a sufficient share of that, it is the venous compensation that directly corrects for hypovolemia (autotransfusion will not even begin until venous compensation can no longer correct, and capillary pressure starts to drop). We propose that this is why low albumin concentration is associated with poor outcome (19, 20).

This analysis identifies improved venous constriction as the means by which OA-sat BSA improves survival and fluid requirements, but it makes no judgments as to *how* it does this. In our previous report, we made a case that the NEFA caused hemolysis and the released hemoglobin scavenged just enough nitric oxide to make veins easier to constrict without affecting arteries enough to worsen tissue perfusion (8). This is consistent with reports stating that changes in nitric oxide affect the venous system prior to affecting the arterial system (22-24). However, it is also possible that

venous constriction is affected by oleic acid through some other mechanism, e.g. prevention of catecholamine refraction.

It is worth emphasizing that permeability to water is not the same thing as permeability to large molecules like albumin. Literature suggests albumin resuscitation increases albumin leakage into the extravascular space, resulting in edema (25). However, total albumin in the vasculature did not decrease in either Plasmalyte or FA-free BSA groups, suggesting that the vasculature did *not become leaky to larger molecules from hemorrhagic shock alone or from albumin resuscitation*. In contrast, a net decrease in total albumin suggests leakage did occur in the OA-sat BSA group, despite the lower fluid extravasation. Possibly, fatty acids damaged the vasculature, increasing the permeability to large molecules. These findings suggest that fatty acids on pharmaceutical albumin could lead to permeability increase.

Decompensation Analysis

In this model, all acute decompensations were lethal, i.e., no animal became unresponsive to fluids and then later recovered. Prior to becoming unresponsive, all 21 acute decompensators had already met the definition for slow decompensation. Therefore, acute decompensators are a *subset* of slow decompensators. Of the 9 slow decompensators that did not transition to acute, only 1 died within the 3-hour post-injury period. Together, these imply that high and early mortality was a consequence of the large number of transitions to acute decompensation.

After determining their incidence, we characterized the decompensatory outcomes. Qualitatively, decompensation appears to accelerate over time based on fluid requirements to reach the target MAP. Also, acute decompensators needed more fluid than stable animals and as much fluid per phase as slow decompensators. While PP increased with fluid administration regardless of outcome

type, the DAP response to fluid administration had some predictive power, as it was lower than stable animals in acute decompensators *before* they transitioned to unresponsiveness. In general, stable animals maintained a higher DAP than slow decompensators and slow decompensators maintained a higher DAP than acute decompensators. This suggests that the drops and recoveries in the group analysis were mostly due to the pressure drops and deaths of acute decompensators. Since MAP was held constant and HR was similar in both the stable subjects and the acute decompensators, our findings suggest that DAP may be the most useful of the standard hemodynamic vital signs for predicting decompensation.

Acute decompensation and the sympathetic nervous system

Acute decompensations have been rare in our previous hemorrhagic shock models (26, 27). However, we frequently observed similar acute pressure drops in a prolonged splanchnic arterial occlusion (SAO) model (7). The complete ischemia of the small intestine in SAO triggers a strong response from the SNS, resulting in hypertension (7, 28). This response was unsustainable, however. Frequently, blood pressure would drop by 80 mmHg or more in just a few minutes without any change in HR or PP, suggesting a sudden loss of venous return due to sympathetic signaling failure. Sudden drops in sympathetic signaling occur after hemorrhage in rabbits, supporting this hypothesis (7, 29, 30). After 2-3 hours of occlusion, rats that had *not* died from spontaneous, acute pressure drop would start slowly decreasing pressure until death, suggesting that the sympathetic signal was still present but that the vasculature was slowly losing the ability to respond. The incidence of acute death in SAO was significantly increased in groups with parasympathetic activators (7, 13) and inhibited by a parasympathetic inhibitor or sub-diaphragmatic vagotomy, leading us to hypothesize that

parasympathetic activity was forcing the SNS to increase its signal output to compensate, causing subsequent SNS exhaustion and signal lapses.

Those outcomes were similar to the acute and slow decompensations observed here, and may share similar underlying mechanisms. In a pilot study using 50% HV, 6 of 8 animals acutely decompensated in the Ischemic phase before treatment bolus ($p=0.002$ vs 8 of 53 with 45% HV), suggesting incidence of acute decompensation is increased by greater ischemia. This fits our argument because the SNS responds to ischemia with increased activity. The increased ischemia caused by TQ and PHR, on top of a 45% HV, may be the reason acute decompensation was prevalent in this model. TQs are needed to stop hemorrhage and improve survival (1), so even if humans are also subject to this effect, forgoing TQ use is not an option. We need, however, to develop ways to achieve earlier conversions to hemostatic dressing, identify casualties at the greatest risk of decompensation, and develop treatments that specifically target decompensation. Oleic acid saturated albumin may be one treatment option.

Unlike the parasympathetic inhibitors in the SAO study that only prevented slow decompensations from becoming acute, OA-sat BSA reduced the *total* incidence of decompensation. By aiding venous constriction, OA-sat BSA may have conserved the vasculature's ability to respond to the SNS signal, reducing slow decompensations. Since a lower SNS signal is then needed to achieve the same output, acute decompensations are also avoided.

Frequency Analysis

We hypothesized that since tourniquets increase ischemia and blood pressure (31), we would detect increased SNS efferent activity after TQ inflation in APV analysis. Unfortunately, the lack of Mayer waves prevented this analysis. This left us with only variability in the time domain and HF

power in the frequency domain. Variability in the time domain is associated with better compensation and outcome (7, 32, 33). We saw significantly increased HRV in the stable outcome subjects at early time points compared to decompensators, but this did not last, decreasing enthusiasm for HRV as a predictive tool. In contrast, variability in arterial pressure, particularly DAP, recovered early in stable outcome subjects and stayed elevated compared to acute decompensators, which never recovered. DAP variability in slow decompensators behaved like acute decompensators early on but looked more like stable subjects later, suggesting high variability indicates an acute decompensation is unlikely, but does not ensure a good outcome. HF spectral power was qualitatively similar to the variability in the time domain (not surprising considering that essentially the entire measured spectral power was in this frequency range).

Limitations

This analysis demonstrated that valuable information about compensation and decompensation can be extracted from measures commonly taken in hemorrhagic shock models. The main limitation is that it is qualitative data, based on indirect measures. For example, plasma albumin concentration is an indirect indicator of oncotic pressure. Direct measurement would require uncommon, specialized apparatus. Likewise, there are logical connections among venous constriction, albumin concentration, mortality, and fluid requirements. However, direct measurements of venous diameters or capillary pressure were not performed.

Military Relevance

Our findings may be of particular importance to the military because TQ and PHR are recommended combat casualty care treatments and there is currently no way to distinguish pre-hospital

deaths from occult hemorrhage from deaths due to acute decompensation. Likewise, there has been a recent push to give whole blood or blood products early. While this change should reduce edema and improve coagulation, our findings with albumin suggest that in treatment of severe hemorrhage with TQ and delayed PHR, volume replacement, even with whole blood, may not be sufficient to prevent death, if the blood only increases unstressed volume. Treatment to directly address decompensation may also be required.

Conclusions

The high incidence of early mortality and fluid requirements in a model of T/HS with TQ and PHR was prevented by treatment with OA-sat BSA but not FA-free BSA. In this model, mortality was due primarily to acute decompensations. Decompensation generally accelerated and was most associated with decreased diastolic pressure and variability. Of the cardiovascular compensatory mechanisms investigated, the most critical appeared to be venous constriction. Therefore, resuscitation strategies for preventing decompensation without inducing organ injury should support venous constriction without exacerbating arterial constriction. OA-sat BSA may achieve this, though the net loss of albumin from the vasculature suggests it may not be without complications. Future studies of resuscitation fluids in shock should consider measurements of vascular volume and albumin concentration in addition to tracking hemodynamics and fluid input/output as useful tools in interpreting outcomes. Our work here and our previous work with the splanchnic arterial occlusion model support a view that, though related, acute decompensations are distinct from slow decompensations, and may be the more immediate threat in a prolonged pre-hospital scenario, with the odds of a conversion from slow to acute being dependent upon the severity of ischemia (i.e. sympathetic stimulation). Our hypothesis that acute decompensation represents exhaustion of

sympathetic efferent signaling while slow decompensation represents a progressive failure of the venous vasculature to respond to those signals, may change how we look at early-stage death from hemorrhage and opens up new targets for intervention for the most severely hypovolemic pre-hospital patients.

Acknowledgements

Supported by US Army Medical Research and Development Command.

Figure Legends

Figure 1 Resuscitation fluid effects on albumin, hematocrit, and vascular volume in venous blood. (A) Albumin concentration in plasma. * $p < 0.002$ vs. Plasmalyte; # $p = 0.002$ vs. FA-free BSA. (**B**) Hematocrit. * $p = 0.008$ vs. OA-sat BSA ($p = 0.07$ vs. Plasmalyte). (**C**) Blood volume in the vasculature (normalized to the starting volume). * $p < 0.027$ vs. OA-sat BSA ($p = 0.08$ vs. Plasmalyte). (**D**) Albumin in the vasculature (plasma volume x albumin concentration, normalized to baseline). * $p < 0.02$ vs. Plasmalyte; # $p < 0.02$ vs. FA-free BSA. Data expressed as mean ± standard deviation. N = 15, 15, 13 at Baseline and t = 1 h; 10, 12, 13 at t = 2 h; and 8, 10, 12 at t = 3 h for Plasmalyte, FA-free BSA, and OA-sat BSA, respectively, for all four graphs.

Figure 2 Fluid volume, as percentage of hemorrhage volume (HV), administered to each animal over time. Treatment bolus and volume given to reach the pressure goal of next phase (MAP of 50 mmHg at 1 h or a MAP of 60 mmHg at 2 h) are excluded. I.e. volumes shown are those *needed* by the animal, not volumes received by the animal. To allow comparison among different experiments, time is normalized so that the moment each animal first *needs* fluid is t=0. (**A**) Stable animals. One animal recovered after a high rate of fluid administration (arrow). Eleven animals not shown needed no fluid beyond the bolus or what was infused to transition to the next pressure goal. (**B**) Acute decompensators. (**C**) Slow decompensators. While similar in appearance to acute decompensators, these animals have a decreased maximum slope. They also had greater number of on/off cycles of the fluid infusion pump, reflecting greater pressure responsiveness to fluids: less fluid was needed to reach the threshold for turning the pump off than acute decompensators.

Figure 3 Fluid volume required in each phase of the experiment (Ischemia, Pre-Hospital, and In-Hospital) for each decompensation category. Data expressed as median ± interquartile range. "% of HV" data can be converted to "ml/kg BW" by multiplying values by 27 ml/kg (i.e. the HV). Stable animals: N = 23 (all phases). Slow decompensation animals: N= 9 (all phases). Acute decompensation: N= 21 (initial), 13 (Pre-Hospital phase), 5 (In-Hospital phase). Kruskal-Wallis test: $p = 0.007$ (Ischemia), $p = 0.0003$ (Pre-Hospital), $p < 0.0001$ (In-Hospital). * significant vs. stable by Dunn's test.

Figure 4 Typical blood pressure traces from each decompensation category. (**A**) Stable animal, OA-sat BSA group. No fluid needed after treatment bolus (first arrow) in pre-hospital period and only a small amount required in In-hospital period. In the final 20 minutes, a pressure drop triggered fluid administration (second arrow), but it likely was not needed, as pressure continued to rise even after fluids were stopped (third arrow). (**B**) Acute decompensator, Plasmalyte group. After blood sample, bolus, and initial resuscitation at 60 min (first arrow), pressure begins to fall. Once the MAP hits ~49 mmHg (second arrow), Plasmalyte is given until MAP reaches ~53 mmHg. The process is repeated as needed, giving the MAP its jagged appearance. DAP decreases, balanced by increasing PP, to maintain a constant MAP, until MAP becomes fluid unresponsive (third arrow) when DAP and SAP drop precipitously. (**C**) Slow decompensator, Plasmalyte group. A small amount of fluid (21% of HV) was needed prior to resuscitation (first arrow) to avoid further MAP drop. After initial resuscitation, little fluid was required until 105 min when the rate of fluid requirement increased. Fluid was needed regularly until the maximum allowable was reached at 161 min. After that, DAP (previously relatively stable) and PP began to drop.

Figure 5 Change in pressure per minute during fluid administration for diastolic and pulse pressure from all animals of each decompensation category. Data collected in the 20 min prior to becoming unresponsive to fluid for acute decompensators or in the last 20 min that fluid was allowed for stable or slow decompensators. Calculated for each animal by adding the total change in pressure during each interval that the infusion pump was on and dividing by the total time that the pump was on within the 20 min period. Data expressed as mean ± standard deviation. * $p = 0.009$ vs. acute decompensators. N = 13, 9, and 11 for acute decompensators (that survived past the start of resuscitation), slow decompensators, and stable animals, respectively. In animals that received no fluid in the final 20 min, we used the last time segment after treatment bolus, if any, in which fluid was given.

Figure 6 (A) Mean arterial pressure, (B) diastolic arterial pressure, (C) pulse pressure, and (D) heart rate at baseline (BSL), start of hemorrhage (SH), and from t = 0 (mean arterial pressure equal to 35 mmHg) until t = 180. Data expressed as mean ± standard deviation. * $p < 0.025$ Stable vs. Slow Decompensators, ^ $p < 0.025$ Slow vs. Acute Decompensators, # $p < 0.025$ Stable vs. Acute Decompensators for all graphs. N=23, 9, and 13 for Stable, Slow Decompensators, and Acute Decompensators, respectively, through t = 60 min (analysis excludes 8 Acute Decompensators that died prior to treatment bolus). N decreases as animals die, to final values of 23, 8, and 0 for Stable, Slow Decompensators, and Acute Decompensators, respectively, at t = 180 min.

Figure 7 Heart period (HP) variability (A) and diastolic (B), mean (C), and pulse pressure (D) variability in the time domain. The standard deviation of each parameter measured over a 2-min period starting at the indicated time was used as an index of variability. For clarity, values are

expressed as mean ± standard deviation. MAP = Mean arterial pressure; DAP = Diastolic arterial pressure; PP = Pulse pressure. * $p<0.025$ acute vs stable; ^ $p<0.025$ acute vs slow; # $p<0.025$ slow vs stable. $N_{stable} = 20$. $N_{slow} = 8$, then 7 @ t=175. $N_{acute} = 20$ at $t\leq5$; 16 at t=55; 12 at t=65; 6 at t=115; 5 at t=125; and 0 at t=175.

Figure 8 High Frequency (HF, 0.75 – 2.5 Hz) spectral power of two-minute segments of heart period (HP) (A) and diastolic (B), mean (C), and pulse (D) pressure starting at the times shown. Values are expressed as mean ± standard deviation. MAP = Mean arterial pressure; DAP = Diastolic arterial pressure; PP = Pulse pressure. * $p<0.025$ acute vs stable; ^ $p<0.025$ acute vs slow; # $p<0.025$ slow vs stable. $N_{stable} = 20$. $N_{slow} = 8$, then 7 @ t=175. $N_{acute} = 20$ at $t\leq5$; 16 at t=55; 12 at t=65; 6 at t=115; 5 at t=125; and 0 at t=175.

Figure 9 Schematic of the connection between venous constriction, albumin concentration, and maximal cardiac output. Because fluid flux into or out of the vasculature takes time, changes in osmotic pressure may lag behind changes in capillary pressure. However, once steady state has been reached, a change in venous constriction will be reflected by a change in albumin concentration.

References

1. Eastridge BJ, Mabry RL, Seguin P, Cantrell J, Tops T, Uribe P, Mallett O, Zubko T, Oetjen-Gerdes L, Rasmussen TE, Butler FK, Kotwal RS, Holcomb JB, Wade C, Champion H, Lawnick M, Moores L, Blackbourne LH: Death on the battlefield (2001-2011): implications for the future of combat casualty care. *J Trauma Acute Care Surg* 73(6 Suppl 5):S431-437, 2012.

2. Krausz MM: Initial resuscitation of hemorrhagic shock. *World J Emerg Surg* 1:14, 2006.

3. Butler FK, Holcomb JB, Schreiber MA, Kotwal RS, Jenkins DA, Champion HR, Bowling F, Cap AP, Dubose JJ, Dorlac WC, Dorlac GR, McSwain NE, Timby JW, Blackbourne LH, Stockinger ZT, Strandenes G, Weiskopf RB, Gross KR, Bailey JA: Fluid Resuscitation for Hemorrhagic Shock in Tactical Combat Casualty Care: TCCC Guidelines Change 14-01--2 June 2014. *J Spec Oper Med* 14(3):13-38, 2014.

4. Kendrick CL, Edens JW, Christy RJ, Dubick MA, Wade CE, Holcomb JB, Wenke JC, Walters TJ: Fresh frozen plasma reduces edema in skeletal muscle following combined limb ischemia-reperfusion injury and hemorrhagic shock in rats. *J Trauma Acute Care Surg* 79(4 Suppl 2):S110-115, 2015.

5. Moller PW, Hana A, Heinisch PP, Liu S, Djafarzadeh S, Haenggi M, Bloch A, Takala J, Jakob SM, Berger D: The Effects of Vasoconstriction And Volume Expansion on Veno-Arterial ECMO Flow. *Shock* 51(5):650-658, 2019.

6. Chien S: Role of the sympathetic nervous system in hemorrhage. *Physiol Rev* 47(2):214-288, 1967.

7. Penn AH, Schmid-Schönbein GW: Severe intestinal ischemia can trigger cardiovascular collapse and sudden death via a parasympathetic mechanism. *Shock* 36(3):251-262, 2011.

8. Penn AH, Williams CE, Walters TJ, Dubick MA, Filho IPT: Fatty Acid-Saturated Albumin Reduces High Mortality and Fluid Requirements In A Rat Model of Hemorrhagic Shock Plus Tourniquet and Hypotensive Resuscitation. *Shock*, 2019.

9. Levick JR, Michel CC: Microvascular fluid exchange and the revised Starling principle. *Cardiovasc Res* 87(2):198-210, 2010.

10. Mazzoni MC, Borgstrom P, Arfors KE, Intaglietta M: Dynamic fluid redistribution in hyperosmotic resuscitation of hypovolemic hemorrhage. *Am J Physiol* 255(3 Pt 2):H629-637, 1988.

11. Kheirabadi BS, Crissey JM, Deguzman R, Perez MR, Cox AB, Dubick MA, Holcomb JB: Effects of synthetic versus natural colloid resuscitation on inducing dilutional coagulopathy and increasing hemorrhage in rabbits. *J Trauma* 64(5):1218-1228; discussion 1228-1219, 2008.

12. Penn AH, Dubick MA, Torres Filho IP: Fatty Acid Saturation of Albumin Used in Resuscitation Fluids Modulates Cell Damage in Shock: In Vitro Results Using a Novel Technique to Measure Fatty Acid Binding Capacity. *Shock*, 2017.

13. Aletti F, Gambarotta N, Penn AH, Ferrario M, Schmid-Schonbein GW: Heart period and blood pressure characteristics in splanchnic arterial occlusion shock-induced collapse. *J Clin Monit Comput* 31(1):167-175, 2017.

14. Pagani M, Lombardi F, Guzzetti S, Rimoldi O, Furlan R, Pizzinelli P, Sandrone G, Malfatto G, Dell'Orto S, Piccaluga E, et al.: Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog. *Circ Res* 59(2):178-193, 1986.

15. Julien C: The enigma of Mayer waves: Facts and models. *Cardiovasc Res* 70(1):12-21, 2006.

16. Bighamian R, Hahn JO: Relationship between stroke volume and pulse pressure during blood volume perturbation: a mathematical analysis. *Biomed Res Int* 2014:459269, 2014.

17. Rutili G, Arfors KE: Protein concentration in interstitial and lymphatic fluids from the subcutaneous tissue. *Acta Physiol Scand* 99(1):1-8, 1977.

18. Suarez JI, Martin RH, Hohmann SF, Calvillo E, Bershad EM, Venkatasubba Rao CP, Georgiadis A, Flower O, Zygun D, Finfer S: Human Albumin Use in Adults in U.S. Academic Medical Centers. *Crit Care Med* 45(1):e16-e22, 2017.

19. Goldwasser P, Feldman J: Association of serum albumin and mortality risk. *J Clin Epidemiol* 50(6):693-703, 1997.

20. Sung J, Bochicchio GV, Joshi M, Bochicchio K, Costas A, Tracy K, Scalea TM: Admission serum albumin is predicitve of outcome in critically ill trauma patients. *Am Surg* 70(12):1099-1102, 2004.

21. Pang CC: Measurement of body venous tone. *J Pharmacol Toxicol Methods* 44(2):341-360, 2000.

22. Benzing A, Geiger K: Inhaled nitric oxide lowers pulmonary capillary pressure and changes longitudinal distribution of pulmonary vascular resistance in patients with acute lung injury. *Acta Anaesthesiol Scand* 38(7):640-645, 1994.

23. Nelson SH, Ehardt JS, Lingnau W, Dehring DJ, Traber L, Traber D: Differential effects of prolonged septicemia on isolated pulmonary arteries and veins from sheep. *Shock* 5(6):440-445, 1996.

24. Ogilvie RI, Zborowska-Sluis D: Effects of nitroglycerin and nitroprusside on vascular capacitance of anesthetized ganglion-blocked dogs. *J Cardiovasc Pharmacol* 18(4):574-580, 1991.

25. Busuito CM, Ledgerwood AM, Lucas CE: Colloid with high fresh frozen plasma/red blood cell resuscitation does not reduce postoperative fluid needs. *J Trauma Acute Care Surg* 76(4):1008-1012, 2014.

26. Altshuler AE, Penn AH, Yang JA, Kim GR, Schmid-Schonbein GW: Protease activity increases in plasma, peritoneal fluid, and vital organs after hemorrhagic shock in rats. *PLoS One* 7(3):e32672, 2012.

27. Altshuler AE, Richter MD, Modestino AE, Penn AH, Heller MJ, Schmid-Schonbein GW: Removal of luminal content protects the small intestine during hemorrhagic shock but is not sufficient to prevent lung injury. *Physiol Rep* 1(5):e00109, 2013.

28. Longhurst JC, Rotto DM, Kaufman MP, Stahl GL: Ischemically sensitive abdominal visceral afferents: response to cyclooxygenase blockade. *Am J Physiol* 261(6 Pt 2):H2075-2081, 1991.

29. Schadt JC: Sympathetic and hemodynamic adjustments to hemorrhage: a possible role for endogenous opioid peptides. *Resuscitation* 18(2-3):219-228, 1989.

30. Bertolini A, Ferrari W, Guarini S: The adrenocorticotropic hormone (ACTH)-induced reversal of hemorrhagic shock. *Resuscitation* 18(2-3):253-267, 1989.

31. Aurora A, Roe JL, Umoh NA, Dubick M, Wenke JC, Walters TJ: Fresh Whole Blood resuscitation does not exacerbate skeletal muscle edema and long-term functional deficit after ischemic injury and hemorrhagic shock. *J Trauma Acute Care Surg* 84(5):786-794, 2018 May.

32. Convertino VA, Wirt MD, Glenn JF, Lein BC: The Compensatory Reserve For Early and Accurate Prediction Of Hemodynamic Compromise: A Review of the Underlying Physiology. *Shock* 45(6):580-590, 2016.

33. Barnaby DP, Fernando SM, Herry CL, Scales NB, Gallagher EJ, Seely AJE: Heart Rate Variability, Clinical and Laboratory Measures to Predict Future Deterioration in Patients Presenting With Sepsis. *Shock* 51(4):416-422, 2019.

Main Figures and Tables

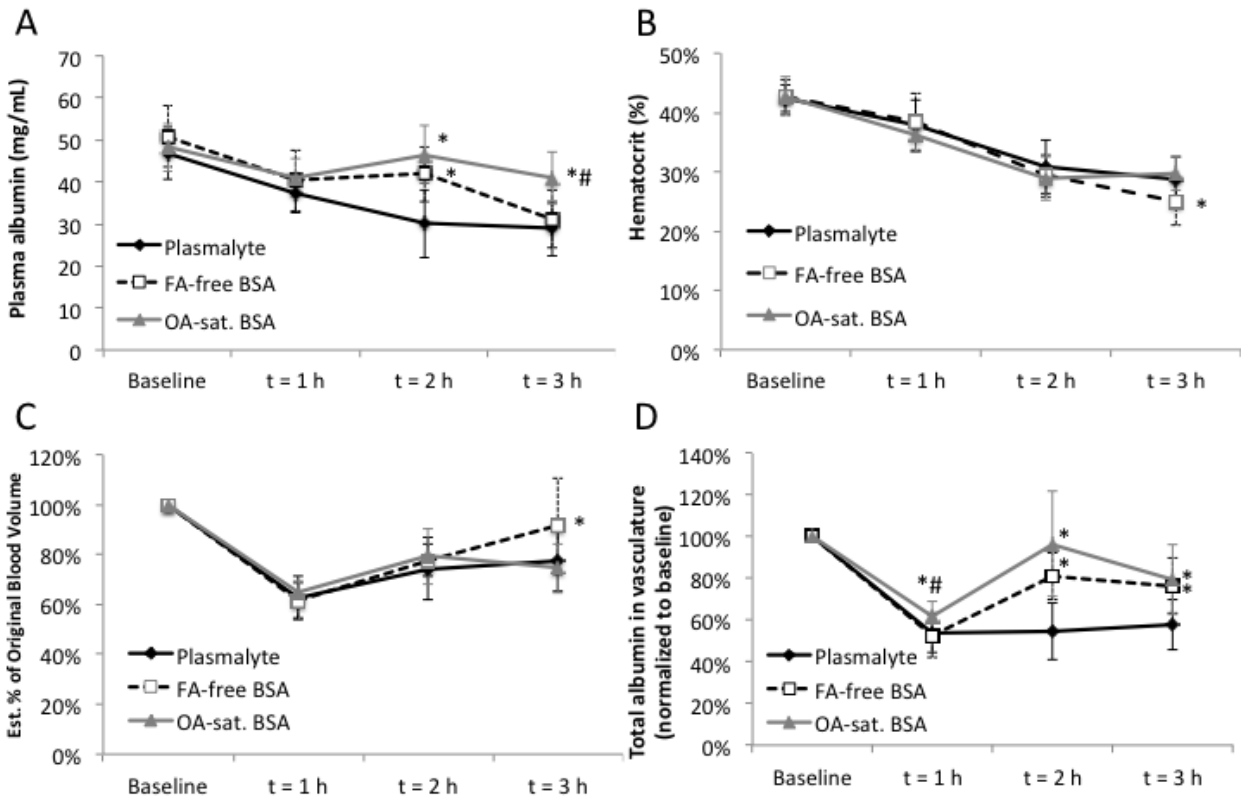


Figure 1 Resuscitation fluid effects on albumin, hematocrit, and vascular volume in venous blood. (A) Albumin concentration in plasma. * $p < 0.002$ vs. Plasmalyte; # $p = 0.002$ vs. FA-free BSA. (**B**) Hematocrit. * $p = 0.008$ vs. OA-sat BSA ($p = 0.07$ vs. Plasmalyte). (**C**) Blood volume in the vasculature (normalized to the starting volume). * $p < 0.027$ vs. OA-sat BSA ($p = 0.08$ vs. Plasmalyte). (**D**) Albumin in the vasculature (plasma volume x albumin concentration, normalized to baseline). * $p < 0.02$ vs. Plasmalyte; # $p < 0.02$ vs. FA-free BSA. Data expressed as mean ± standard deviation. N = 15, 15, 13 at Baseline and t = 1 h; 10, 12, 13 at t = 2 h; and 8, 10, 12 at t = 3 h for Plasmalyte, FA-free BSA, and OA-sat BSA, respectively, for all four graphs.

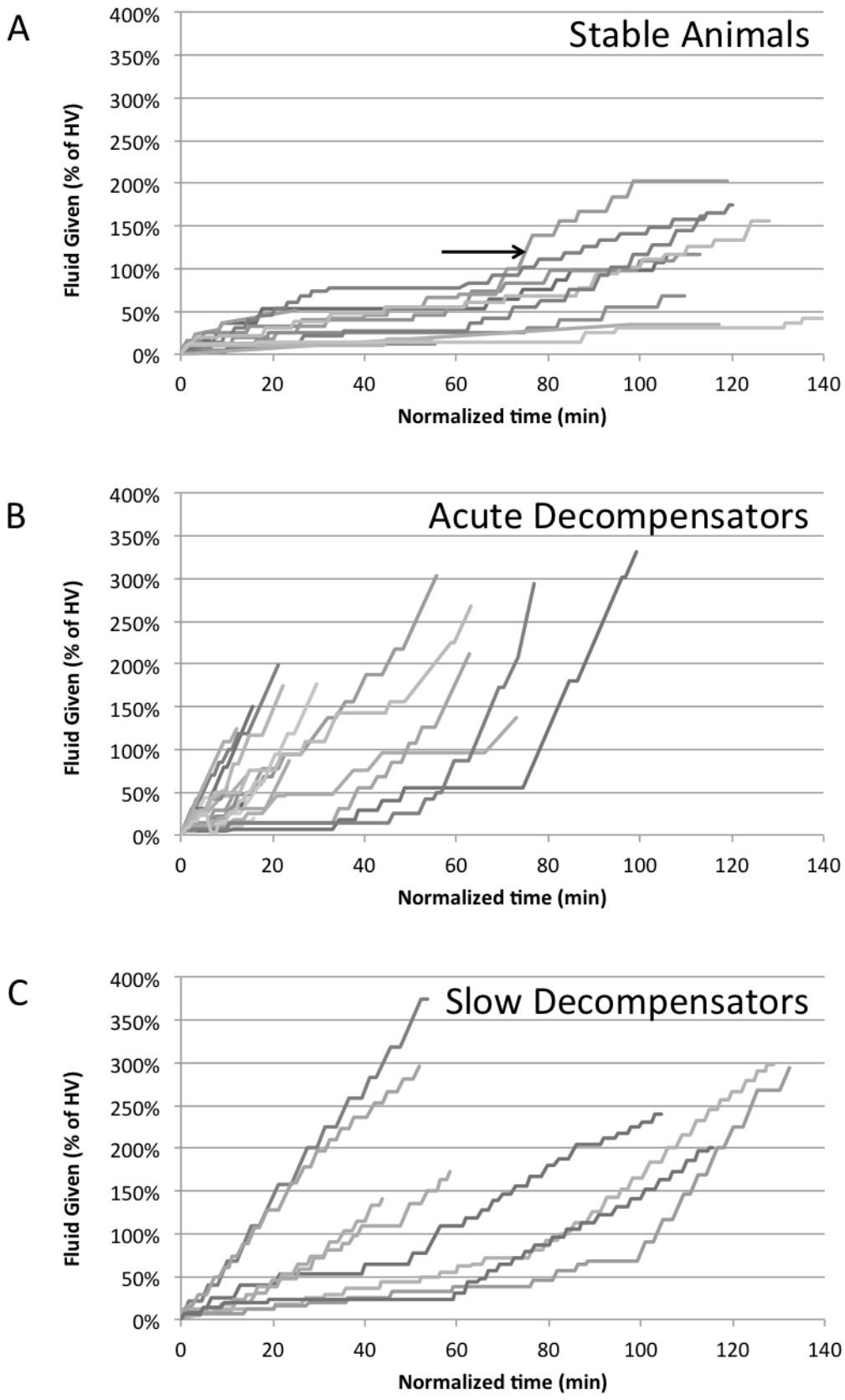

A
Stable Animals
Fluid Given (% of HV)
400%
350%
300%
250%
200%
150%
100%
50%
0%
0
20
40
60
80
100
120
140
Normalized time (min)
B
Acute Decompensators
Fluid Given (% of HV)
400%
350%
300%
250%
200%
150%
100%
50%
0%
0
20
40
60
80
100
120
140
Normalized time (min)
C
Slow Decompensators
Fluid Given (% of HV)
400%
350%
300%
250%
200%
150%
100%
50%
0%
0
20
40
60
80
100
120
140
Normalized time (min)

Figure 2 Fluid volume, as percentage of hemorrhage volume (HV), administered to each animal over time. Treatment bolus and volume given to reach the pressure goal of next phase (MAP of 50 mmHg at 1 h or a MAP of 60 mmHg at 2 h) are excluded. I.e. volumes shown are those *needed* by the animal, not volumes received by the animal. To allow comparison among different experiments, time is normalized so that the moment each animal first *needs* fluid is t=0. (**A**) Stable animals. One animal recovered after a high rate of fluid administration (arrow). Eleven animals not shown needed no fluid beyond the bolus or what was infused to transition to the next pressure goal. (**B**) Acute decompensators. (**C**) Slow decompensators. While similar in appearance to acute decompensators, these animals have a decreased maximum slope. They also had greater number of on/off cycles of the fluid infusion pump, reflecting greater pressure responsiveness to fluids: less fluid was needed to reach the threshold for turning the pump off than acute decompensators.

Table 1: Decompensation Categories by Treatment Group

	N	Death before bolus	Treatment Group		
			Plasmalyte	FA-free BSA	OA-sat BSA
Stable	23	0% [0]	35% [6]	40% [6]	85%^ [11]
Slow Decompensation	9	0% [0]	24% [4]	27% [4]	8% [1]
Acute Decompensation	21	100% [8]	41% [7]	33% [5]	8% [1]

^ $p < 0.02$ higher incidence of Stable outcome vs. both Plasmalyte and FA-free BSA.

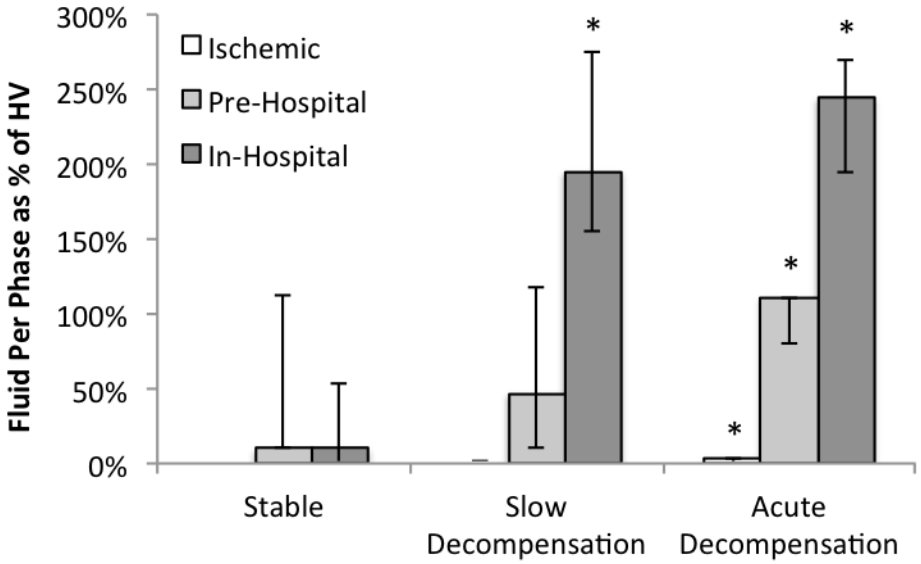


Figure 3 Fluid volume required in each phase of the experiment (Ischemia, Pre-Hospital, and In-Hospital) for each decompensation category. Data expressed as median ± interquartile range. "% of HV" data can be converted to "ml/kg BW" by multiplying values by 27 ml/kg (i.e. the HV). Stable animals: N = 23 (all phases). Slow decompensation animals: N= 9 (all phases). Acute decompensation: N= 21 (initial), 13 (Pre-Hospital phase), 5 (In-Hospital phase). Kruskal-Wallis test: $p = 0.007$ (Ischemia), $p = 0.0003$ (Pre-Hospital), $p < 0.0001$ (In-Hospital). * significant vs. stable by Dunn's test.

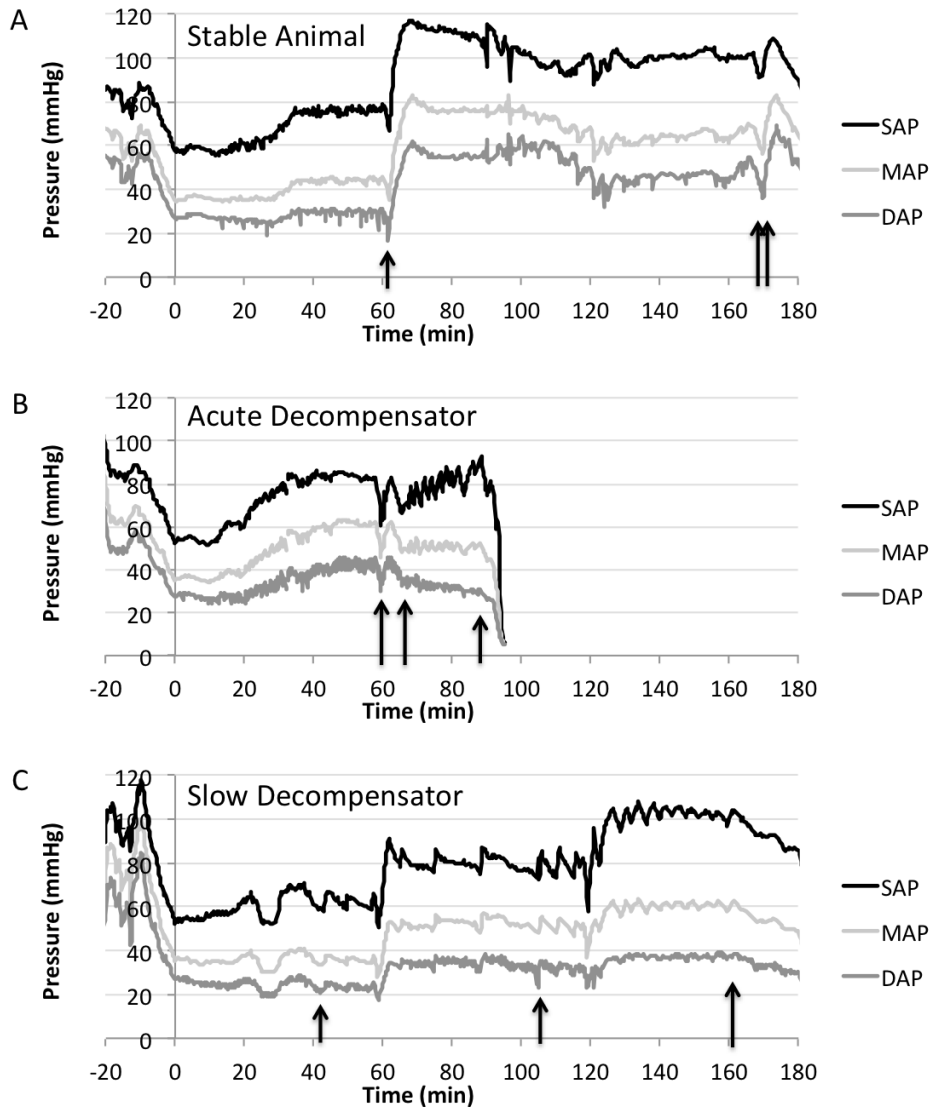


Figure 4 **Typical blood pressure traces from each decompensation category**. (**A**) Stable animal, OA-sat BSA group. No fluid needed after treatment bolus (first arrow) in pre-hospital period and only a small amount required in In-hospital period. In the final 20 minutes, a pressure drop triggered fluid administration (second arrow), but it likely was not needed, as pressure continued to rise even after fluids were stopped (third arrow). (**B**) Acute decompensator, Plasmalyte group. After blood sample, bolus, and initial resuscitation at 60 min (first arrow), pressure begins to fall. Once the MAP hits ~49 mmHg (second arrow), Plasmalyte is given until MAP reaches ~53 mmHg. The process is repeated as needed, giving the MAP its jagged appearance. DAP decreases, balanced by increasing

PP, to maintain a constant MAP, until MAP becomes fluid unresponsive (third arrow) when DAP and SAP drop precipitously. (**C**) Slow decompensator, Plasmalyte group. A small amount of fluid (21% of HV) was needed prior to resuscitation (first arrow) to avoid further MAP drop. After initial resuscitation, little fluid was required until 105 min when the rate of fluid requirement increased. Fluid was needed regularly until the maximum allowable was reached at 161 min. After that, DAP (previously relatively stable) and PP began to drop.

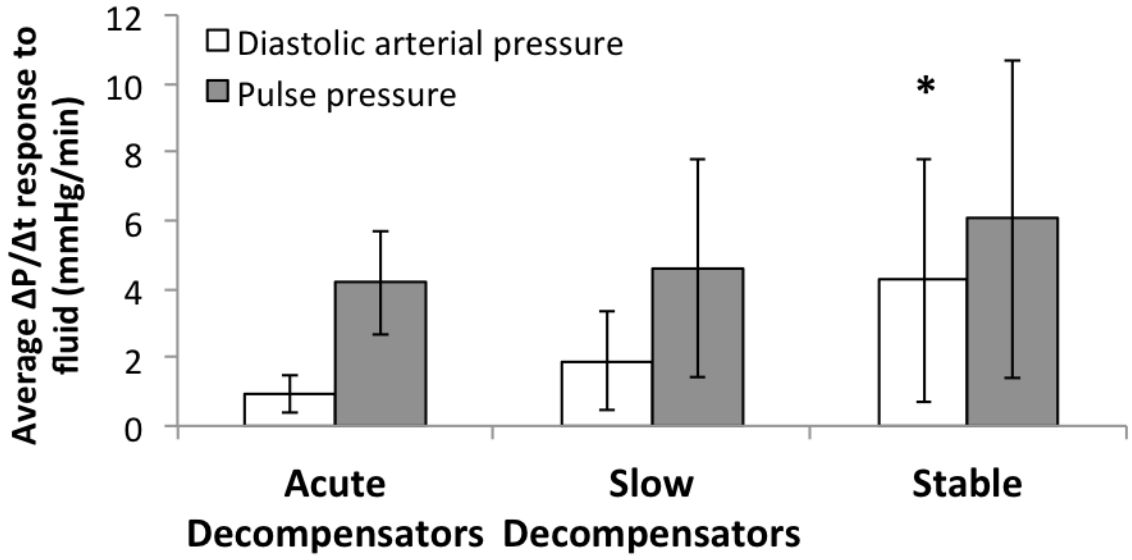


Figure 5 Change in pressure per minute during fluid administration for diastolic and pulse pressure from all animals of each decompensation category. Data collected in the 20 min prior to becoming unresponsive to fluid for acute decompensators or in the last 20 min that fluid was allowed for stable or slow decompensators. Calculated for each animal by adding the total change in pressure during each interval that the infusion pump was on and dividing by the total time that the pump was on within the 20 min period. Data expressed as mean ± standard deviation. * $p = 0.009$ vs. acute decompensators. N = 13, 9, and 11 for acute decompensators (that survived past the start of resuscitation), slow decompensators, and stable animals, respectively. In animals that received no fluid in the final 20 min, we used the last time segment after treatment bolus, if any, in which fluid was given.

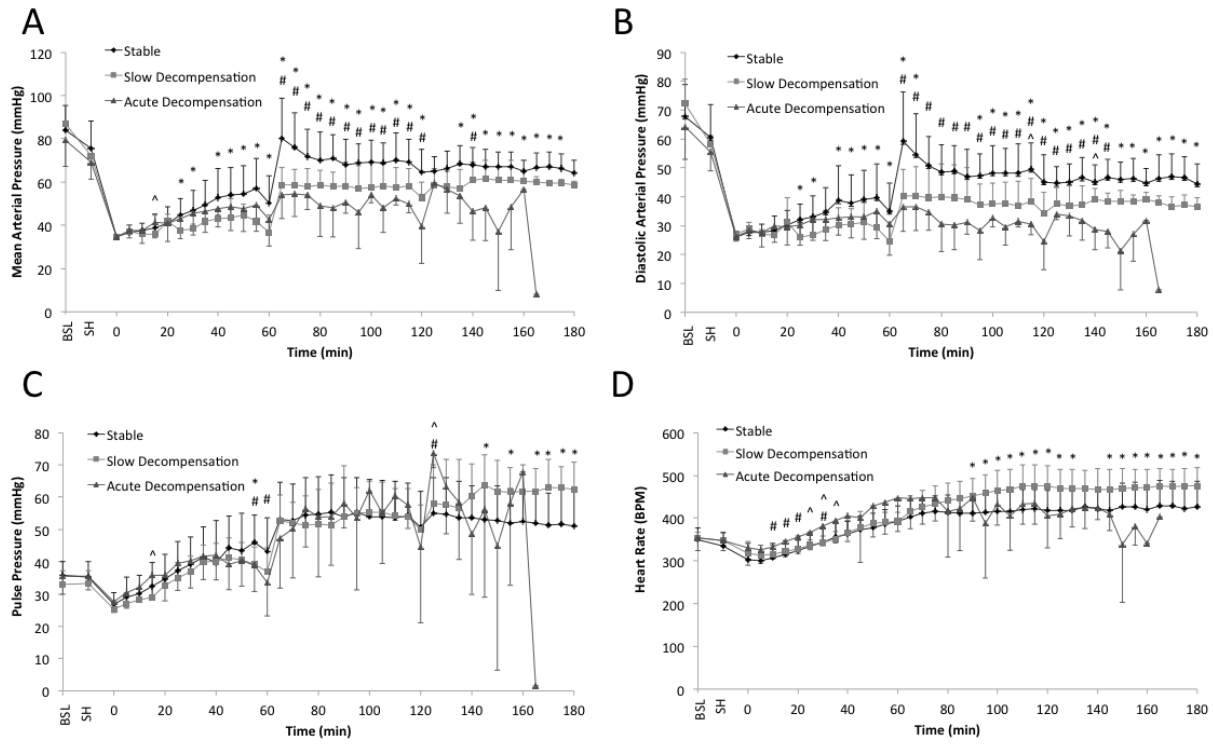


Figure 6 (A) Mean arterial pressure, (B) diastolic arterial pressure, (C) pulse pressure, and (D) heart rate at baseline (BSL), start of hemorrhage (SH), and from t = 0 (mean arterial pressure equal to 35 mmHg) until t = 180. Data expressed as mean ± standard deviation. * $p < 0.025$ Stable vs. Slow Decompensators, ^ $p < 0.025$ Slow vs. Acute Decompensators, # $p < 0.025$ Stable vs. Acute Decompensators for all graphs. N=23, 9, and 13 for Stable, Slow Decompensators, and Acute Decompensators, respectively, through t = 60 min (analysis excludes 8 Acute Decompensators that died prior to treatment bolus). N decreases as animals die, to final values of 23, 8, and 0 for Stable, Slow Decompensators, and Acute Decompensators, respectively, at t = 180 min.

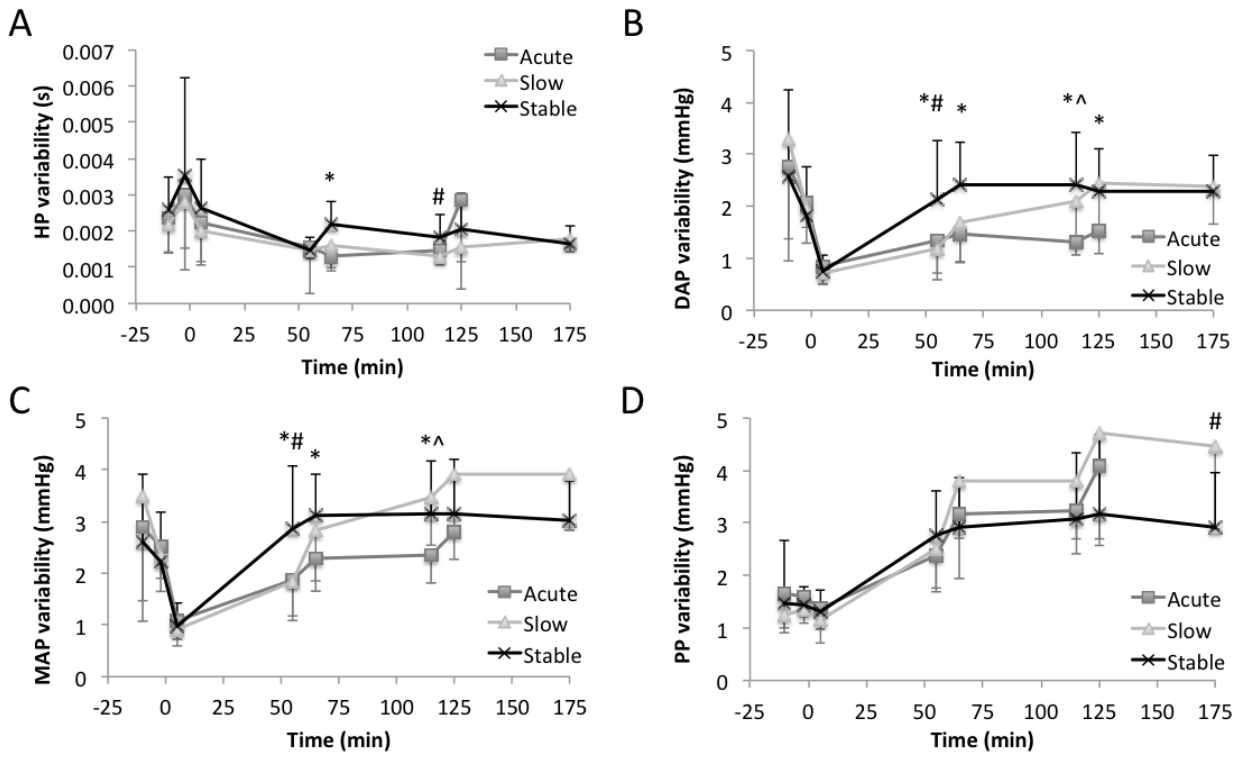


Figure 7 Heart period (HP) variability (A) and diastolic (B), mean (C), and pulse pressure (D) variability in the time domain. The standard deviation of each parameter measured over a 2-min period starting at the indicated time was used as an index of variability. For clarity, values are expressed as mean ± standard deviation. MAP = Mean arterial pressure; DAP = Diastolic arterial pressure; PP = Pulse pressure. * $p<0.025$ acute vs stable; ^ $p<0.025$ acute vs slow; # $p<0.025$ slow vs stable. $N_{stable} = 20$. $N_{slow} = 8$, then 7 @ t=175. $N_{acute} = 20$ at $t\leq5$; 16 at t=55; 12 at t=65; 6 at t=115; 5 at t=125; and 0 at t=175.

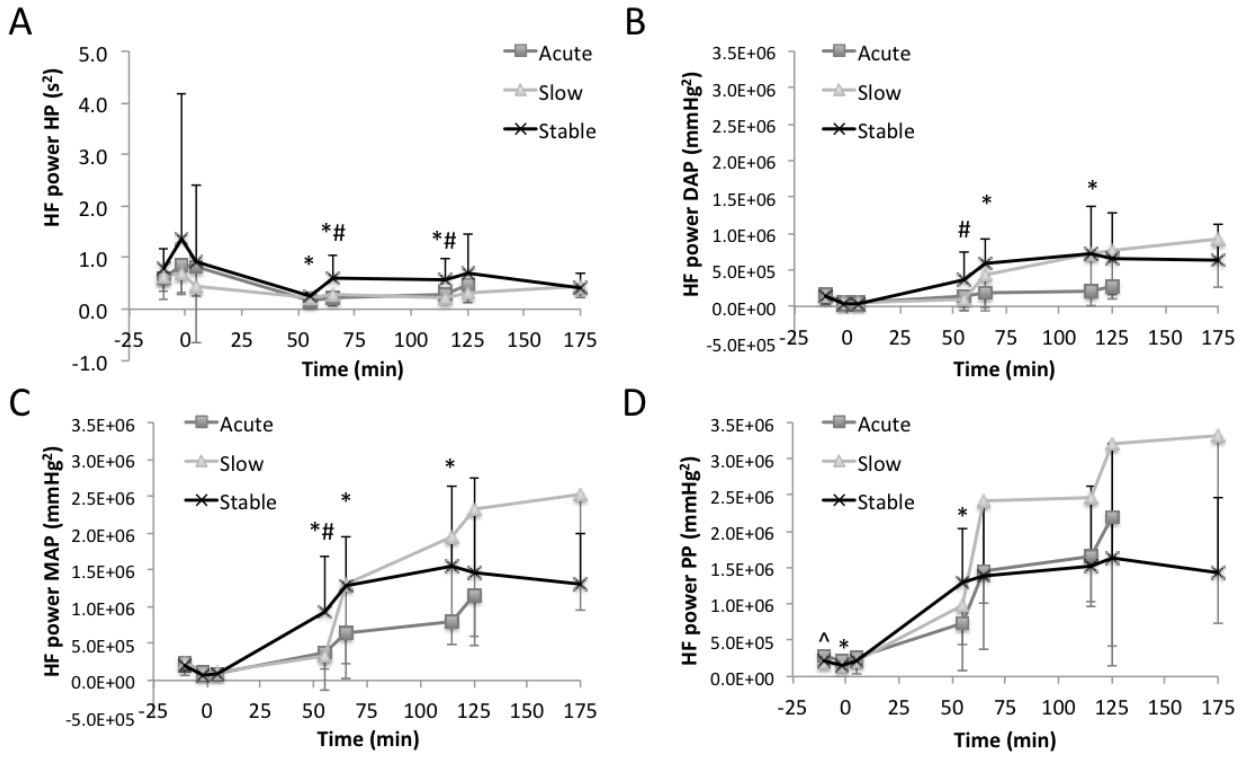


Figure 8 High Frequency (HF, 0.75 – 2.5 Hz) spectral power of two-minute segments of heart period (HP) (A) and diastolic (B), mean (C), and pulse (D) pressure starting at the times shown. Values are expressed as mean ± standard deviation. MAP = Mean arterial pressure; DAP = Diastolic arterial pressure; PP = Pulse pressure. * $p<0.025$ acute vs stable; ^ $p<0.025$ acute vs slow; # $p<0.025$ slow vs stable. $N_{stable} = 20$. $N_{slow} = 8$, then 7 @ t=175. $N_{acute} = 20$ at $t \leq 5$; 16 at t=55; 12 at t=65; 6 at t=115; 5 at t=125; and 0 at t=175.

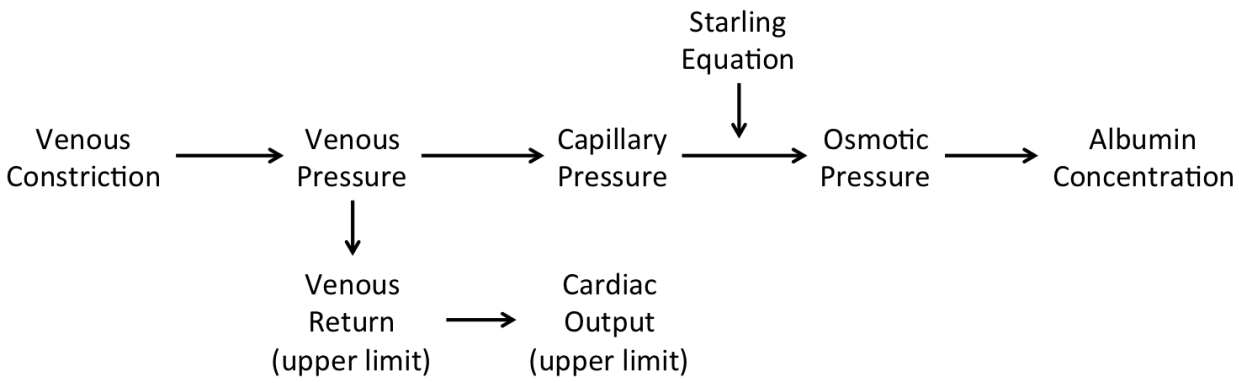
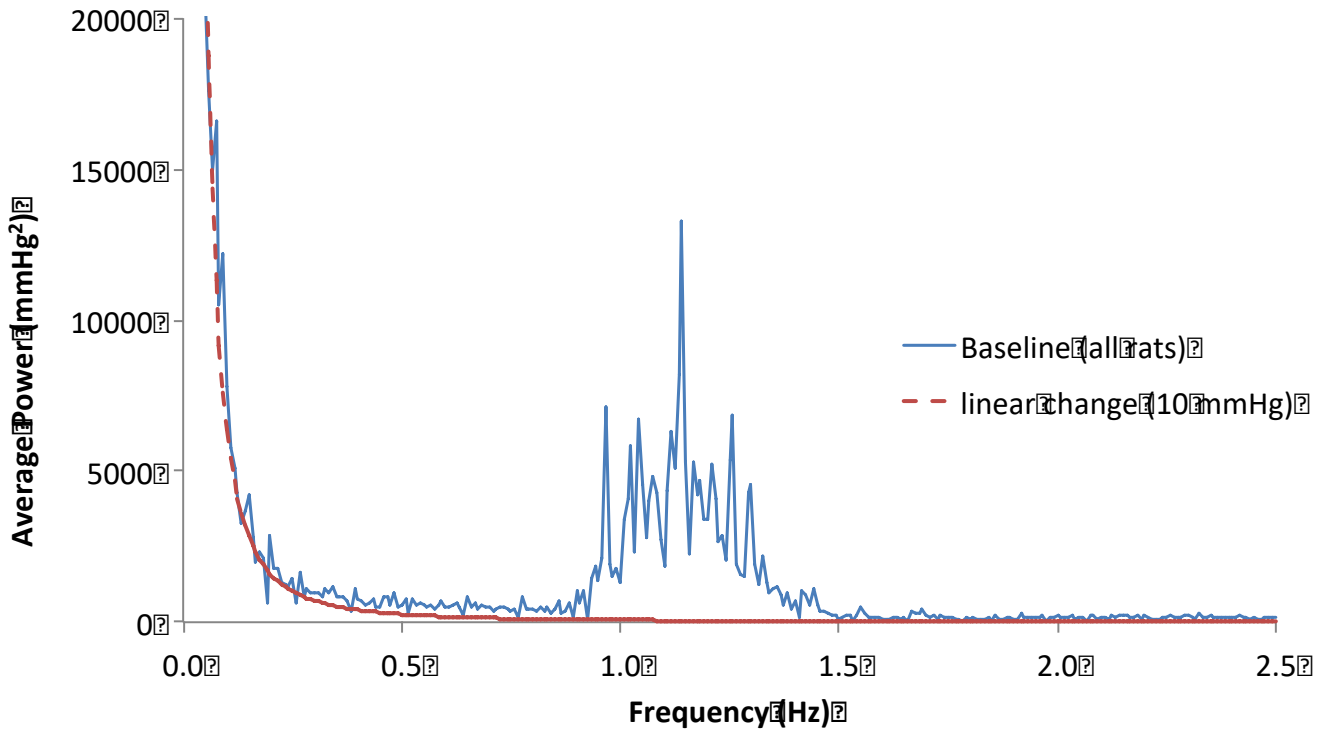


Figure 9 Schematic of the connection between venous constriction, albumin concentration, and maximal cardiac output. Because fluid flux into or out of the vasculature takes time, changes in osmotic pressure may lag behind changes in capillary pressure. However, once steady state has been reached, a change in venous constriction will be reflected by a change in albumin concentration.

Supplemental Material



Supplemental Figure 1 Mean power spectrum for mean arterial blood pressure of all subjects at baseline compared to the power spectrum of a line with slope 5 mmHg/min (i.e. a 10-mmHg difference between starting and ending pressure in a 2 min period). The Fast-Fourier Transfer assumption of repeating data causes an artifact in data segments that end at a different value than they begin, resulting in high power at the low frequency range, as demonstrated by the power spectrum for the line segment. The absence of peaks in the low frequency range suggests that Mayer waves (reported to appear around 0.4 Hz) were either suppressed or shifted into very low or high frequency ranges.

Supplemental Calculations

Can autotransfusion of fluid <u>alone</u> account for the albumin concentration drop at T = 1 h?

Total starting blood volume (TBV): assumed to be 60 ml/kg body mass
Hematocrit at Baseline (BL): 0.426 (42.6%)
Vascular volume (at BL): 1.00 (100% of starting volume)
Albumin concentration ([Albumin]) at BL: 48.6 mg/ml

Plasma Fraction (PF): 1 – 0.97 * hematocrit

Hematocrit is determined by capillary tube centrifugation method.

Vascular albumin at BL (mg per kg body mass) = $[Albumin]_{BL}$ * PF_{BL} * $Vascular\ volume_{@BL}$ * TBV

= 48.6 mg/ml * (1 – 0.97 * 0.426) * 1.00 * 60 ml/kg

= 1711 mg/kg

Vascular albumin after 45% hemorrhage = 1711 * (1 - 0.45) = 941 mg/kg

If no net albumin enters or leaves vasculature between hemorrhage and resuscitation at T = 1 h (i.e. vascular volume change due to autotransfusion is the only reason for change in albumin concentration), what would be the calculated [Albumin] at T = 1 h, given the measured changes in vascular volume and hematocrit?

Vascular volume (at T = 1 h): 0.63 (63% of starting volume)

Hematocrit (at T = 1 h): 0.376 (37.6%)

Calculated [Albumin] = Vascular albumin / [$PF_{@T=1}$ * $Vascular\ volume_{@T=1}$ * TBV]
= 941 mg/kg / [(1 – 0.97 * 0.376) * 0.63 * 60 ml/kg]
= **39.2 mg/ml**

Measured [Albumin] (at T = 1 h): **39.4 mg/ml**

Conclusion: the measured autotransfusion of fluid is sufficient to account for the change in albumin concentration.