Direct Peritoneal Resuscitation Improves Inflammation, Liver Blood Flow, and Pulmonary Edema in a Rat Model of Acute Brain Death

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BACKGROUND: Brain death in organ donors alters central hemodynamic performance, impairs physiology, exaggerates inflammation, and causes end-organ microcirculatory dysfunction and hypoxia. A new treatment, direct peritoneal resuscitation (DPR), might improve these derangements in acute brain death (ABD).

STUDY DESIGN: We studied a standardized rodent model of brain death with matched controls to assess the efficacy of DPR as a resuscitation strategy after ABD. Anesthetized Sprague-Dawley rats were randomized as follows: ABD (supradural balloon inflation) with minimal IV fluid (IVF; 2 mL/h, n = 12); ABD + adequate IVF (5 mL/h, n = 12); ABD with aggressive IVF (goal: mean arterial pressure [MAP] >80 mmHg, n = 15); or ABD + IVF + DPR (goal: MAP >80 mmHg, n = 12). Ventilation support, IVF, and DPR were started at loss of reflexes, and MAP, heart rate, and effective hepatic blood flow were recorded.

RESULTS: High IVF and DPR prevented mortality (0%) compared with low IVF (81.8%) or mid IVF (16.7%). Effective hepatic blood flow was decreased in low and mid IVF (2.8 ± 0.3 mL/min/g body weight and 4.0 ± 0.5 mL/min/g body weight, respectively) vs baseline, but was stable in high IVF (6.2 ± 0.5 mL/min/g body weight; NS) or improved with DPR (8.6 ± 0.7 mL/min/g body weight). The high-IVF group had significant organ edema, which was prevented in the DPR group. The mid-IVF and low-IVF groups had higher serum markers of organ injury compared with high-IVF or DPR groups. The high-IVF group had elevated inflammatory cytokines compared with the DPR group.

CONCLUSIONS: Direct peritoneal resuscitation improved survival and effective hepatic blood flow, required less IVF to stabilize blood pressure, prevented organ edema, and normalized fluid electrolyte balance compared with IVF-alone groups. Direct peritoneal resuscitation in animals reduced inflammatory response after ABD compared with IVF-alone controls. These data suggest a potential role for DPR in organ donors to stabilize donors and possibly increase the number of organs suitable for transplantation per donor. (J Am Coll Surg 2014;219:79–89. © 2014 by the American College of Surgeons)

Disclosure Information: Nothing to disclose.
Research was sponsored by a grant from the Kentucky Organ Donor Affiliates.
Presented at the Western Surgical Association 121st Scientific Session, Salt Lake City, UT, November 2013.
Received January 3, 2014; Revised March 25, 2014; Accepted March 25, 2014.
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Organ transplantation is the curative treatment for multiple end-stage diseases, unfortunately, the availability of organs for transplantation has not been sufficient to meet the high demand. Nationally, the number of patients waiting for a transplant is >100,000, and the number of organ donors is approximately 8,000. Problems with organ procurement include family refusal to donate, inability to recognize slowly evolving brain death, and physiologic instability after brain death.1,2 This has led to strategies for expanding the limited deceased donor pool, increasing living organ donation, and pushing to increase organ donation after cardiac death.1,2 Although these strategies expand the pool of potential organ donors, donation of thoracic organs and liver continue
to be a problem across the nation. Donor management goals, or the aggressive medical management of hemodynamic instability in the brain-dead donor before organ procurement, have been successful in increasing the number of organs donated per donor. This simple idea has opened the realm of research into how manipulation of the brain-dead donors’ physiology can increase or enhance the number and functions of the organs donated at time of procurement.

Brain death is associated with substantial hemodynamic and hormonal instability, as well as an increase in inflammatory processes throughout the body. After brainstem herniation, an autonomic storm occurs, lasting hours, which causes transient increases in systemic vascular resistance, cardiac force of contraction, and cardiac output. After the catecholamine surge, sympathetic vascular tone decreases substantially, resulting in profound hypotension and organ hypoperfusion. Unfortunately, conventional resuscitation with blood products, crystalloid, and vasopressors, although allowing a restoration of central hemodynamics, sacrifices peripheral organ perfusion. This organ hypoperfusion can lead to tissue hypoxia, cellular necrosis, and inflammation, which can compromise graft viability and reduce function in the organ recipient.

Additionally, changes in electrolyte composition in the serum and cells due to the initiation of hyperosmolar therapy before brain death and in response to physiologic derangements can have a profound effect on organ-specific blood flow. Endothelial and cellular edema can lead to reduced capillary diameter and a reduction in effective blood flow to the organs. Also, the hypernatremia in potential donor organs can cause intracellular water accumulation, cell lysis, and potential organ damage when the organs are transplanted in patients with normal sodium levels.

New and better treatment options for organ donors to preserve organ function are needed to increase the suitability of organs for donation. Direct peritoneal resuscitation (DPR) is a novel treatment for hypovolemic shock that reverses visceral organ hypoperfusion and dysfunction to prevent development of multiple organ failure in hemorrhagic and septic shock models. In resuscitated hemorrhagic shock, DPR prevents edema formation, prevents systemic inflammatory response syndrome, stabilizes intestinal and liver blood flow, and improves survival in animals. In a small nonrandomized clinical trial in trauma patients with hemorrhagic shock, DPR decreased time to primary wound closure and prevented septic complications. Acute brain death (ABD) in trauma patients presents a similar low-flow condition in the visceral circulation, and we propose that DPR might provide stability to visceral blood flow in ABD with ventilator and IV fluid (IVF) support similar to that found in resuscitated hemorrhagic shock.

In the current study, we hypothesized that liver perfusion, organ function, inflammatory cytokine expression, and organ-specific edema are worsened in a slow-onset model of ABD, and that the addition of DPR using warm 2.5% Delflex solution (Fresenius) would reverse that event and minimize end-organ dysfunction. To test this hypothesis, we studied a rat model of ABD produced by supradural angioplasty balloon catheter inflation to increase intracranial pressure.

**METHODS**

**Animals**

Rats were maintained in the American Association for the Accreditation of Laboratory Animal Care—approved Veterinary Medical Unit of the Robley Rex Veterans Affairs Medical Center in Louisville, Kentucky. The research protocol was approved by the Institutional Animal Care and Use Committee and the Biohazard Safety Committee at the Robley Rex Veterans Affairs Medical Center. Fifty-one Sprague-Dawley rats (198 to 222 g) were acclimated at the Robley Rex Veterans Affairs Medical Unit of the Robley Rex Veterans Affairs Medical Center. Fifty-one Sprague-Dawley rats (198 to 222 g) were acclimated for 2 weeks before experimental use, during which time the animals received standard rat chow (20 g/d) and water ad libitum. Rats were randomly assigned to one of the following groups: ABD plus minimal IVF resuscitation (low IVF, n = 12); ABD plus fixed IVF resuscitation (mid IVF, n = 12); ABD plus aggressive IVF to maintain blood pressure >80 mmHg (high IVF, n = 15); or ABD plus IVF management and DPR (IVF + DPR, n = 12). Direct peritoneal resuscitation was given as 30 mL warm (37.0°C) 2.5% Delflex solution by intraperitoneal injection at the time of the start of IVF and mechanical ventilation. The electrolyte composition per liter of the Delflex is as follows: dextrose 2.5 g, sodium chloride 576 mg, calcium chloride 26.1 mg, magnesium chloride 15.4 mg, sodium lactate 353 mg, and sodium bicarbonate 29.4 mg. This concentration was chosen based on earlier laboratory and clinical studies outlined in this article.
Earlier laboratory evidence demonstrates that this fluid is not absorbed via the peritoneal cavity. In the first group, the only IVF resuscitation that the animals received was the fluid necessary to perform the galactose clearance technique, which was a 1-mL bolus before baselines and 1 mL/h during the experimental protocol, which amounts to 4 to 5 mL normal saline total. In the mid-IVF group, IVF administration after ABD included the fluid for the galactose clearance plus 6 mL/h, or approximately 15 to 16 mL normal saline total. The high-IVF group’s animals received as much fluid as necessary to maintain mean arterial pressure (MAP) >80 mmHg.

**Acute brain death model**

Anesthesia was induced with pentobarbital (50 mg/kg intraperitoneally) and supplemented with subcutaneous injections (25 mg/kg) to maintain a surgical plane throughout the protocol. Before surgery, animals received 1 mL subcutaneous normal saline to maintain body fluid homeostasis during the surgery. Body temperature was monitored and maintained at 37.0 ± 0.5°C using a heating pad, rectal thermistor, and temperature feedback controller. A tracheotomy was performed and animals spontaneously breathed room air. The left femoral artery was cannulated for monitoring MAP and heart rate, as well as the left femoral vein for IVF infusion. A burr hole was created in the skull at the paramedian space near the frontal transverse sinus and a 4F angioplasty balloon catheter was placed into the supradural space. Slow infusion (1 mL normal saline/h) into the balloon catheter induced brain death with the rat positioned prone with head down and hind end elevated (ie, Trendelenburg position).11

Once the blood pressure began to rise, indicating the sympathetic surge, the animal was mechanically ventilated (rodent ventilator; Harvard Apparatus) with tidal volume of 1.6 mL and rate of 80 breaths/min. These settings were determined in preliminary experiments that analyzed arterial blood gas measurements in rats undergoing this model of ABD. Balloon catheter inflation was stopped when MAP peaked during the sympathetic surge and loss of blink and withdrawal reflexes occurred. Anesthesia was withheld after initiation of brain death. Brain death was confirmed after completion of each experiment by apnea test. Rats in the second group had IV normal saline infusion started at a rate of 0.1 mL/min for the duration of the protocol in addition to the saline in the galactose infusion. In the third group that received aggressive IVF administration, normal saline infusion was started to maintain MAP >80 mmHg at an initial rate of 0.2 mL/min, which was increased up to 0.5 mL/min as required.

**Effective hepatic blood flow**

The galactose clearance method that estimates effective hepatic blood flow (EHBF) has been used in both experimental animals and humans. Earlier laboratory work demonstrates that IV-infused galactose is cleared by first-order kinetics within the liver. It is not secreted within the urine and evaluation of the peritoneal fluid of the experimental animals demonstrates that it is not present in this fluid. An initial bolus of galactose (2.6 mg/mL normal saline/5 min) was infused via the femoral vein before initiation of brain death. After completion of the bolus, a constant galactose infusion (13 mg/mL/h) was maintained until completion of the protocol (Fig. 1). Steady-state systemic galactose concentration ([Gal]SS) was readily achieved 30 to 40 minutes after the initial bolus and [Gal]SS was verified with repeated blood samples (0.2 mL) 15 minutes apart. When variation in MAP, heart rate, and [Gal]SS between samples was <10%, the brain-death protocol was initiated. Effective hepatic blood flow was determined in triplicate at 40 and 120 minutes post...
brain death using the equation: \( EHBF = \frac{I}{[\text{Gal}]_{SS}} \), where 
I is infusion rate (13 mg/mL/h) and EHBF is expressed in mL/min/g body weight. Figure 2 demonstrates the linear response in galactose clearance related to EHBF. Correlation with other accepted techniques, such as laser Doppler flowmetry, are included for verification and confirmation of this technique.

Wet-to-dry weight ratio calculation
Organs were weighed and homogenized (after addition of 1 mL distilled water), and the homogenate was weighed. A portion of the homogenate (approximately 0.6 mL) was centrifuged (16,000 \( g \) for 8 minutes) for assay of hemoglobin concentration in the supernatant. The remainder of the organ homogenate was desiccated in an oven (70°C for 24 hours) for determination of dry weight. Organ wet-to-dry weight ratio was computed from wet and dry weights, organ and blood hemoglobin concentrations, and blood wet and dry weights by standard procedures.12,13

Serum protein and electrolytes
In addition to the MAP, heart rate, and EHBF, a complete metabolic panel was measured at 120 minutes post brain death (VS2 Blood Chemistry Analyzer; Abaxis Inc). The measured chemistries were Na\(^+\), K\(^+\), Ca\(^{++}\), PO\(_4\)\(^{3-}\), glucose, albumin, total protein, globulins, total bilirubin, alkaline phosphatase, alanine aminotransferase (ALT), amylase, blood urea nitrogen, and creatinine. Also, Luminex 23-plex cytokine panel was used to measure serum cytokine profiles in the 4 groups using a MapPix Magnetic multiple analyte machine (EMD Millipore).

Statistical analysis
All data are expressed as mean ± SEM. Differences between groups and time points were determined by 2-way ANOVA for repeated measures. Differences between groups at a single time point were determined by 1-way ANOVA. When differences were found using 2-way ANOVA for repeated measures or ANOVA, the post-hoc Tukey-Kramer honestly significant difference multiple range test was applied. The null hypothesis that there were no differences between time points or groups was rejected a priori at \( p < 0.05 \). A priori power analysis demonstrated that \( n = 12 \) per group would be sufficient to detect previously measures difference in hepatic blood flow at >80% confidence.

| Table 1. Preprocedural Body Weights and Baseline Hemodynamic Data |
|------------------|------------------|------------------|------------------|------------------|
|                  | Low IV fluid \((n = 12)\) | Mid IV fluid \((n = 12)\) | Aggressive IV fluid \((n = 15)\) | IV fluid + DPR \((n = 12)\) |
| Preprocedure variables |                  |                  |                  |                  |
| Body weight, g  | 215 ± 3          | 211 ± 3          | 217 ± 4          | 218 ± 4          |
| MAP, mmHg      | 107.3 ± 3.0      | 114.7 ± 4.1      | 109.7 ± 3.1      | 117.9 ± 4.5      |
| HR, beats/min  | 368.1 ± 11.0     | 368.6 ± 15.0     | 361.7 ± 10.5     | 354.4 ± 9.0      |
| LBF, mL/min/g body weight | 6.2 ± 0.4        | 6.7 ± 0.4        | 6.3 ± 0.4        | 6.9 ± 0.4        |
| Post procedure variables |                  |                  |                  |                  |
| MAP, mmHg      | 55.3 ± 3.0       | 68.9 ± 4.4       | 84.3 ± 4.1       | 81.3 ± 3.6       |
| Total IV fluids, mL | 3.6 ± 0.4        | 16.6 ± 1.1\(^*\) | 46.0 ± 3.5\(^{**}\) | 12.7 ± 1.6\(^{**}\) |

Values are mean ± SEM.
The intracranial balloon catheter volume needed to induce acute brain death and total IV fluid administered in all groups. Additionally, the ending mean arterial pressure and total IV fluid infused at the conclusion of the experiment.

\(^*\)\( p < 0.05 \) vs low-IV fluids group.
\(^{**}\)\( p < 0.05 \) vs mid-IV fluids group.
\(^{***}\)\( p < 0.05 \) vs high-IV fluids group by 1-way ANOVA and Tukey-Kramer honestly significant difference test.
DPR, direct peritoneal resuscitation; HR, heart rate; LBF, liver blood flow; MAP, mean arterial pressure.
RESULTS

Table 1 shows the body weights and baseline values for blood pressure, heart rate, and liver blood flow for the 4 groups. There were no differences in baseline values between groups for body weight, MAP, heart rate, liver blood flow, or intracranial balloon catheter volume to induce ABD among groups. Intravenous fluid volumes were different between groups, with the mid-IVF and high-IVF groups receiving significantly more IVF than the low-IVF group per the experimental design. Within the DPR group, the peritoneal fluid was collected at the end of the procedure. Volume was slightly higher than, but significantly different from the instillation volume (30 ± 0.1 mL instilled vs 30.8 ± 1.7 mL collected; p = 0.20). This finding is consistent with previous data demonstrating nonabsorption of the dialysis fluid. Additionally, galactose levels were nondetectable within the peritoneal fluid, demonstrating conservation of intravascular galactose for EHBF determination. The DPR group had significantly decreased IVF requirements compared with the high-IVF group and was similar to the mid-IVF group.

Figure 3 shows that inflation of the intracranial catheter induced the classic sympathetic surge, with significantly elevated heart rate and MAP in all groups, which subsided after the intracranial inflation was stopped, and rats then quickly developed hypotension. Heart rate remained elevated in the low-IVF (until 40 minutes post brain death) and mid-IVF (until 60 minutes post brain death) groups and MAP dropped significantly at 5 minutes post brain death in the low-IVF fluids group and at 80 minutes post brain death in the mid-IVF group. In the high-IVF group, heart rate was considerably elevated shortly after the start of the IV saline infusion at 5 minutes post brain death, and MAP remained near baseline levels throughout the protocol. Urine output appeared to be grossly increased in the group receiving aggressive IVF administration, but it was not quantified in any experimental group. Liver blood flow decreased significantly in the low-IVF group at 40 minutes post brain death compared with either mid-IVF or high-IVF groups, and animals in the low-IVF groups continued until 59 ± 4 minutes post brain death on average (range 45 to 80 minutes post brain death). However, in the high-IVF group, liver blood flow was protected throughout the 120-minute experimental protocol. The addition of DPR to the resuscitation treatment significantly improved liver blood flow at 40 and 120 minutes after brain death compared with baseline levels or with low-, mid-, or high-IVF groups.

Only 4 of the animals in the low-IVF group avoided cardiovascular collapse to the completion of the 120-minute protocol, so only these 4 were available for complete metabolic panel data from this group. Table 2 shows that in both the mid- and high-IVF groups, calcium, total protein, albumin, and globulins were decreased compared with historic control values, which is likely due to increased plasma volume. This finding is corroborated by the “normal” total protein, globulin, and albumin levels in the DPR group compared with sham controls. Serum glucose was not significantly different between the mid-IVF and high-IVF groups. The DPR group did have a higher serum glucose level compared with the other resuscitation groups, however, this was not significantly different than the sham animals tested earlier, indicating that these values all fall within normal physiologic parameters. Alanine transaminase was elevated in the mid-IVF group (107.8 ± 4.1 U/L) compared with the high-IVF group, DPR group, or historic control data, suggesting that aggressive fluid
administration and DPR both stabilized liver blood flow and also protected against liver damage. Similarly, the mid-IVF group’s animals also exhibited elevated creatinine and blood urea nitrogen, which was prevented by both aggressive IVF administration and administration of DPR. Neither the DPR group nor the high-IVF group was different from controls.

In evaluating organ-specific edema, wet-to-dry weight ratio was calculated. Intravenous fluid resuscitation after onset of ABD was associated with edema formation in the lung, liver, and ileum. The addition of DPR prevented or decreased the incidence of edema formation compared with the high-IVF resuscitation groups at 2 hours post brain death (Fig. 4). There was no difference in organ-specific edema formation between the mid-IVF and DPR groups.

Serum cytokine levels from multiple analyte protein assays are shown in Table 3. With regard to anti-inflammatory cytokines, the low-IVF group’s animals had high levels of interleukin (IL)-4 and IL-10 compared with the other groups. The addition of DPR to the resuscitation regimen increased levels of interferon-γ and IL-2, but decreased IL-4, IL-10, and IL-13 compared with the high-IVF group. Pro-inflammatory cytokines were decreased in the IVF + DPR group with regard to IL-1β, IL-5, IL-6, IL-17, and IL-18, suggesting that DPR modulates the systemic inflammatory response in this model of ABD. In comparison with the mid-IVF group, the high-IVF group’s animals had higher levels of IL-1α, IL-1β, IL-6, and IL-18. There was no statistical difference in tumor necrosis factor–α in any group, most likely due to the time point in the experiment when the sample was drawn. These findings suggest that DPR improves the cytokine and chemokine status of animals with ABD to moderate the systemic inflammatory response compared with what occurs in this model when resuscitated with IVF and mechanical ventilation alone.

**DISCUSSION**

Experiments in dogs and rodents have demonstrated the classic catecholamine response associated with ABD.6,7 Peripheral organs exposed to this intense sympathetic response often demonstrate decreased perfusion and organ dysfunction, which provides a potential explanation for the high rate of nonviable organs for transplantation after brain death.6,7 The rate of induction of brain death influences the degree of autonomic instability produced. Shivalker and colleagues examined the rate of brain-death induction and its effect on catecholamine response, cardiac function, and organ viability for transplantation.6

They found that induction of brain death resulted in animals with a large sympathetic surge (1,000-fold increase in circulating catecholamine compared with normal levels). In addition, evaluation of myocardial viability (via histopathology) showed that 93% of the myocardium was severely ischemic.

In the current study, we used an established model of ABD to characterize physiologic responses in the absence of pharmacologic support.5,11,14 This model mirrors clinically observed physiologic responses in potential organ
donors with ABD and demonstrated the classic sympathetic surge that occurs during brain stem herniation. The novelty of the current study is the comparison of 3 levels of IVF administration with the use of DPR on blood pressure, heart rate, liver blood flow, and liver enzyme levels, and cytokine response to therapy. Our findings with this model showed vascular collapse shortly after the catecholamine surge, which required aggressive IVF administration to forestall cardiovascular collapse.

If aggressive normal saline infusion was not started immediately in those earlier animals, MAP rapidly collapsed to <80 mmHg. In those earlier studies, animals with MAP that remained <80 mmHg had compromised liver blood flow, elevated ALT levels, and ischemic hepatitis (data not shown). Therefore, in our current study, aggressive IVF normal saline administration was used to maintain MAP and effectively preserve both liver perfusion (ie, EHBf) and hepatocyte integrity (ie, ALT).

Figure 4. Wet-to-dry weight ratios in lung, liver, and ileum after the acute brain-death protocol at 2 hours post brain death. *p < 0.05 vs low IV fluids; †p < 0.05 vs mid IV fluids; ‡p < 0.05 vs high IV fluids by 1-way ANOVA and Tukey-Kramer honestly significant difference test. DPR, direct peritoneal resuscitation; IVF, IV fluids.

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<th>Table 3. Changes within the Inflammatory Response as Indicated by Circulating Serum Cytokine Data</th>
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*p < 0.05 vs low-IV fluids group.
†p < 0.05 vs mid-IV fluids group.
‡p < 0.05 vs high-IV fluids group by 1-way ANOVA and Tukey-Kramer honestly significant difference test.
DPR, direct peritoneal resuscitation; IFN, interferon; IL, interleukin; MCP-1, monocyte chemotactic protein-1; RANTES, regulated on activation normal T cell expressed and secreted; TNF, tumor necrosis factor.
However, the preservation of liver blood flow and hepatocyte integrity comes at the cost of the large fluid volume needed to maintain pressure (43.6 ± 4.4 mL/2 h). This high crystalloid resuscitation volume leads to substantial electrolyte abnormalities and organ edema, particularly in the ileum, lung, and liver. This edema can lead to a reduction in organ acceptance for donation. For example, increased pulmonary edema can reduce PO2 and lead to elevated pulmonary vascular resistance. These 2 parameters help guide a thoracic surgeon in making decisions about the adequacy of donor’s lungs for procurement. To “recruit” the lungs for donation, an intensivist might increase the positive end expiratory pressure on a patient or begin to treat the hemodynamic instability with IV pressors. Both of these steps can lead to reduction in organ blood flow and end-organ ischemia, which compromise organ function in the recipient. A familiar clinical conundrum exists: Do we risk poor organ perfusion to enhance thoracic organ transplantability or do we sacrifice pulmonary function to enhance renal, hepatic, and gastrointestinal organ blood flow?

Direct peritoneal resuscitation presents a novel technique in organ-specific resuscitation and allows us to find a middle ground. The success of donor-management goals demonstrated that targeted organ-specific resuscitation in donors can have a profound effect on the number of organs transplanted per donor.15-19 In this study, animals treated with DPR were able to maintain a systolic blood pressure at >80 mmHg with vastly reduced volumes of fluid infusion, leading to a reduction in organ edema. Additionally, markers for organ injury were not significantly increased over the high-IVF group, indicating a preservation of visceral organ perfusion, despite a reduction in resuscitation volumes. Indemnifying the high-IVF group is the substantial reduction in serum albumin and globulins, indicating that perceived reductions in metabolic parameters are most likely explained by hemodilution rather than a preservation of organ perfusion/function. In essence, DPR allowed the experimenters to have the best of both worlds, effective visceral blood flow, stabilization of central hemodynamic parameters, and a minimization of organ edema. Yet, the reason for this impact on organ-specific edema goes beyond simple IVF infusion rates.

The role of donor inflammation on organ function in the recipient is not well understood, however, studies have postulated that the progressive detrimental effects of brain death on donor organ quality might be linked to elevated inflammatory processes.20-25 Direct peritoneal resuscitation appeared to have a profound effect on the inflammatory response within this model. Compared with both mid- and high-IVF groups, DPR-treated animals showed a substantial reduction in several circulating monokines (ie, IL-1α, IL-β, IL-4, IL-6, IL-10, IL-13, and IL-18), and a general reduction in the proinflammatory response and enhancement of the anti-inflammatory response. Interestingly, interferon-γ levels were significantly increased in the DPR group compared with both the high- and mid-IV fluid groups. The reasons for this are unclear. In other models using DPR, we have shown that a reduction in these proinflammatory cytokines leads to a reduced immune-mediated neutrophil infiltration into ischemic organs, a reduction in ROS-mediated cellular injury, and a reduction in immune-mediated endothelial cell injury.24-25 Speculatively, this could be due to a reduction in damage-associated protein formation due to enhanced organ blood flow or possibly a “wash out” of these proinflammatory mediators, leading to a reduction in systemic inflammation. Regardless, additional investigations into these unique findings are warranted.

CONCLUSIONS
We present DPR as an innovative technique in the resuscitation of acute brain-dead donors. We have shown that DPR augments visceral blood flow and reduced organ edema, and allows for a more stable MAP using considerably less IVF for resuscitation. Direct peritoneal resuscitation also reduced systemic inflammatory proteins and can mitigate the inflammatory response after brain death. These findings offer hope for a new avenue of research in the resuscitation of brain-dead organ donors, and might offer a method for increasing future organ donation rates.

Author Contributions
Study conception and design: Smith, Hurt, Garrison, Matheson
Acquisition of data: Smith, Ghazi, Cain, Matheson
Analysis and interpretation of data: Smith, Matheson
Drafting of manuscript: Smith, Matheson
Critical revision: Hurt, Garrison

REFERENCES
Discussion

INVITED DISCUSSANT: DR ALAN HEMMING (San Diego, CA): Dr Smith and his colleagues from Louisville presented an elegant study on the effects of direct peritoneal resuscitation (DPR) on liver blood flow and pulmonary edema in a rat model of brain death. They demonstrated that in this model, DPR improves liver blood flow and reduces pulmonary edema. Additionally, DPR appears to reduce the proinflammatory cytokine release that is also characteristically observed in human brain dead donors.

Why is this important? One of the more common dilemmas faced in managing brain dead donors until time of organ allocation and procurement is balancing the conflicting needs of the various organ teams. Lung teams generally require the donor to be run dry to minimize pulmonary edema and maintain oxygenation; however, this may require additional inotropic support and be detrimental to other organs. Heart and abdominal organ teams prefer optimizing tissue perfusion and reducing inotropic requirements by giving additional volume. A fine line is walked to maximize organ use while optimizing organ quality. Direct peritoneal resuscitation, at least in this model, may make this line wider.

Additionally, there is growing evidence that suggests that the state of brain death activates surface molecules on peripheral organs by the massive release of macrophage and T cell-associated cytokines and adhesion molecules into the circulation. This leads to nonspecific endothelial and complement activation, which in turn, lead to organ injury and reduced function. This proinflammatory release of cytokines has also been associated with increased organ immunogenicity and subsequent rejection. In this model, DPR abrogates the massive release of cytokines and one can at least postulate that there may be multiple downstream benefits as well as the potential to reduce the proinflammatory cytokine release and one can at least postulate that there may be multiple downstream benefits.

The authors are to be congratulated on their innovation in applying their findings from previous work in DPR and trauma to a perplexing problem for transplantation. I have several questions:

1. Liver blood flow was the highest in the DPR group, yet the alanine aminotransferase (ALT) was approximately 1.5 times that in the sham animal group, while the high IV fluid animals had normal values for ALT. Why is this happening?

2. You did not find an increase in tumor necrosis factor (TNF) alpha in this model although in other models of brain death it has clearly been shown to increase. Can you explain?

3. If this were to be attempted in human brain dead donors, how would the logistics work? Do you have any data on whether the hepatic blood flow by direct peritoneal resuscitation improves survival and prevents hepatic inflammation following hemorrhagic shock. Am J Physiol Gastrointest Liver Physiol 2012;303:G1144—G1152.