A Pilot Study of Four Intraosseous Blood Transfusion Strategies

Jonathan Auten*,1; Julie B. Mclean, PhD, CTR2; Jean D. Kemp3; Paul J. Roszko4; Grady A. Fortner2; Alyssa L. Krepela6; Alexandra C. Walchak, MS, CTR7; Chemely M. Walker, CTR8; Travis G. Deaton9; Joanna E. Fishback10

ABSTRACT

Background: Intraosseous (IO) access is used by military first responders administering fluids, blood, and medications. Current IO transfusion strategies include gravity, pressure bags, rapid transfusion devices, and manual push-pull through a three-way stopcock. In a swine model of hemorrhagic shock, we compared flow rates among four different IO blood transfusion strategies. Methods: Nine Yorkshire swine were placed under general anesthesia. We removed 20 to 25mL/kg of each animal’s estimated blood volume using flow of gravity. IO access was obtained in the proximal humerus. We then autologously infused 10 to 15mL/kg of the animal’s estimated blood volume through one of four randomly assigned treatment arms. Results: The average weight of the swine was 77.3kg (interquartile range, 72.7kg–88.8kg). Infusion rates were as follows: gravity, 5mL/min; Belmont rapid infuser, 31mL/min; single-site pressure bag, 78mL/min; double-site pressure bag, 103mL/min; and push-pull technique, 109mL/min. No pulmonary arterial fat emboli were noted. Conclusion: The optimal IO transfusion strategy for injured Servicemembers appears to be single-site transfusion with a 10mL to 20mL flush of normal saline, followed immediately by transfusion under a pressure bag. Further study, powered to detect differences in flow rate and clinical complications, is required.

Keywords: blood transfusion; operational medicine; intraosseous infusion; intraosseous transfusion; hemorrhagic shock

Introduction

Intraosseous (IO) access is used by military first responders administering fluids, blood, and medications during remote damage-controlled resuscitation (rDCR).1 Prehospital blood transfusions for severely battle-injured military personnel have been associated with an improvement in mortality in the austere environment.2 Unfortunately, multisystem trauma, such as dismounted complex blast injury, presents a vascular access challenge to even the most seasoned medical teams attempting to initiate rDCR.1 In cases where access is difficult, IO catheters provide a noncollapsible method that serves as a bridge to therapy while preparations are made for central venous access.3,4

IO blood transfusion can present technical challenges, because medullary pressure is approximately one-third of systemic pressure.5 To improve IO transfusion flow rates, the pressure gradient between bone and systemic circulation must be increased.6 The existing literature has shown few associations between increasing IO transfusion pressure gradients and significant clinical complications like pulmonary arterial fat embolism, hemolysis, or coagulopathy.7,8

However, after a decade of use in combat settings, the broad acceptance of IO blood transfusions in adult DCR is still questioned.9 Clinical concern stems from foundational studies that used a skeletally immature swine model to determine the safety of IO blood transfusions.9,10,11 The bone density of a healthy man 20 to 40 years old is roughly double that of skeletally immature swine used in those studies.9,12 Bone density or media permeability is a critical physical property in Darcy’s law, which describes the relationship of fluid flow through porous media.13 Darcy’s law predicts increased transfusion pressures are required to maintain or improve flow rates with increasing bone densities and fluid viscosity.14 Current pressurized military IO transfusion strategies include using gravity, pressure bags, rapid-transfusion devices, and manual push-pull of a syringe with a three-way stopcock.1,7 Although swine are an established model for human bone and cardiovascular physiology, the clinical effects of different pressurized IO transfusion strategies in skeletally mature adults is not fully understood and requires further investigation.9,15–17

In this pilot study, we compared four different IO blood transfusion strategies with varying degrees of transfusion pressure in a swine model with similar bone density to that of an adult Military Servicemember. We hypothesized that increasing transfusion pressures would lead to increased flow rates with nonsignificant differences in rates of pulmonary arterial fat embolism, coagulopathy, and periosteal damage at the transfusion site.

Methods

All activities were approved by the Naval Medical Center Portsmouth Institutional Animal Care and Use Committee and conducted in compliance with the Animal Welfare Act

9Correspondence to Naval Medical Center Portsmouth, Department of Emergency Medicine, 620 John Paul Jones Circle, Portsmouth, VA 23708; or jonathan.d.auten.mil@mail.mil
1LCDR Auten, MC USN; 2Dr Mclean; 3LCDR Roszko, MC USN; 4LT Fortner, MC USN; 5LT Krepela, MC, USN; 6Ms Walchak; and 7Ms Walker are at Naval Medical Center Portsmouth, Department of Emergency Medicine, Portsmouth, VA. 8LCDR Kemp, MC USN, is at Naval Medical Center Portsmouth, Department of Pathology, Portsmouth, VA. 9CDR Deaton, MC, USN, is at Naval Medical Center San Diego, Department of Emergency Medicine, San Diego, CA. 10MAJ Fishback, VC, USA, is at Naval Medical Center Portsmouth, Clinical Investigations Department, Portsmouth, VA.
and Department of Defense regulations. Animals were maintained in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International in accordance with the Guide for the Care and Use of Laboratory Animals.18 Our study included nine Yorkshire swine (Sus scrofa), weighing between 70kg and 90kg. This weight range was chosen because it represents the 50th percentile bone density range of the average adult male 20 to 39 years old, the group that constitutes the majority of our Combat Forces.12,19 All animals were healthy, intact females. No animals were excluded because of disease, injury, or illness before commencement of the study. Females were chosen because prior research reported minimal differences in bone density among male, female, and barrow swine.20

Transfusion Strategies
Animals were randomly assigned to one of the following four transfusion strategies (Figure 1): (1) gravity (n = 2); (2) pressure bag (n = 2); (3) rapid-transfusion device (n = 2; Belmont Rapid Infuser pump; Belmont Instrument Corp., http://www.belmontinstrument.com/); and (4) manual push-pull using a syringe and three-way stopcock (n = 3).12,19,22

FIGURE 1 Intraosseous transfusion strategies.

Left to right: Gravity transfusion; pressure bag transfusion; Belmont Rapid Infuser; and push-pull transfusion.

Gravity
In the gravity arm, transfusion in two swine occurred through a single IO access in the left proximal humerus via the pressure generated from the weight of the blood hanging from a standard IV pole for hanging intravenous (IV) fluids and medications. The IV pole was positioned lateral to the proximal humerus IO insertion site prior to transfusion.

Pressure bag
In the pressure-bag arm, the first animal had a single IO access placed in the left proximal humerus, a bag of blood was hung from a standard IV pole, and a pressure bag was inflated to and maintained at or above 300mmHg by a member of the research team. The second animal in the pressure-bag arm had IO access placed in the left and the right proximal humeri and blood was transfused simultaneously through both sites using a pressure bag.

Rapid infusion
In the Belmont Rapid Infuser arm, the rapid infuser was set up according to manufacturer guidelines. The transfusion rate was set at 100mL/min in the first animal and 50mL/min in the second animal to detect any difference in frequency of machine alarm signals due to overpressure. The Belmont is designed to sound an alarm, display a “High Pressure” message, and stop the transfusion at the factory-determined maximum pressure limit at or above 300mmHg. When the high-pressure alarm sounded, the research assistant silenced the machine and manually restarted the transfusion. The number of pressure alarms during a 5-minute interval was recorded.

Push-pull transfusion
In the manual push-pull arm, three swine were transfused with either a 10mL, 20mL, or 60mL syringe connected to a three-way stopcock. The 50mL syringe transfusion method was previously described by the British Medical Emergency Response Team during Operation Enduring Freedom.22 Pediatric resuscitation literature advocates for 10mL or 20mL syringes to decrease hand fatigue.23

Anesthesia
Activities were conducted in a controlled, designated veterinary surgical suite. Animals were fasted before anesthesia and premedicated with glycopyrolate (0.05mg/kg intramuscularly [IM]), ketamine (20mg/kg), and xylazine (2mg/kg). Once endotracheal intubation was achieved, the animals were mechanically ventilated with a mixture of isoflurane and oxygen, using a large animal veterinary anesthesia machine (Hallowell EMC, www.hallowell.com/). Butorphanol (0.2mg/kg IM) was given for analgesia. Vital signs were continuously monitored every 15 minutes. All animals were euthanized at the completion of the protocol under general anesthesia.

Experimental Hemorrhage
To establish baseline clotting strength, three thromboelastogram (TEG) measurements were performed for each animal before inducing hemorrhage. In addition, three TEG measurements were performed at the conclusion of transfusion, and at time of death or 1-hour after transfusion, whichever occurred first. The left carotid artery was exposed using a standard cutdown technique and cannulated using a Seldinger technique with a 9F introducer catheter for arterial, invasive blood pressure monitoring during the experimental procedures. Using a similar cutdown technique, the femoral artery was cannulated with a 9F introducer catheter to induce a controlled hemorrhage. After instrumentation, there was a 10-minute stabilization period during which baseline hemodynamic data, including hemoglobin, hematocrit, an electrolyte panel, and lactate were collected. These values were repeated immediately after transfusion and at time of death or 1-hour after transfusion, whichever occurred first.

A controlled hemorrhage was performed by collecting blood through the force of gravity into a 450mL blood-bag system containing 63mg of citrate phosphate dextrose adenine. The blood-container bag was located on a weight scale and rocker (Genesis BPS, http://www.genesisbps.com/) below the animal. An intended range of 20–25mL/kg blood was removed over 15 to 40 minutes. Hemorrhage and blood collection were halted if the mean arterial pressure (MAP) reached 30mmHg or less. After experimental hemorrhage, specimens were collected for testing and analysis. After hemorrhage completion, each animal was given 30 minutes to allow time for vital signs to stabilize.

IO Access
After the stabilization period, an IO catheter was placed according to the manufacturer’s instructions. A 15-gauge, 45mm,
IO needle (EZ-IO; Teleflex Medical, www.teleflex.com) was inserted in the proximal humeral head of the animal with a device driver (EZ-IO) from the same manufacturer. Placement was confirmed by successful bone marrow aspiration verification of patency by flushing with 10mL of normal saline (0.9% sodium chloride) and, finally, by positively identifying the IO insertion site on fluoroscopy. The IO was flushed with a second 10mL saline flush before initiating the transfusion strategy by treatment group. Approximately 10–15mL/kg of autologous blood was transfused per animal per treatment group. Transfusion flow rates were calculated during the first 5 minutes of transfusion. The remainder of the transfusion was completed after this flow rate was documented.

**Tissue Sample Collection and Analysis**
Surviving animals were euthanized while under general anesthesia and tissue samples were collected for analysis. The humerus from subject 5 was analyzed via dual energy x-ray absorptiometry (DEXA) for bone density (Figure 2). The IO needles from the second subject of each study arm were collected and the residual effluent material within the needle was submitted to pathology for analysis under microscopy. The humerus of the second animal in each treatment arm was removed for analysis and cross-sectioned proximal to transfusion site. These specimens were submitted to the laboratory, decalcified, and evaluated for changes to the bony matrix. Architectural changes, periosteal hemorrhage, bone debris, or necrosis within the matrix were reported descriptively.

Representative 2cm × 2cm segments of the upper and lower left lung were collected and placed in 10% formalin and submitted for pathologic assessment for gross evidence of fat embolism. The lung samples submitted in formalin were grossly examined for areas of infarct or hemorrhage. Representative sections were processed for routine hematoxylin-and-eosin (H/E) staining. The resultant slides were examined after H/E staining for evidence of fat or bone marrow emboli (i.e., fat and/or marrow elements in the lumen of a vessel). The second subject in each transfusion-strategy arm had an additional sample from the left upper lung and lower lung transported directly to the Pathology Department in a sterile container for staining with Oil Red O stain to examine for fat embolism. The lung samples sent for Oil Red O staining were serially sectioned and a random section was selected for analysis. Half of the section was snap frozen in optimal cutting temperature compound for sectioning and subsequent staining with Oil Red O. The other half was submitted in formalin for routine processing as a control. The highest density of parenchymal fat globules on stained slides was located and the globules were quantified by counting the number of globules in this area per 10 contiguous high-power fields. Analysis was conducted by a staff pathologist at Naval Medical Center Portsmouth who was blinded to transfusion strategy group.

**Results**

**Characteristics of Study Subjects**
Nine swine were evaluated during this pilot study. Data from the push-pull arm with 60mL syringe were excluded from data analysis. On tissue collection for the swine undergoing this technique with the 60mL syringe, it was noted that the IO access had migrated through the posterior aspect of the bony cortex during push-pull transfusion initiation. Given the accurate position of the IO on fluoroscopy before push-pull transfusion, the research team thought direct transfusion from syringe into the needle caused needle migration from its original pretransfusion position. Our protocol was refined to transfuse the remaining subjects in the push-pull group through accompanying IV tubing and not directly into the needle hub. The remaining two push-pull subjects were transfused with 10mL and 20mL syringes through IV tubing connected to the IO access and no needle migration was observed.

This resulted in a total of eight swine in which the study protocol was completed successfully—two swine in each study arm. Median weight of the swine was 77.3kg (interquartile range [IQR], 72.7–88.8kg). Median volume of hemorrhage was 1,231mL (IQR, 1,143–1,382), which corresponded to an estimated median 24.2% blood loss (IQR, 21.5–25.2). The density of the proximal humerus of the study subject undergoing DEXA scan was 1.027g/cm². Baseline laboratory data and characteristics of each animal were collected (Table 1).

**Main Results**
Flow rates in the two subjects in the gravity arm were 5mL/min. In the rapid-transfusion group, flow rates were 31mL/min with an average of seven overpressure alarms per 5 minutes and pressure average of 280mmHg in both subjects. A single-pressure

<table>
<thead>
<tr>
<th>TABLE 1 Laboratory Results (Mean Values)</th>
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<tr>
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<tr>
<td><strong>Hematocrit (%) PCU</strong></td>
</tr>
<tr>
<td><strong>Grav</strong></td>
</tr>
<tr>
<td>Baseline</td>
</tr>
<tr>
<td>Postinfusion</td>
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<tr>
<td>Postobservation</td>
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<tr>
<td><strong>Ionized calcium (mmol/L)</strong></td>
</tr>
<tr>
<td><strong>Grav</strong></td>
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<tr>
<td>Baseline</td>
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<tr>
<td>Postinfusion</td>
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<tr>
<td>Postobservation</td>
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<tr>
<td><strong>Lactate (mmol/L)</strong></td>
</tr>
<tr>
<td><strong>Grav</strong></td>
</tr>
<tr>
<td>Baseline</td>
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<tr>
<td>Postinfusion</td>
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<td>Postobservation</td>
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<td><strong>pH</strong></td>
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<td><strong>Grav</strong></td>
</tr>
<tr>
<td>Baseline</td>
</tr>
<tr>
<td>Postinfusion</td>
</tr>
<tr>
<td>Postobservation</td>
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</table>
bag in the proximal humerus infused at a rate of 70mL/min in subject 1 of the pressure-bag arm. The flow rate of the double IO site strategy was 103mL/min in subject 2 of the pressure-bag arm. Push-pull transfusion provided infusion rates of 109mL/min (Figure 3). The first subject in the push-pull arm experienced a decreased MAP to 20mmHg at minute 7 of transfusion, but recovered and completed the observation period. Subject 2 had a decreased MAP to 20mmHg at minute 7 and subsequently went into pulseless electrical activity. The animal died before completion of the 1-hour observation period. There were no other significant hemodynamic variations noted among the additional arms (Figure 4).

FIGURE 3 Flow rates by transfusion strategy.

<table>
<thead>
<tr>
<th>Delivery Method</th>
<th>Subject 1</th>
<th>Subject 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gravity</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Belmont</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Pressure Bag</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td>Push Pull</td>
<td>120</td>
<td>120</td>
</tr>
</tbody>
</table>

Belmont, Belmont Rapid Infuser; PB, pressure bag.

TEG values were taken on samples from each animal and those values were averaged by study arm. Baseline and 1-hour posttransfusion TEG values were reported for time of latency from the start of the test to initial fibrin formation, time taken to achieve a certain level of clot strength, measure of speed at which fibrin builds up and cross linking takes place, ultimate strength of the clot, and degree of fibrinolysis. No physiologically significant changes among transfusion strategies were noted on baseline TEG or TEG drawn at 60 minutes after transfusion (Table 2).

None of the 32 examined H&E-stained slides of lung showed any arterial fat or bone marrow emboli. There were no pulmonary arterial fat emboli noted on Oil Red O staining. There were pulmonary fat globules within the lung parenchyma on Oil Red O staining in each of the transfusion strategies (Table 3); however, no pulmonary arterial fat emboli were noted. Decalcified cross-sections of the infusion site showed no evidence of abnormal bony architecture, periosteal hemorrhage, necrosis, or bone debris. The effluent from the IO catheters of those same study subjects contained only fragmented red blood cells on microscopic analysis. No cortex or bone marrow was identified within the effluent.

Discussion

IO access serves a critical role in combat medical care delivered in the prehospital environment.2,3 During Operation Enduring Freedom, medical providers used IO access more than 1,000 times during combat operations.4 However, concern still exists regarding the use of IOs in DCR.5 To our knowledge, this pilot study is one of the first to study IO blood transfusion flow rates and potential complications in a swine model with bone density similar to the active-duty military population.

The flow rates measured in the gravity arm of our study cannot meet the clinical demands of remote DCR. With flow rates of 5L/min, it would take over 3 hours to transfuse 1,000mL of autologous whole blood. Our study suggests that the Belmont Rapid Infuser system is a suboptimal method for transfusing blood through an IO route. The flow rates were higher than those in the gravity arm; however, transfusion was interrupted by overpressure alarms in both animals seven times over 5 minutes. It would take over 30 minutes to transfuse 1,000mL of whole blood via the rapid infuser and this device requires the provider to continuously restart the machine after an alarm. Push-pull, single-site, and double-site pressure-bag transfusion strategies achieved flow rates that would allow for 2,100mL or more to be transfused over 30 minutes. However, in the push-pull arm, one subject died and the other displayed significant hemodynamic changes. Evidence of pulmonary fat or bone marrow globules were noted within the lung parenchyma of all study subjects analyzed. There was no evidence of pulmonary arterial fat emboli or architectural changes to the bone cortex or marrow with any of the four transfusion strategies.

Maximizing blood transfusion flow rates is vital in the first hour of care delivered to a critically ill trauma patient, and combat practice guidelines suggest a potential role for IO access in the care of bilateral lower extremity amputations secondary to dismounted complex blast injuries.6 Research in humans and animals has shown that IO infusion rates with pressure bags through sternal or proximal humeral access are superior to gravity alone.25–29 Our study found similar results, suggesting that pressure bags confer an advantage over gravity by increasing flow rates through an IO. The frequency of overpressure alarms with the Belmont machine was similar that reported in previous research.30 Although we were able to continue transfusion by silencing the overpressure alarm and restarting the machine, this may not be desired in an austere medical environment. The findings of our pilot study concur with prior findings that double-site IO infusion strategy produces higher fluid flow rates than a single-site IO infusion.30

The bone density reported in our study subject that underwent DEXA scanning was similar to that reported in prior animal research evaluating the humeral head of 60–90kg swine.19 The bone density of our study subject was similar to that in adult studies reporting the average bone density of the upper arm of human men 20–40 years old.31 The density was double the predicted bone density of the <10kg swine used to initially establish safety of IO blood transfusion.8 Prior studies evaluating
TABLE 2 Pre- and Posttransfusion Thromboelastogram Values

<table>
<thead>
<tr>
<th>Transfusion Strategy</th>
<th>R (s)</th>
<th>K (s)</th>
<th>(°)</th>
<th>M (mm)</th>
<th>Lys30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gravity Pressure bag (LF)</td>
<td>BL</td>
<td>4.85± 2.3</td>
<td>4.2± 2.6</td>
<td>1.3± 0.6</td>
<td>1.3± 0.9</td>
</tr>
<tr>
<td>Gravity Pressure bag (UF)</td>
<td>T+60</td>
<td>5.65± 3.9</td>
<td>6.1± 2.2</td>
<td>1.7± 0.6</td>
<td>2.0± 0.1</td>
</tr>
<tr>
<td>Belmont Rapid Infuser (LF)</td>
<td>BL</td>
<td>5.5± 3.0</td>
<td>6.1± 1.8</td>
<td>1.25± 0.8</td>
<td>1.2± 0.1</td>
</tr>
<tr>
<td>Push-pull (LF)</td>
<td>T+60</td>
<td>6.35± 1.8</td>
<td>5.65± 4.3</td>
<td>1.6± 0.5</td>
<td>1.4± 0.8</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation. All n values based on two subjects. α, measure of speed at which fibrin builds up and cross linking takes place; BL, baseline averages (three samples per subject); K, time taken to achieve a certain level of clot strength; Lys30, degree of fibrinolysis; M, ultimate strength of the clot; R, time of latency from the start of the test to initial fibrin formation; T+60, 60 minutes postinfusion (three samples per subject).

TABLE 3 Pulmonary Pathology Findings

<table>
<thead>
<tr>
<th>Transfusion Strategy</th>
<th>Pulmonary Arterial Fat Emboli (Histology or Oil Red O Stain)</th>
<th>Fat Droplets/10 HPF (Oil Red O Stain)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gravity/pressure bag (UF)</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Gravity/pressure bag (LF)</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Belmont Rapid Infuser (UF)</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Belmont Rapid Infuser (LF)</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Pressure bag single site</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Pressure bag single site</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>Pressure bag double site</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Pressure bag double site</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>Push-pull (UF)</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Push-pull (LF)</td>
<td>0</td>
<td>22</td>
</tr>
</tbody>
</table>

HPF, high-power field; LF, lower lung segment tissue sample; UF, upper lung segment tissue sample.

Research has suggested IO transfusion may result in pulmonary fat embolism. In the current study, no evidence of pulmonary arterial fat embolism was found. However, in subjects in each transfusion-strategy group, we did find varying degrees of fat globules within the lung parenchyma.

Future research should include plasma-free hemoglobin to test for rates of hemolysis and be powered to detect these differences among transfusion strategies that vary by pressure and anatomic site. Another limitation is that results from a swine model may not directly translate to humans. However, swine have very similar bone, cardiovascular, and blood physiology, and serve as an excellent model for this type of research.15,17

The major limitation of this pilot study is that it was not powered to detect differences in flow rate or hemolytic, osseous, or pulmonary complications among the transfusion strategies. We also lacked the logistic capability to evaluate rates of hemolysis or renal inflammation as performed in prior research.8,17 Future research should include plasma-free hemoglobin to test for rates of hemolysis and be powered to detect these differences among transfusion strategies that vary by pressure and anatomic site. Another limitation is that results from a swine model may not directly translate to humans. However, swine have very similar bone, cardiovascular, and blood physiology, and serve as an excellent model for this type of research.15,17

Our study also differs from prior studies that used platelet, fibrinogen, or plasma-free hemoglobin levels to determine rates of hemolysis secondary to transfusion.8,17 We cannot comment directly on hemolysis among our strategies. However, we evaluated for clotting ability by testing TEG values and found no physiologically significant difference among strategies. We report a faster infusion rate than another study on IO blood transfusion in animals with higher bone density.17 Our methodology differs in that we placed the IO access, flushed 10mL of saline, and immediately began to transfuse. The previous study delayed transfusion until 20 minutes after initial IO insertion. Tactical Combat Casualty Care (TCCC)currently advocates for gravity as an IO transfusion strategy. This practice is not supported by the findings of our pilot study. TCCC instructors anecdotally have reported the resistance encountered before IO infusion as a “bone plug” that needs to be cleared.3 In our assessment of the bone matrix and effluent from the IO needle, we found no destruction of the matrix or marrow content that suggested a bone plug. However, findings in the literature support the TCCC recommendation of a 10–20mL flush of normal saline before infusion. The physiologic differences between systemic pressure and the pressure within the marrow is likely causal in the resistance encountered before IO transfusion, not a bone plug.35

Conclusion

IO blood transfusion by gravity alone cannot meet the requirements for rDCR. The optimal strategy currently appears to be IO blood transfusion with a 10–20mL flush of normal saline followed immediately by transfusion under 300mmHg via a pressure bag with a member of the resuscitation team inflating...
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the bag to keep pressures at or above 300mmHg. Although this was a pilot study, one of the two nonexcluded animals in the push-pull group died during infusion and the other had a concerning hypotensive episode. The push-pull and double-barrel methods may confer a benefit in speed of transfusion but should be further studied to determine the effect these increased transfusion pressures have on clinical end points like hemolysis, fat emboli, hemodynamic stability, and shear stress injury to the bony matrix. addition, given the future need for prolonged field care and the growing body of literature on IO blood transfusion, a combat practice guideline for IO use in the early echelons of care may be beneficial.

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We thank Dr Greg Zarrow of the Combat Trauma Research Group for his mentorship, assistance with data interpretation, and formal presentation. Research data were derived from an approved Naval Medical Center, Portsmouth, Virginia, Institutional Review Board protocol No. NMCP 2016.0040.

Presentation
This study was presented at the 2017 Navy Wide Academic Research Competition, 25 May 2017, in Portsmouth, Virginia.

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Disclosure
The authors have indicated they have no financial relationships relevant to this article to disclose.

Author Contributions
JDA, JBM, PJR, CMW, JEF, and TGD designed this study. All authors contributed to the literature search. JDA and CMW managed institutional review board submission. JDA, CMW, ACW, GAF, and ALK collected data. JDA, JLM, and MRG contributed to data analysis. JDA, JBM, JDK, PJR, CMW, and TGD performed data interpretation. JDA JDK, JBM, PJR, CMW, and TGD contributed to writing the manuscript. All authors participated in critical revision. All authors approved the final version of the manuscript.

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