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TITLE: Preclinical Evaluation of the Effects of Aeromedical Evacuation on Military-Relevant Casualties

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14. ABSTRACT

Aeromedical evacuation is associated with several stressors that may cause harm during casualties' transport. In addition to these stressors, timing, oxygen supplementation and altitude may have additional effects that are currently unknown. The purposes of this proposal are to better evaluate the effect of these additional variables and define an adequate timing, oxygen supplementation level and best appropriate altitude to maintain normal organ physiology. This proposal includes the use of two different animal models, rats and swine, using different and complement strategies to better understand the effects of aeromedical evacuation. So far, our results showed the feasibility of our models to monitor the effects of aeromedical evacuation on neurobehavioral damage, inflammatory response and hemodynamic changes. Specifically, we showed no changes in behavioral or pathological changes in the short term of transport after injury in the rat model but noticed modification in the inflammatory response. In addition, we established a swine model that can be used to monitor hemodynamic changes during aeromedical evacuation with different levels of oxygen and altitude. Overall, we were able to show that restricted oxygenation (21%) is not sufficient for a wounded warrior. However, it was not clear if 100% oxygenation was necessary due to the limited number of experimental animals.

15. SUBJECT TERMS

Traumatic brain injury; hemorrhagic shock; aeromedical evacuation; oxygenation; altitude; timing of evacuation

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1. INTRODUCTION:

Current practice for the aeromedical evacuation of combat casualties during recent US military conflicts has included transport of the critically injured patient to the Continental United States (CONUS) after stabilization. On average, service members return to role V care in five to seven days. Aeromedical transport exposes service members to additional environmental exposures such as hypobaria, hypoxemia, localized pressure differential related to air trapped within a body cavity, vibration, and in some cases, hypothermia. Current guidelines for critical care air transport teams (CCATT) include considerations for these exposures including flight specific guidelines for adequate oxygen saturation parameters, ventilation parameters, and goals for blood pressure. In flight, these parameters may be more difficult to achieve than in a conventional ICU setting. The physiologic, cellular, and molecular impacts of hypobaria on the transport of critically ill patients remain relatively unknown and the current guidelines for aeromedical transport are based on observation of physiologic parameters during patient transport.

This grant incorporates three projects that address specific operational issues regarding optimization of aeromedical evacuation standards. In established animal models of combat polytrauma, we will investigate the 1) impact of the timing, 2) altitude, and 3) oxygen supplementation during aeromedical evacuation.

The relevant hypotheses tested will include the following:

1) Aeromedical evacuation closer in proximity to point of injury will result in worse histologic and inflammatory outcomes than delayed aeromedical evacuation.
2) Aeromedical evacuation at 4,000ft will result in decreased inflammatory outcomes of polytraumatically injured swine when compared to simulated aeromedical evacuation at 8,000ft.
3) During simulated aeromedical evacuation of polytraumatically injured swine, increasing oxygen supplementation at fixed levels will result in incrementally improved inflammatory outcomes on histologic evaluation.

2. KEYWORDS:

Traumatic brain injury; hemorrhagic shock; aeromedical evacuation; oxygenation, altitude; timing of evacuation

3. ACCOMPLISHMENTS:

The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.

During the time period for this funding opportunity, initial investigation into the timing of aeromedical evacuation on polytraumatically injured rats was performed. Based on the data collected from the rat model, no definitive conclusions could be made from the parameters proposed and studied. The focus of investigation for the additional swine work moved to the other named aims in the project with the investigation into the differences when oxygen supplementation is varied at fixed levels during simulated aeromedical evacuation. This project has provided the opportunity to investigate outcomes using conventional critical care invasive monitoring techniques and has allowed for investigation of new technology to measure tissue perfusion percutaneously using the Lumee device developed by Profusa.

What were the major goals of the project?

	Timeline	Method	NMRC
Specific Aim 1: Evaluation of the timing of aeromedical evacuation in rat and swine models of TBI and polytrauma	Months		
Major Task 1: IACUC/ACURO approval	1-3	Writing	Complete
Major Task 2: Rat blast/AE timing experiments	4-20	Animal experiment	Complete
Major Task 3: Swine TBI/polytrauma AE timing experiments	50-62	Animal experiment	Cancelled
Major Task 4: Data analysis/manuscript/final report	68-74	Statistics/ writing	
Specific Aim 2: The effects of oxygen supplementation during aero-medical evacuation on brain oxygenation in swine with fluid-percussion (FP) - traumatic brain injury (TBI)			
Major Task 1: IACUC/ACURO approval	6-9	Writing	Complete
Major Task 2: Swine supplemental O_2 /AE experiments	10-50	Animal experiment	Completed
Major Task 3: Data analysis/manuscript/final report	50-62	Statistics/ writing	Completed
Specific Aim 3: Physiological consequences of 4,000 and 8,000 ft. altitude aeromedical evacuation on swine with traumatic brain injury and hemorrhagic shock			Cancelled
Major Task 1: IACUC/ACURO approval	45-55	Writing	Completed
Major Task 2: Swine AE/altitude experiments	55-68	Animal experiment	Cancelled
Major Task 3: Data analysis/manuscript/final report	68-74	Statistics/ writing	Ongoing

Aim 1/ Major Task 3: Swine TBI/polytrauma AE timing experiments

Aim2/ Major Task 2 Swine supplemental O2/AE experiments.**:** Assess the impact of different levels of fixed oxygen supplementation during aero-medical evacuation on brain oxygenation in swine with fluid-percussion (FP) - traumatic brain injury (TBI).

Aim 3: Physiological consequences of 4,000 and 8,000 ft. altitude aeromedical evacuation on swine with traumatic brain injury and hemorrhagic shock

What was accomplished under these goals?

Aim 1: The swine projects proposed in this study were intended to be based on rat studies that would provide a preliminary insight on differences in organ specific damage related to the timing of aeromedical evacuation following polytrauma. During the data review, there were no clearly observed differences between groups within the parameters examined. The team of investigators decided to suspend additional study of this aim until revisions to the experimental design could be examined in additional rat studies.

Aim 2: Investigation was performed for 21%, 40%, 54% oxygen supplementation in sham and polytraumatically injured animals with some pilot studies in a few animals who received 100% oxygen supplementation during SAE. Initial investigation on the use of the Profusa Lumee device was performed to assess tissue oxygenation as an additional parameter for this study beyond conventional invasive hemodynamic techniques. A revised statement of work specifically with revisions to the number of animals per group and eliminating 70% oxygen supplementation as a group was submitted and the revised experimental proposal was completed.

Aim 3: This aim was eliminated in a recent revision to the statement of work. There were several factors that contributed to this decision which will be detailed extensively in the subsequent report.

(a) Human Use Regulatory Protocols

"No human subjects research will be performed to complete the Statement of Work."

(b) Use of Human Cadavers for Research Development Test & Evaluation (RDT&E), Education or Training

"No human cadavers will be used to complete the Statement of Work."

PROTOCOL (1 of 3 total):

Protocol USUHS-FY17-001.03

Title: Evaluation of the timing of aeromedical evacuation in rat and swine models of TBI and polytrauma.

Title of the Protocol: Evaluation of the timing of aeromedical evacuation in rat (Rattus norvegicus) and swine (Sus scrofa domestica) models of TBI and polytrauma

Target required for statistical significance:
For the rats: Power calculation were performed using a statistical program (IBM SPSS Statistics 23.0; IBM Corporation, 2015 or others software) written to calculate power/sample size.
Based on our previous work, with a normal distribution of differences in hypobaric and normobaric exposed animals, we observed a difference in Mean Arterial Pressure of 16.0 and Standard Deviation 10. Given these values, a sample size of 8 animals per group are necessary to achieve the power of .84 and alpha of .05 by using a two- tail test. The significance level (alpha) of the test is 0.05.

Target approved for statistical significance:

For the rats: 8 animals per group. A total of 264 rats.

Target approved for statistical significance:

For the swine: Power calculation were performed using a statistical program (IBM SPSS Statistics 23.0; IBM Corporation, 2015 or others software) written to calculate power/sample size. Based on our previous work, with a normal distribution of differences in hypobaric and normobaric exposed animals, we observed a difference in Mean Arterial Pressure of 16.0 and Standard Deviation 10. Given these values, a sample size of 8 animals per group are necessary to achieve the power of .84 and alpha of .05 by using a two- tail test.

Target approved for statistical significance:
For the swine: 8 animals per group. A total of 88 swine.

SUBMITTED TO AND APPROVED BY:

- WRAIR/NMRC IACUC 21-SEP-2017
- ACURO approval 22-NOV-2017

Status:

- This protocol was completed under the new protocol number 20-OUMD-28LS

PROTOCOL (2 of 3 total):

Protocol [ACURO Assigned Number]: USUHS-FY17-001.02
Title: Evaluation of supplemental oxygen delivery during aeromedical evacuation in a complex polytrauma porcine (Sus scrofa domestica) model.
Target required for statistical significance:
For the swine: Power calculation were performed using a statistical program (IBM SPSS Statistics 23.0; IBM Corporation, 2015 or others software) written to calculate power/sample size. A decrease of oxygenation below 40 mmHg will be considered significant to detect a change related to oxygen in the brain and/or tissue from baseline to end point. A sample size of 8 animals per group will achieve 80% power to detect a difference between the groups using a one-way ANOVA for this parameter. The significance level (alpha) of the test is 0.05.

Target approved for statistical significance:
For the swine: 8 animals per group. A total of 174 swine

Submitted to and Approved by:
IACUC 20-OUMD-28LS

- IACUC on 15 Nov 2020
- ACURO on 22 Dec 2020

Status:

- Protocol Approved and active

- **Summary of findings**

Maintenance of normoxia is a key tenant of trauma evacuation protocols to prevent a "second hit" injury. It is common to administer 100% oxygen to patients during aero-evacuation to prevent the impact of hypobaric hypoxia during flight; however, hyperoxia has been associated with its own set of deleterious consequences with other groups demonstrating tissue inflammation and free radical formation in early pre-clinical models. The impact of different fixed levels of oxygen supplementation during medical aero-evacuation are being addressed in a swine non-survival model and will help examine an adequate range of oxygen supplementation levels for altitude transport. The injured swine will be exposed to fixed levels of oxygen supplementation: FiO2 of 21, 40, 54, and 100% during simulated aeromedical evacuation at 8000ft (~2500m) compared to ground level (300ft). To help assess tissue oxygenation beyond traditional critical care methods of using invasive and non-invasive technology, a subcutaneous tracking probe based on the principles of near infra-red spectroscopy and phosphorescence quenching of metalloporphyrins (Lumee), has been developed for tracking tissue oxygenation by the company Profusa. This technology was used as an additional method for evaluating tissue hypoxia in the skin and subcutaneous tissue.

- Design and Methods

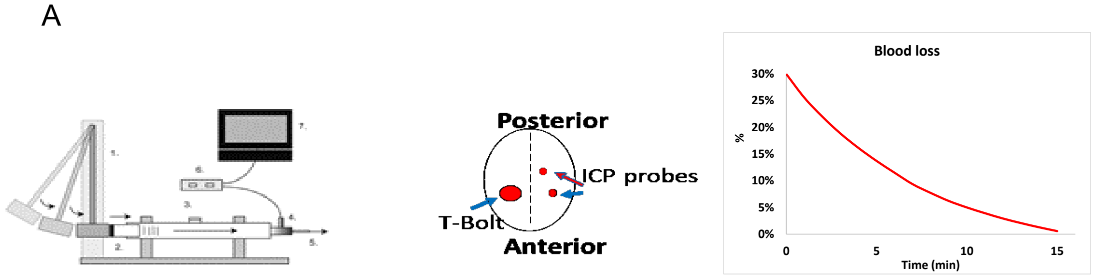
Preparation:
The day of the surgery, anesthesia was induced with ketamine/midazolam and switched to gas anesthesia (2-5% isoflurane) for surgery with the animal being mechanically ventilated with (FiO2 = 0.4) (Apollo® ventilator, Draeger Medical Inc. Telford, PA, USA). A Seldinger modified technique for blunt and sharp dissection was used to expose and catheterize the right external jugular vein (9Fr, Starflex, InSitu) and the right carotid artery (5Fr, Starflex, InSitu). The left femoral arterial catheter (5Fr, Starflex, InSitu) was used for blood pressure (Mean arterial pressure, MAP) monitoring and arterial blood sampling. The left femoral venous catheter (4Fr, Starflex, InSitu) was used for infusion of intravenous anesthetics and maintenance fluids. A Swan-Ganz catheter (7Fr, Edwards Lifesciences LLC, Irvine, CA, USA) was inserted in the jugular and used for measuring pulmonary pressure (PAP) and cardiac output (CO); it was also used to record core temperature. The bladder was identified, isolated, and a 10 Fr foley catheter was inserted. Percutaneous tissue oxygenation was measured using the Profusa implantable Lumee device (Lumee probe, Fig 1B). Using large bore needles according to manufacturer instructions, probes were implanted on Day -5. Four sites were uniformly used including the right lower leg, left lower back, left shoulder, and right upper leg. Probes were placed on the skin to confirm that the device was functional and after 10 min of continuous signal confirmation, the readers were removed, and the animal recovered. On the day of experimental protocol, the readers were placed back on the skin animal and continuous readings were measured throughout the duration of the protocol until euthanasia.

Fluid percussion cortical impact:
Three craniotomies were performed on the animals, one on the mid-parietal skull bone to connect the fluid-percussion device and the other two to quantify the impact of brain injury and intracranial pressure (ICP).

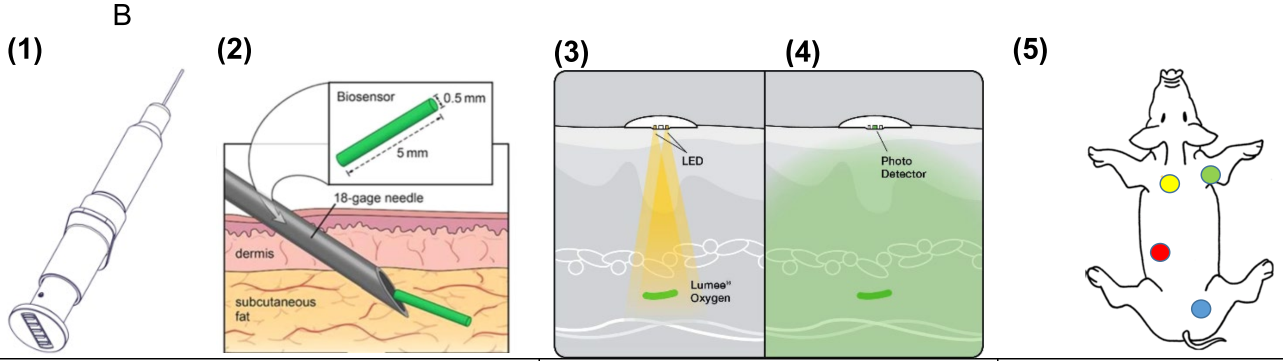
Three craniotomies were performed in the supine position at the mid-parietal skull bone. The first craniotomy measured 16 mm and was used to place an extradural 3-way T-bolt to facilitate connection of the fluid percussion device and pressure transducer (SenSym®, Sunnyvale, CA), used to quantify the TBI impact. (Figure 2). The second and third craniotomies were used to place intracranial probes to record intracranial pressure (ICP) (Codman & Shurtleff Inc., Maynham, Massachusetts, and Rammedic Inc, Mills River, NC). At the time of injury, the pendulum from the percussion device was released from a 35° angle to hit a cylinder filled with water. The pressure that was transmitted to the animal through the hose to the T-bolt caused the TBI impact on the brain.

Hemorrhagic shock: Immediately after TBI, hemorrhage was started (T0) to remove 30% of estimated blood volume (EBV) over 15 min from the femoral artery in a controlled arterial model with half of the blood removed within 5 min and the other half over 10 min (Fig 1A: injury illustration). Resuscitation occurred at T30 and T60 after the initial injury start time with 2 boluses of hetastarch (250 ml over 10 min) that were given through the femoral vein or normal saline. Sham animals that were not injured did not receive resuscitation fluids.

Experimental protocol:
Swine were divided into an injury (TBI+HS) group and a sham group. TBI occurred at time 0 (T0) at the same time as a hemorrhage phase started to remove 30% of EBV over 15 min in a controlled arterial model (Fig 1A: injury illustration). At T30 and T60 after the initial injury start time, resuscitation 2 boluses of hetastarch (250 ml over 10 min) were given. Sham animals did not receive resuscitation fluids. At T120, all animals were transported to the hypobaric chamber for a 4-hour simulated evacuation and an additional hour of stabilization. Swine were divided into normobaria (300ft) and hypobaria (8000ft) groups. TIVA was administered via the CVL with a tapered exchange rate of isoflurane to maintain stable blood pressure. During evacuation, the fixed levels of oxygen supplementation provided to the experimental groups included 21, 40, 54, and 100%. Normobaria was set at baseline 21% FiO2 level to represent the ambient O2; in the hypobaria groups, the FiO2 was adjusted to account for the reduced partial pressure of oxygen at 8000ft and was set to 29%. At the end of the experimental period, the animals were euthanized, and necropsy was performed (Figure 2). Physiologic measurements, including heart rate (HR), MAP, ICP, and end-tidal carbon dioxide (EtCO2), were collected throughout the study. Blood samples, arterial and venous, were collected for blood gas (ABL, Radiometer) and blood cell count (Proccyte, Idexx), and blood chemistry (Alera, Alfawasserman). Additional measures of oxygen delivery (DO2), oxygen consumption (VO2), and oxygen extraction (VO2/DO2) were calculated using standard formula. Immediately following euthanasia, a complete necropsy was performed and select tissues collected for histopathologic analysis. Lungs were inflated and all collected tissues were then immersion fixed in 10% buffered formaldehyde for 24-48 hours. Tissue samples were trimmed, routinely processed, and paraffin embedded. Tissue blocks were then sectioned (4- to 5-μm thick) and stained with hematoxylin and eosin (H&E) for histopathologic analysis. Lesions were identified and graded semi-quantitatively by a board-certified veterinary pathologist blinded toward treatment groups. In addition, immunohistochemistry was performed on lung sections from injured animals following normo or hypobaric evacuation to detect myeloperoxidase (MPO) as an indicator of inflammation and oxidative stress. H&E histology scores were quantified on a 0-5 scale as 0 (not present), 1 (minimal), 2 (mild), 3 (moderate), 4 (marked), 5 (severe). Injury variables were reported based on cellular infiltration, congestion, edema, inflammation and necrosis. For MPO analysis, formalin-fixed, paraffin-embedded sections were deparaffinized by placing the slide in a microwave for 5 min. They were then rehydrated by passage through a graded series of ethanol and distilled water. Slides were incubated in Tris-buffered saline with 0.075% Tween-20 (pH 7.6) for 10 minutes and endogenous peroxidase activity was quenched by incubation of the slides in 0.3% v/v H_2O_2 in methanol for 20 minutes at room temperature. Sections were incubated overnight at 4 degrees with a polyclonal rabbit anti-human myeloperoxidase antibody diluted 1:250 (Agilent Technologies). The sections were subsequently incubated with fluorescein isothiocyanate-labeled anti-mouse IgG and Texas Red-labeled anti-rabbit IgG antibodies (Vector Laboratories) at a dilution of 1:1000 for 1 hour at room temperature, then incubated with DAPI (1:1000) for 5 minutes at room temperature to stain cell nuclei. The slides were then examined using fluorescence microscopy (Zeiss) and images were converted to a binary image and quantified for pixel density analysis conducted using image J software. The number of MPO positive counts on 5 different aeras were averaged after being normalized to DAPI positive counts.

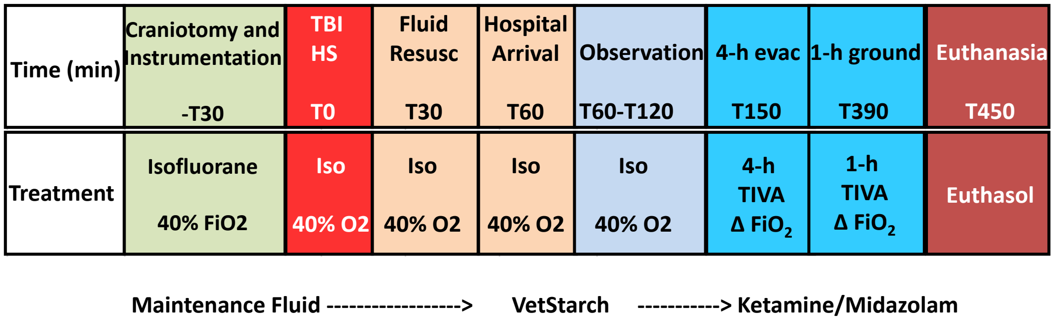


Fluid percussion device Craniotomies Blood loss kinetics



(1) Research-use-only injector pen to place **(2)** the Lumee Oxygen hydrogel (0.5 x 0.5 x 5 mm) in the subcutaneous space using an 18G needle.	**(3)** Lumee Patch reader adheres to the skin above the Lumee Oxygen hydrogel sensor. LEDs on Lumee Patch illuminate Lumee Oxygen hydrogel. **(4)** Lumee Oxygen hydrogel returns a light signal proportional to oxygen concentration which is detected by a photodetector on the Lumee patch.	**(5)** Lumee oxygen hydrogel placement on swine animal model: right lower leg (blue); left lower back (red), left shoulder (yellow), right upper leg (green).

Figure 1: A) Illustration of the injury mechanism of TBI via a fluid percussion device through the craniotomy and a 30% EBV controlled arterial hemorrhage. B) Illustration of the Lumee Oxygen System placement on a swine:1) the Lumee injector, 2) subcutaneous injection, 3) reader, 4) implanted hydrogel and 5) schematic location of the O2 hydrogels in the swine



Time (min)	Craniotomy and Instrumentation -T30	TBI HS T0	Fluid Resusc T30	Hospital Arrival T60	Observation T60-T120	4-h evac T150	1-h ground T390	Euthanasia T450
Treatment	Isofluorane 40% FiO2	Iso 40% O2	Iso 40% O2	Iso 40% O2	Iso 40% O2	4-h TIVA Δ FiO_2	1-h TIVA Δ FiO_2	Euthasol

Maintenance Fluid -----------------> VetStarch -----------> Ketamine/Midazolam

Figure 2: Schematic timeline and experimental design: Day-5: implantation of the Profusa sensors; Day 1: injury by TBI and HS; and resuscitation at T30 and T60; Removal of TBI instrument at T60; monitoring until T120. AE: 4h evacuation – 300 ft or 8000 ft and 1 h stabilization on ground; Euthanasia

Data acquisition, formatting, and statistical analysis

Hemodynamic parameters were measured from the catheter sensors and from pulse oximetry. Blood samples were collected at 60 min regular intervals for blood gas (ABL, Radiometer) and blood cell count (Proccyte, IDEXX). LOI and skin temperature were continuously monitored via the Lumee platform in a subset of animals (sham (n=9), injured (n=14). To adjust for the temperature effect on oxygen measurement, and alignment of the time scale, raw data were transformed using direct input from Profusa personnel. LOI were averaged at each implant site over 3 recording phases: Day-5, in the prep-room, and in the OR. The LOI data were processed, averaged and normalized at T0 using the first 10 min prior to T0. The normalization of the LOI were necessary to extract the pattern observed with each experiment over time. The data were analyzed for normal distribution. The Lumee oxygen microsensors and vitals (CO, MAP, and HR) data were systematically aligned by syncing the time of injury (T0). Pearson-moment correlation coefficients were used to define the strength and direction of a linear relationship between oxygen time traces with simultaneously measured vitals. Briefly, aggregate tissue oxygen traces were calculated by averaging the time traces across all microsensors and by microsensor location for injured. Correlation coefficients were calculated over a 2-hr time window post-injury. For each microsensor location on a pig, correlation coefficients were calculated from the comparison of the microsensor oxygen time trace to each of the vital time traces, and calculated correlation coefficients for all microsensors were reported in terms of average ± 1 standard deviation. Basic descriptive analyses were performed on all physiological and tissue oxygenation variables. A 2-way repeated measures ANOVA was used to determine differences in variables over time, across groups and treatment and to address the relationship among selected physiological variables, LOI and FiO2. Statistical analysis including regression coefficients were obtained using Excel (Microsoft 365), and GraphPad Prism (version 8.0.0 for Windows) software.

•Results

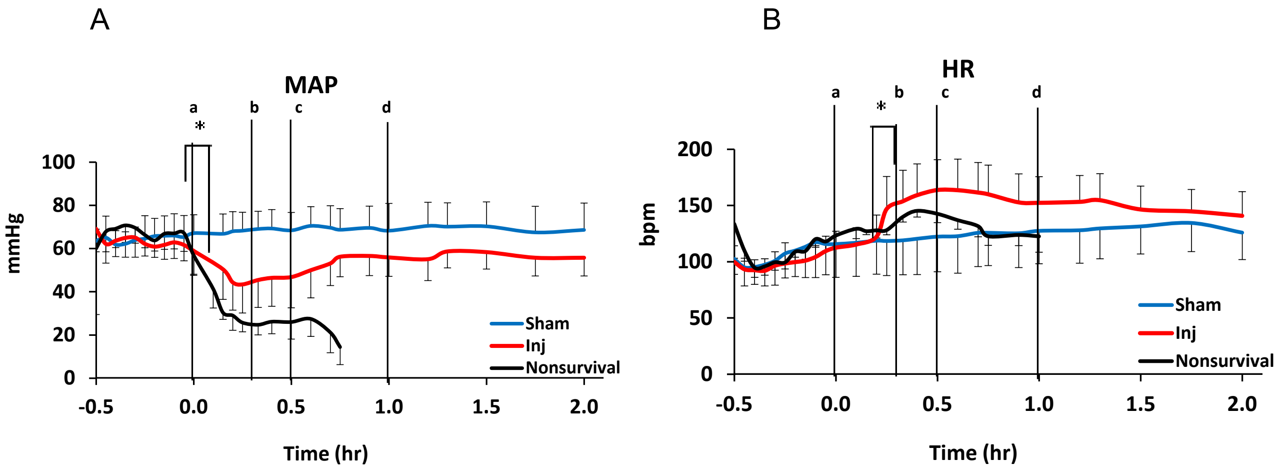
The results are presented in two sections: the surgical phase and the evacuation phase.

•Surgical phase

Forty-three anesthetized Yorkshire swine underwent instrumentation for controlled cortical impact traumatic brain injury and hemorrhagic shock. The average weight of the study animals was 34.6± 3.9 kg. Animals (n=29) were injured via fluid percussion followed by a 30% controlled arterial hemorrhage. Shams (n=14) were instrumented but not injured. The animals were observed for 2 hrs post-injury using MAP, HR, CO, and blood gas. Lumee probes were placed subcutaneously 5 days before catheter placement and craniotomy of the animals. Vital signs and Lumee data were normalized and aligned to a uniform baseline value at the time of injury (t=0). Normalized and aligned Lumee and vitals data were analyzed for correlation (R) during the 2 hrs post-injury. Of the 29 injured animals, 7 expired (non-survival, NS) before the end of the surgical phase.

The fluid-percussion impact on the dura resulted in an average pressure of 24.6 ± 5.0 psi. This resulted in an ICP increase from a baseline of 6.2 ± 2.1 mmHg to a maximum of 21.8 ± 8.4 mmHg ($p<0.001$) occurring immediately after the impact. ICP then decreased and returned to baseline (5.3 ± 3.8 mmHg) by 30 min for all animals.

The hemodynamic parameters are presented in Figure 3. All measured vital signs (MAP, PAP, HR, and CO) demonstrated minimal variation in the Sham group during the 2 hr observation period. In the polytrauma group, MAP and CO trends decreased from the time of injury until the end of the hemorrhage while HR increased consistent with hemorrhagic shock physiology. Injured animals received 2 fluid boluses per protocol resulting in increases in MAP and CO and decrease in HR, though not to baseline. There were no statistically significant differences observed in PAP between groups. The animals in the non-survival group demonstrated decreases in CO and MAP and increase in HR without complete recovery.



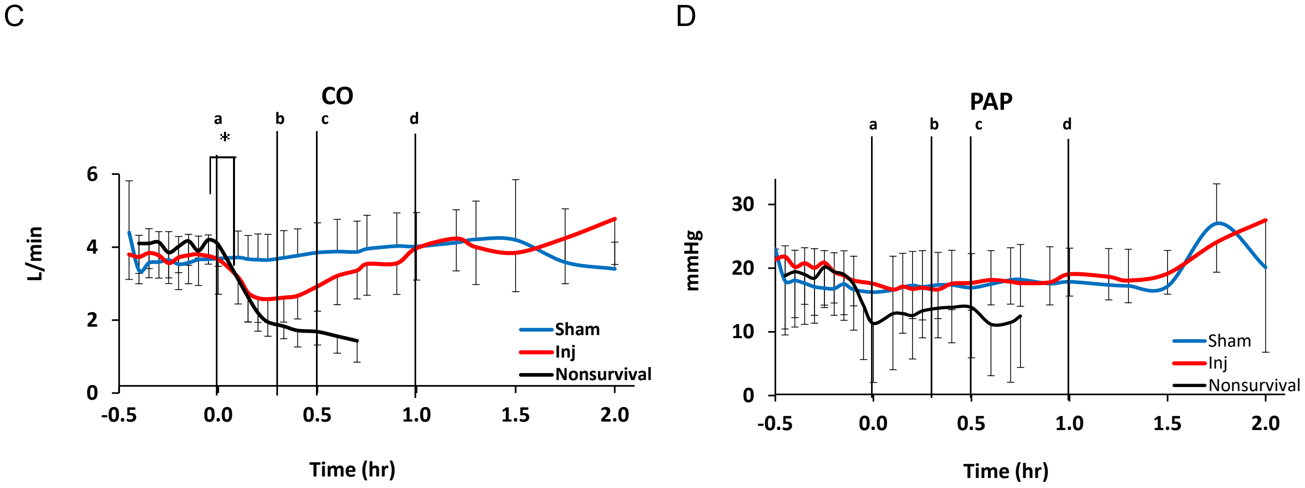


Figure 3: Average hemodynamic parameters per group during the post-injury observational period for 29 animals. Vertical lines indicate the major step in the procedure: (a) start of injury (T0), (b) end of injury (0.25 hr); (c) fluid resuscitation (0.5 hr) and (d) disconnection of the fluid percussion (1 hr). A: MAP Mean arterial Pressure, B: CO Cardiac output, C: HR Heart rate, D: Pulmonary pressure, and E: Temperature. Mean and standard deviation. *p<0.01.

Brain tissue oxygenation (Raumedics) was measured through a probe inserted in the small craniotomy. There was a high variability in the recorded measurements. Data were normalized to the initial reading at T0. No statistically significant differences were observed between groups. The measured oxygenation in the non-survivors trended below baseline after injury.

For the data collected as %LOI from the Lumee platform, across sites and between animals, a high degree of variability was observed. In conjunction with scientific support from Profusa, the data was normalized and trends in tissue oxygenation were observed. Three groups were compared: Sham, injured, and non-survival (NS) (Figure 4). The average of LOI% for each of the 4 sites on animals in the survivor injury group is shown in Fig 7A. LOI% from all sites for each experimental group were compared among all groups (Fig. 4B). LOI% in the Sham group remained stable with an observed average increase over the last 45 min of the injury phase ($p<0.05$). In contrast, the LOI% in the groups of injured animals decreased sharply after injury (T0), then increased after crystalloid resuscitation in the injured group ($p<0.001$). This trend in LOI% for the non-survival group remained depressed ($p<0.05$). The increasing trend of LOI % in the injured group did not reach the Sham level at T120 ($p<0.01$). Vital sign parameters were compared with LOI% to assess for correlation between invasive and non-invasive monitoring methods. There was a strong correlation between the observed trends in LOI% with CO and MAP. Similarly, vital sign trends in CO and MAP correlated with LOI% measured from the Lumee system in the NS group. Peripheral oxygen saturation (SpO2) as measured by pulse oximetry remained normal throughout the duration of this portion of the experimental protocol and averaged 99.8 ± 0.3% for Sham and 99.6 ± 1.0% for injured and 99.4 ± 1.1% NS group.

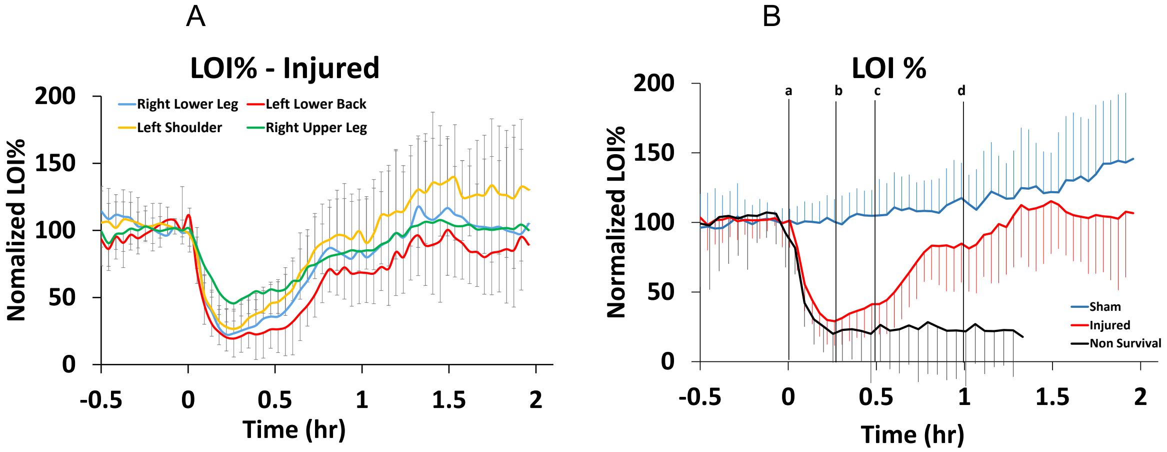


Figure 4: Tissue oxygenation measured by the Lumee device and presented as normalized LOI (LOI%) during the 2-hour observation period. A) Average LOI% for the 4 implant sites for the injured group; right lower leg (blue line); left lower back (red line); left shoulder (yellow line) and right upper leg (green line). B) data representing the combined LOI% of the 4 sites for the 3 groups of animals; sham (blue line); injured (red line) and non-survival (black line). Vertical black lines indicate the major events of the procedure: (a) start of injury (T0), (b) end of injury (0.25 hr); (c) fluid resuscitation (0.5 hr) and (d) disconnection of the fluid percussion (1 hr). Mean and standard deviation.

The vital sign parameters (MAP, CO and HR) measured by invasive monitoring strongly correlated with the tissue oxygenation as measured with %LOI using the Lumee system; correlation was R = 0.76 ± 0.12, and 0.70 ± 0.2 for MAP and CO, respectively, and R=-0.3 ± 0.4 for HR) (Fig 5). In the non-survival group, observed trends of the graphical data from the Lumee closely matched the measured trends of MAP, CO, and HR (R= 0.80 ± 0.3 and 0.68 ± 0.36, for CO and MAP respectively). (Fig 5.)

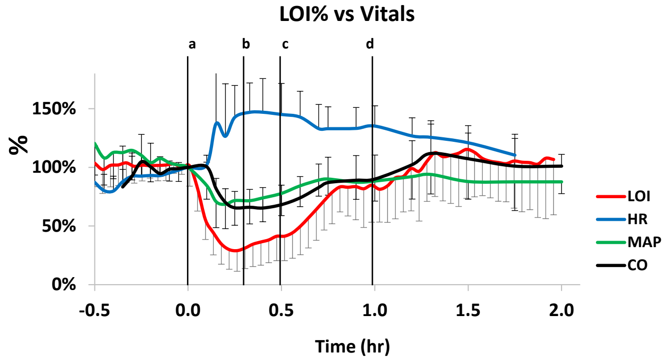


Figure 5: Overlap of the LOI% and the vital signs to illustrate the correlation between each of the vital parameters with LOI% in injured animals: MAP: Normalized Mean arterial Pressure (R=0.70 ± 0.2), CO: Normalized Cardiac output (R= 0.76 ± 0.12), R: Normalized Heart rate (R=-0.30 ± 0.4). Vertical black lines indicate the major events of the procedure: (a) start of injury (T0), (b) end of injury (0.25 hr); (c) fluid resuscitation (0.5 hr) and (d) disconnection of the fluid percussion (1 hr). Mean and standard deviation.

Metabolic parameters measured during this phase include blood gas information as well as liver associated enzymes. Serum lactate increased sharply at T60 as the animals expired in the NS group when compared to injured animals (5.2 ± 1.8 vs 1.4 ± 0.4 mM, respectively; p = 0.0180). There were no other statistically significant differences observed between groups over time for the other parameters: liver enzymes, creatinine, glucose, or lactate.

Complete blood count and coagulation studies were performed at the same time points for all groups. There was a slight decrease of WBC and platelets from T0 in injured animals following the injury compared to Sham (p<0.01) and this also observed in the NS group. Initial coagulation indices measured with Rotem (CT,CFT, MCF) were similar in all groups (343 ± 217 sec, 100 ± 67 sec and 69.9 ± 6.3 % for CT, CFT and MCF, respectively); those that reflect platelet function (MCF) were lower after injury (60 ± 4.8 %; P<0.01). Prothrombin time (PT) was increased in the injured at the end of the 2 hr observation (13.7 ± 0.7 vs 15.3 ± 1.4,sec; p<0.01) and was greater compared to Sham (13.9 ± 0.6; p<0.01) (Figure 6).

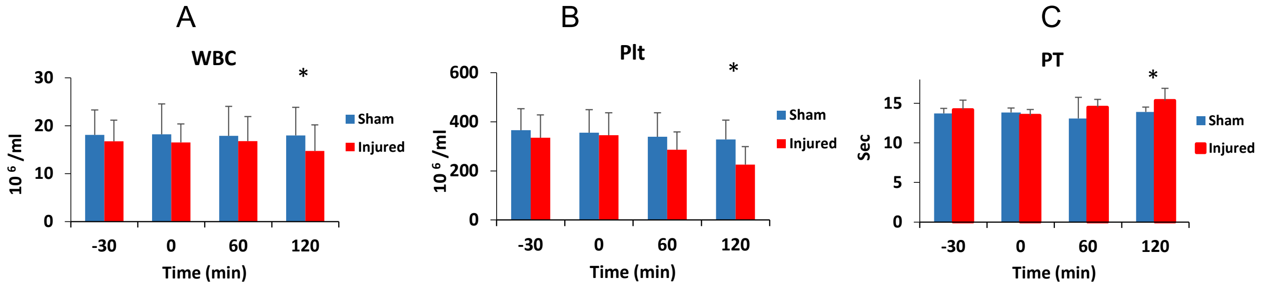


Figure 6: A) WBC B) Platelets and C) Prothrombin time (PT) for Sham and Injured animals. *p<0.01

Pathology:

Necropsy of the non-survival group demonstrated a variety of anatomic and infectious findings that may have impacted the overall survival rates in this study. Anatomic variability in the cardiopulmonary system included: pericarditis, pericardial effusion, massive organ adherence, thickened left ventricle, heart valve stenosis, and congested heart and coronaries.

Represented in the non-survival group was also pulmonary edema suggestive of underlying infectious disease. In the group of animals represented in the NS data, several animals tested positive for PRRSV post-mortem. While this diagnosis is unlikely to impact the data trends related to hemorrhagic shock, it likely impacted affected animals' ability to respond to fluid resuscitation and recover post injury.

The survival rate in this combined TBI and hemorrhage model was 24% (7/29). In our previous models, we reported 100% survival with TBI only, 40% survival for hemorrhage only and increased mortality in rats and swine with combined injury (Arnaud F, et al. 2019 and Proctor JL, et al. 2021). When studied separately, MAP generally increases after the TBI impact and tends to decrease thereafter, in contrast, it decreases to a minimum of 25 mmHg following 55% EBV hemorrhage only for example with all animals surviving. In our polytrauma model, when MAP reached a minimum around 40 mmHg, although this is recoverable for some animals, it is severe enough to be lethal for others. In addition, most of the experimental model uses healthy animals,therefore our design qualifies as a severe injury model which could be challenging for some of our animals that had initial pathology.

During the injury phase of this experiment, the Lumee platform was able to provide consistent data demonstrating distinct patterns that correlated with measured vital sign trends consistent with hemorrhagic shock physiology. This is a novel finding with the skin and subcutaneous tissue serving as a new site of end organ perfusion measurement not previously utilized to provide real time clinical information for resuscitation in a forward or prolonged field care setting. These preliminary findings suggest that with additional refinement of this platform, direct measurement using this device could be used to guide resuscitative efforts before critical care capabilities are reached and conventional monitoring is established.

- Simulated aeromedical evacuation

Thirty-six (n=36) animals were transported to the hypobaric chamber and oxygen supplementation was provided at fixed levels including FiO2 of 21%, 40%, 54%, and 100%. Survival during the evacuation phase was 89%. The remaining 32 animals were divided into groups and underwent normo- or hypobaric evacuation at the oxygen supplementation levels described above (Table 2). The small size per groups is due to the continued shortages in veterinarian staffing, delayed experiments, and changes in research assistant personnel.

Number of animals per group				
Groups FiO2	Sham		Injured	
	Normo	Hypo	Normo	Hypo
21%	3	2	3	3
40%	2	2	3	3
54%	2	1	3	3
100%	1	1	2	3

Table 2: Animal number in different sub-groups during the evacuation phase.

Validation of ventilator oxygen settings, oxygen delivered, and chamber oxygenation were all measured to confirm experimental integrity and are reported in Table 3.

Measured oxygen during evacuation		
FiO$_2$ (%)	Inhaled O$_2$ (%)	Environmental O$_2$ (%)
Normobaria		
21	19.6 ± 1.5	20.1 ± 0.9
40	39.4 ± 1.1	21.4 ± 0.2
54	57.6± 4.2	22.0 ± 1.0
100	100.0 ± 0.0	21.8 ± 0.1
Hypobaria		
21	21.8 ± 2.2	18.6 ± 3.1
40	30.3 ± 2.8	16.4 ± 1.7
54	44.3 ± 4.3	16.8 ± 1.5
100	74.9 ± 2.8	18.2 ± 1.3

Table 3: Mean oxygenation with ventilator settings, direct measurement of inhaled O$_2$, and environmental oxygen during 4-hour evacuation period.

- Measured oxygenation outcomes

Pulse oximetry:

A few animals had SpO2 reaching lower than 92% during the 4 hr evacuation and primarily within the first 2 hours, and then SpO2 increased towards 100%, the average SpO2 over 2 hr were found in the 21% Sham normo group (94.9 ± 2.7; n=1), 21% Sham hypo group (83.4 ± 15.7; (n=1) and 40% sham hypo (94.8 ± 7.0; n=1). Sham animals receiving 21% and being aero-evacuated were more subject to have a low SpO2 during flight; it is unclear why injured animals did not have this susceptibility. Animals in the other groups had their SpO2 at 100% during the entire 5 hr observation period. All animals had SpO2 at 100% when returning on the ground

Partial pressure of oxygen:

PaO2 (mmHg) during evacuation			
	Beginning of the evacuation	After 4 hr evacuation	After 5 h evacuation
21% Sham Normo	73.5 ± 9.3	110 ± 29	95 ± 22
21% Sham Hypo	106 ± 19	126 ± 0	125 ± 0
21% Injured Normo	73 ± 1.4	77 ± 10	58 ± 25
21% Injured Hypo	102 ± 42	110 ± 49	126 ± 16
40% Sham Normo	199	120	
40% Sham Hypo	206	202	210
40% Injured Normo	193 ±11	203 ±14	202 ±5
40% Injured Hypo	170 34	180± 25	169± 14
54% Sham Normo	300	311± 33	316
54% Sham Hypo			
54% Injured Normo	266 ±24	274± 26	273 ±21
54% Injured Hypo	257± 21	270 ±25	286 ±37
100% Sham Normo	490	518	506
100% Sham Hypo	319	285	310
100% Injured Normo	462 ±24	498± 13	507 ±29
100% Injured Hypo	460 ± 88	503 ±33	498 ±44

Table 4: Systemic partial arterial oxygen pressure (PaO$_2$) during the 5hr evacuation under different FiO$_2$ conditions for Sham A) Normo and B) Hypo and injured C) Normo and D) Hypo animals. Averaged data for all animals in each of the groups corresponding to the beginning of the evacuation in the chamber (T0), during the first 4 hr (after 4 hr) and during the last hour (after 5hr) Mean and Standard deviation.

Measured arterial PaO2 was similar for sham and injured in either normo or hypo conditions (Table 4).
As FiO2 during surgery was 40% PaO2 decreased with 21% for all conditions ($p<0.01$), it remained similar with 40%), and to increase with 54% and 100%FiO2 ($p<0.05$). There was no difference between conditions and groups; sham, injured normo or hypo.

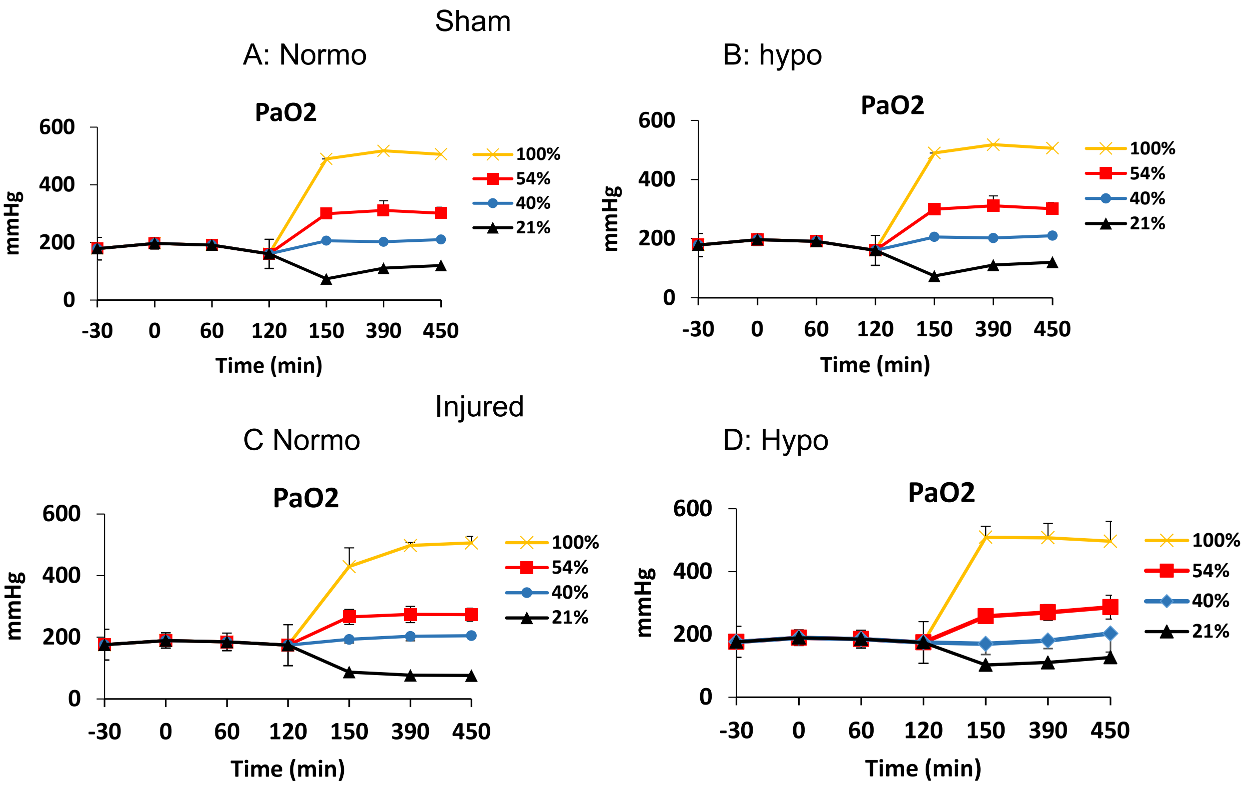


Figure 7: Systemic partial arterial oxygen pressure (PaO_2) for surgery and the 5hr evacuation under different FiO_2 conditions for Sham A) Normo and B) Hypo and injured C) Normo and D) Hypo animals. Mean and Standard deviation.

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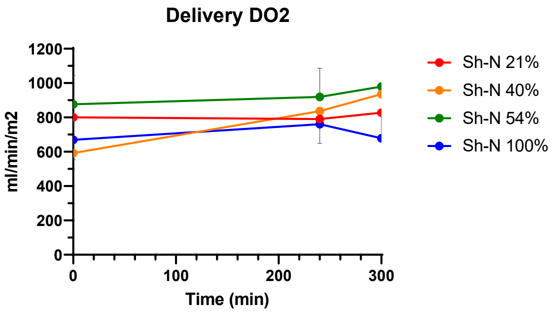
When analyzed per FiO2 conditions (Fig 8), due to small sample size in each subgroup, statistical analysis was not always possible. DO2 was similar at the start and after the 4hr of the evacuation for all animals across groups and FiO2 conditions (643 ± 167 ml/min/m2), at the end of the 5 hr the variation observed per each FiO2 was also not significant (659 ± 197 ml/min/m2). VO2 was similar at the start (22.0± 7.9 ml/min/m2), and after the 4hr of the evacuation and at the end of the 5 hr (despite the span of variability during the evacuation) (22.5± 9.7 ml/min/m2) for animals across groups and FiO2 conditions. ExtO2 was similar at the start and after the 4hr of the evacuation for all animals across groups and FiO2 conditions (3.6 ± 1.5 %), at the end of the 5 hr the variation observed per each FiO2 was also not significant (3.8 ± 2.7 %). As there was no difference due to FiO2 conditions, data were combined and analyzed for Sham and Injured; there was no difference found between normo or hypobaria for DO2, VO2 and ExtO2 parameters respectively. For the injured animals, VO2 and ExtO2 trended towards a reduction without significant difference (Fig 9). Overall, neither FiO2 levels nor hypobaria affected the delivery, consumption or extraction of oxygen.

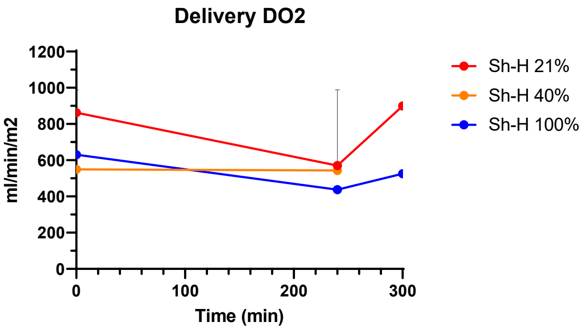
DO_2: Oxygen delivery

Sham

A: Normo

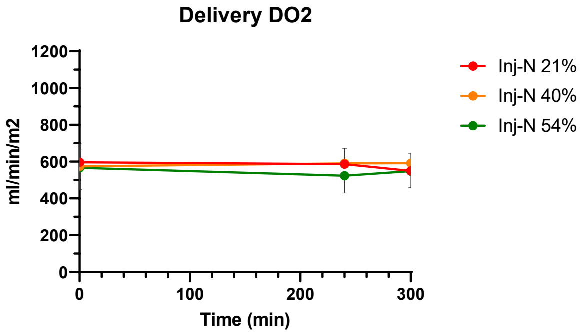

Delivery DO2

B:Hypo

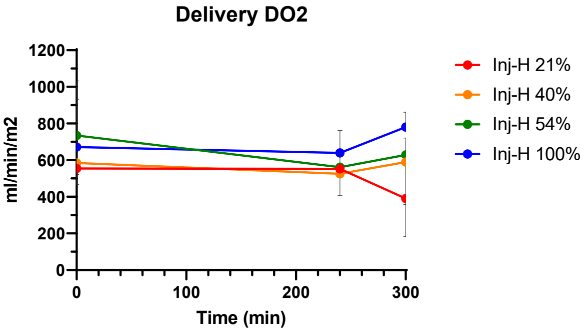

Delivery DO2

Injured

C: Normo


Delivery DO2

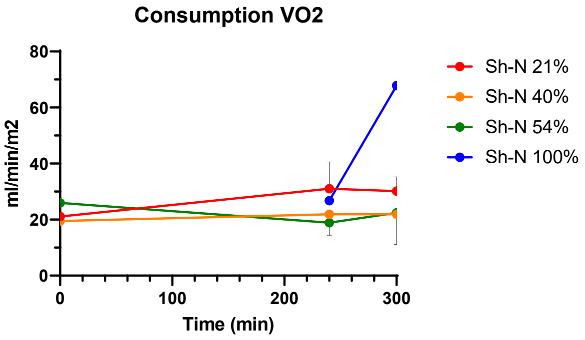
D: Hypo


Delivery DO2

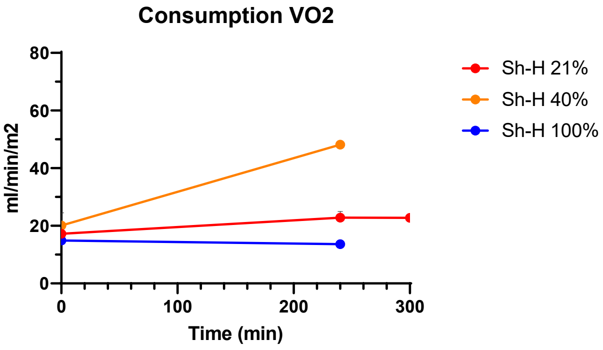
VO_2: Oxygen consumption

Sham

A: Normo

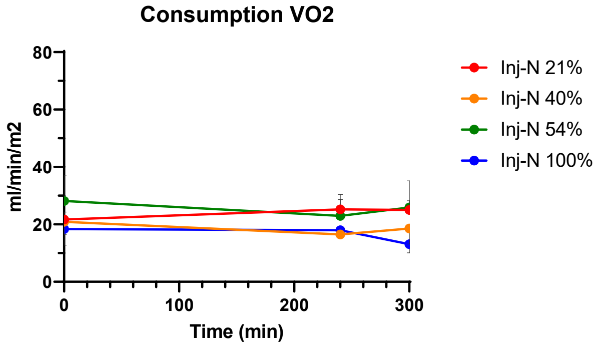

Consumption VO2

B:Hypo

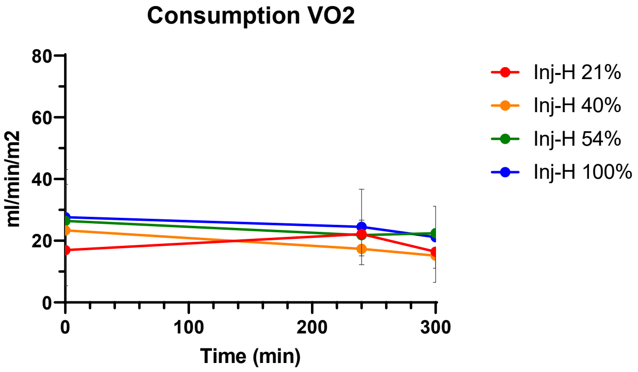

Consumption VO2

Injured

C: Normo



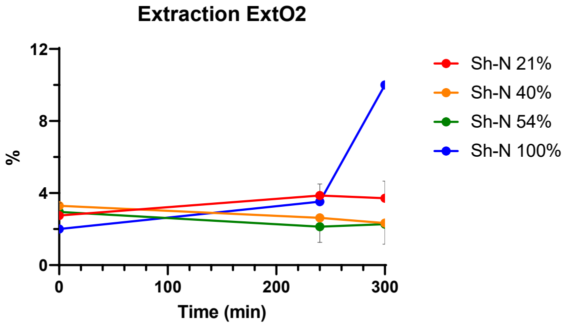
D: Hypo



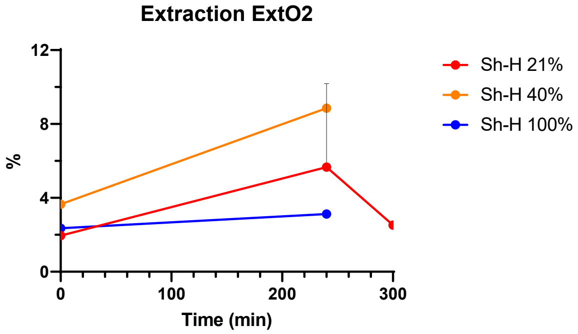
ExtO$_2$: Oxygen extraction

Sham

A: Normo

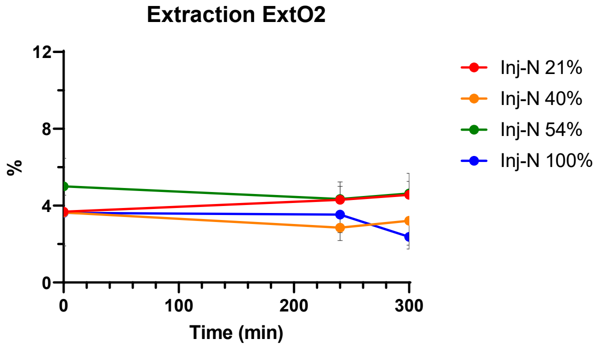


B:Hypo



Injured

C: Normo



D: Hypo

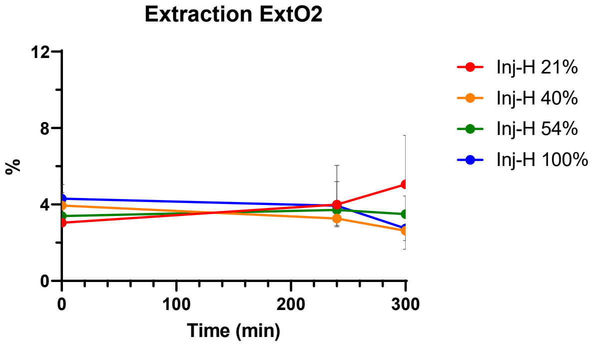
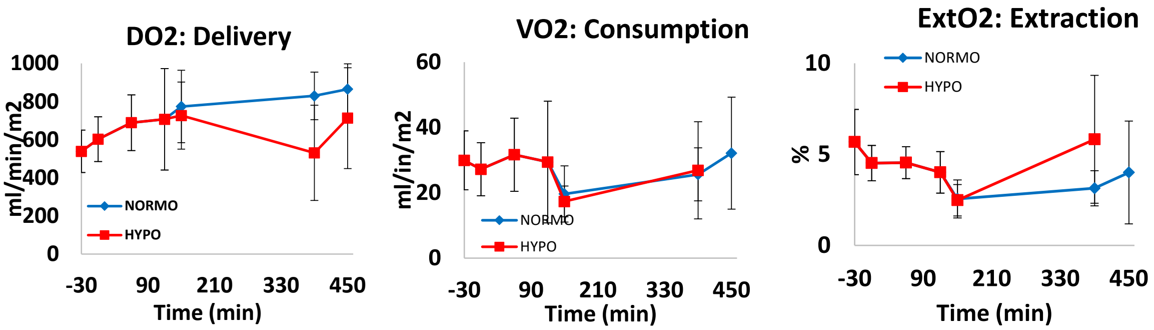


Figure 8: Oxygen derived parameters under different FiO2 conditions during evacuation for A) Sham Normo, B) Sham Hypo, C) Injured Normo, and D) Injured Hypo for respectively DO_2: Oxygen delivery, VO_2: Oxygen consumption, and $ExtO_2$: Oxygen extraction. Mean and SD.

A Sham



B Injured

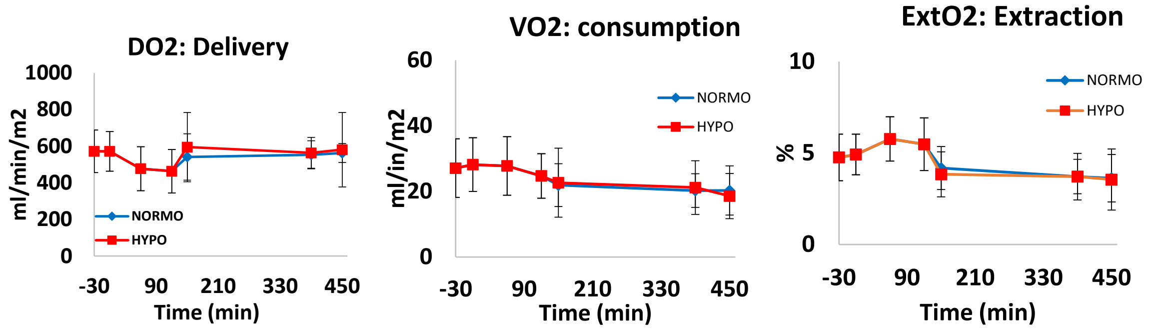
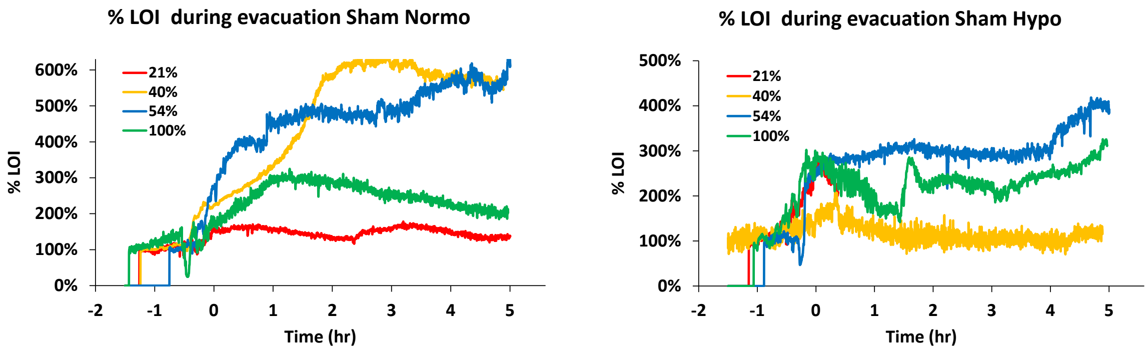


Figure 9: Oxygen derived parameters over the course of the entire experiment for DO_2: Oxygen delivery, VO_2: Oxygen consumption, $ExtO_2$: Oxygen extraction for A) Sham and B) Injured. The time scale is reported in min from the start of the experiment in surgery. Mean and standard deviation.

- Tissue oxygenation LOI

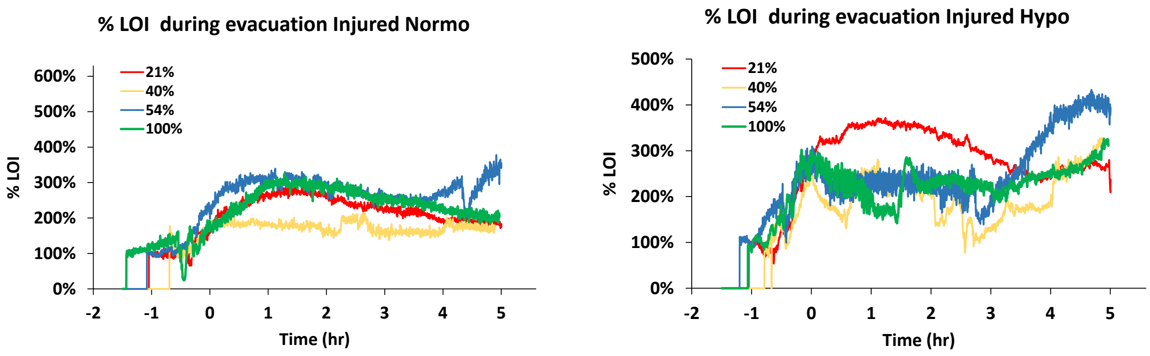
The LOI% demonstrated similar trends at all 4 implant sites and were averaged for subsequent analysis. Regardless of the groups or treatments, there was an approximate 3-fold increase in LOI% during the transition from OR to the start of the flight. This change was temporally related to the transition from inhalational anesthetic to intravenous anesthesia and was observed universally in all animals. Further analysis of the impact inhalational versus intravenous anesthetic on the subcutaneous tissue oxygenation implant readings warrants additional targeted investigation. We observed some trends but no significant difference between the groups and conditions as there was variability due to the small sample size for each condition (Fig 10).

Tissue Oxygenation
Sham


A:Normo

B:Hypo

Injured


C: Normo

D: Hypo

Figure 10: Tissue oxygenation as normalized LOI (LOI%) during the 5 hours evacuation period, respectively for: A) Sham Normo, B) Sham Hypo, C) Injured Normo, and D) Injured Hypo. Average for the different group under the FiO2 levels of interest.

- Hemodynamics

At the end of the surgery period, anesthesia was switched from inhalational anesthesia with isoflurane to TIVA with Ketamine/Midazolam. During this transition, MAP and CO depression was observed across all groups (Figure 11).
There was a trend for increased CO towards the start of the evacuation regardless of the groups, but this was not significant.

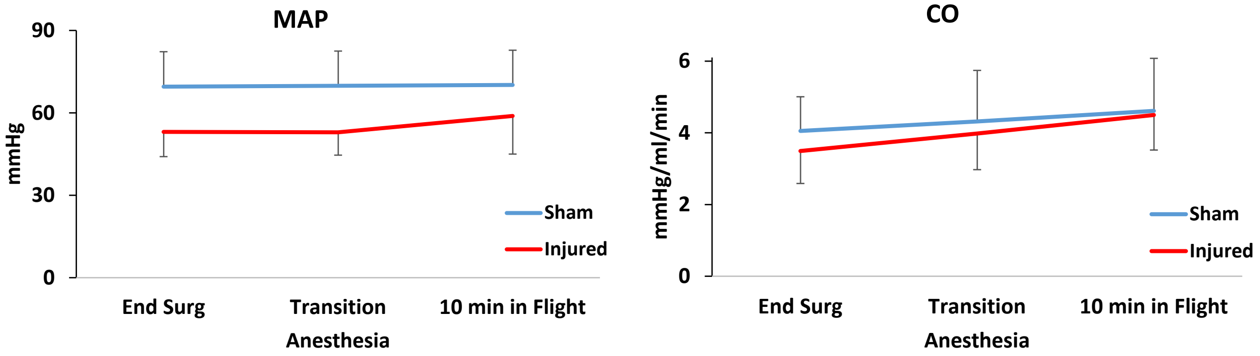
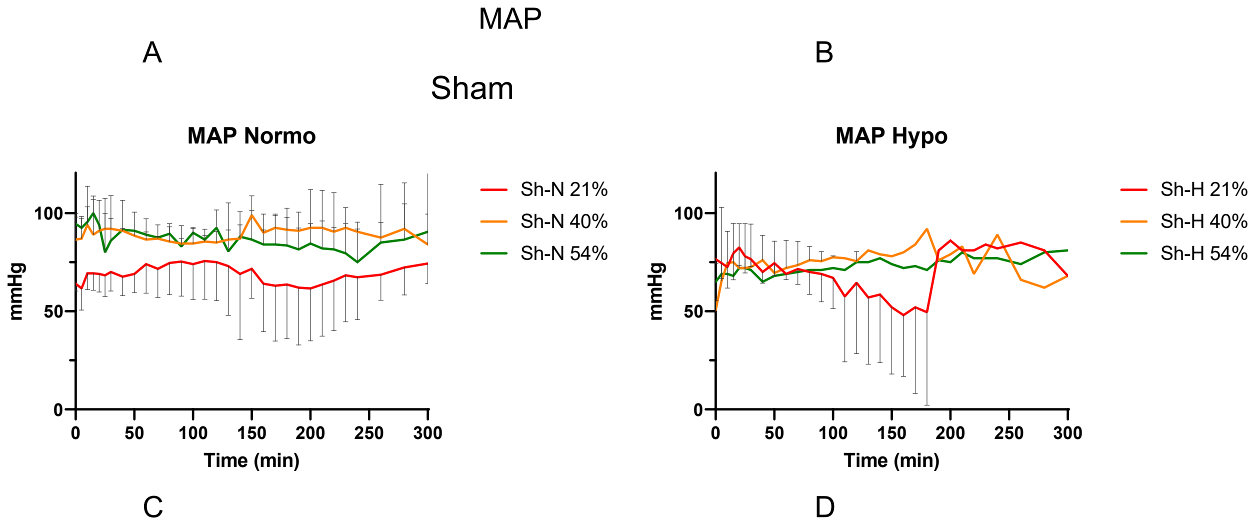
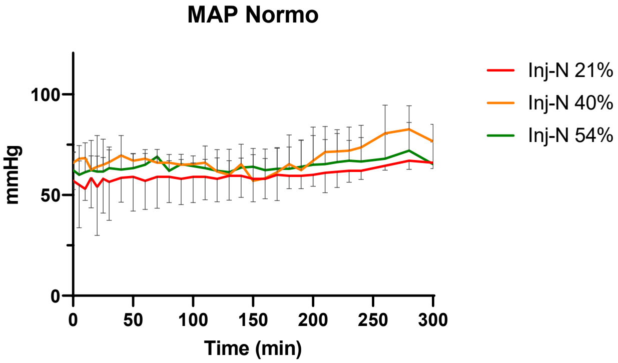


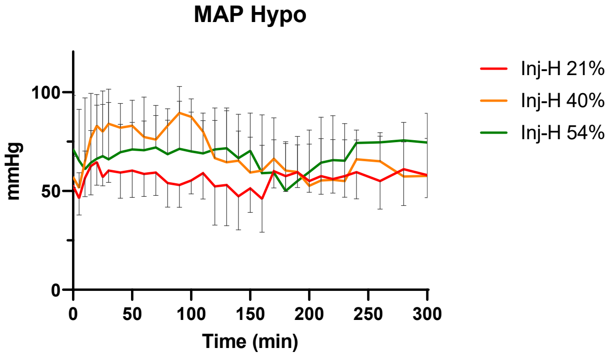
Figure 11: changes of MAP and CO during the transition from isoflurane to ketamine/midazolam from the end of surgery to the beginning of the evacuation.

There was no difference over the 5 hr evacuation for MAP or CO under all FiO2 in the Sham or Injured groups (Fig 12). To compare MAP and HR variability for each group, the delta MAP and delta HR was calculated for each group over the simulated evacuation time period. The variability for MAP and HR across per groups was calculated for the entire evacuation period and for the first half and the second half of the evacuation (Figure 13) There was no significant variability for HR due to FiO2 in any of the Sham, Injured or Normo or Hypo groups.



Injured



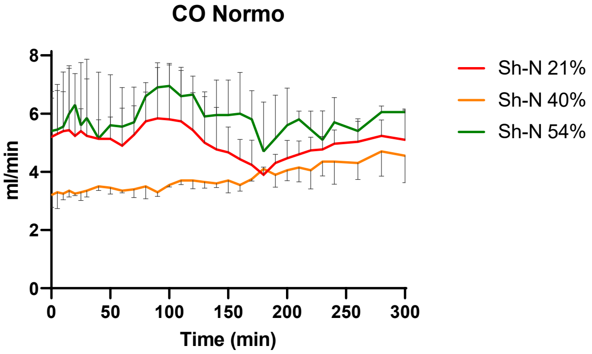


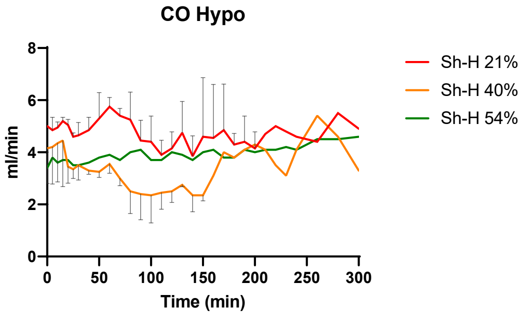
CO

E

F

Sham

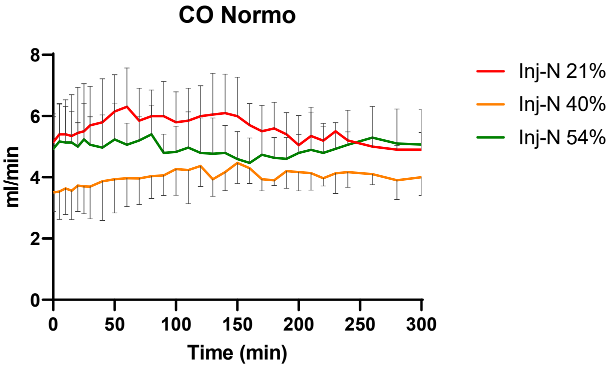


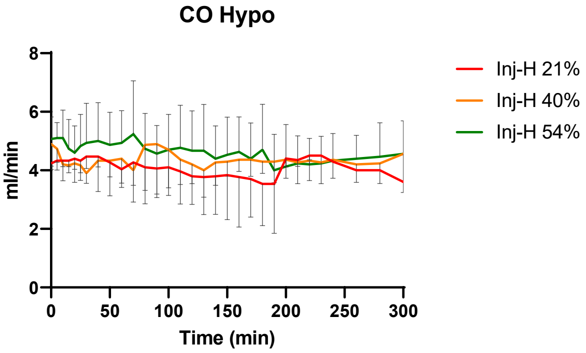


G

H

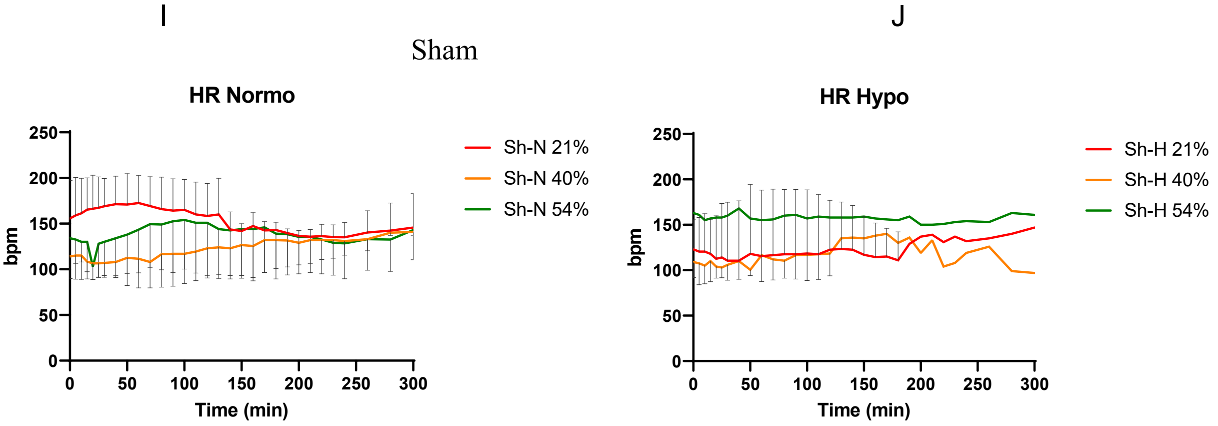
Injured





HR

Sham



Injured

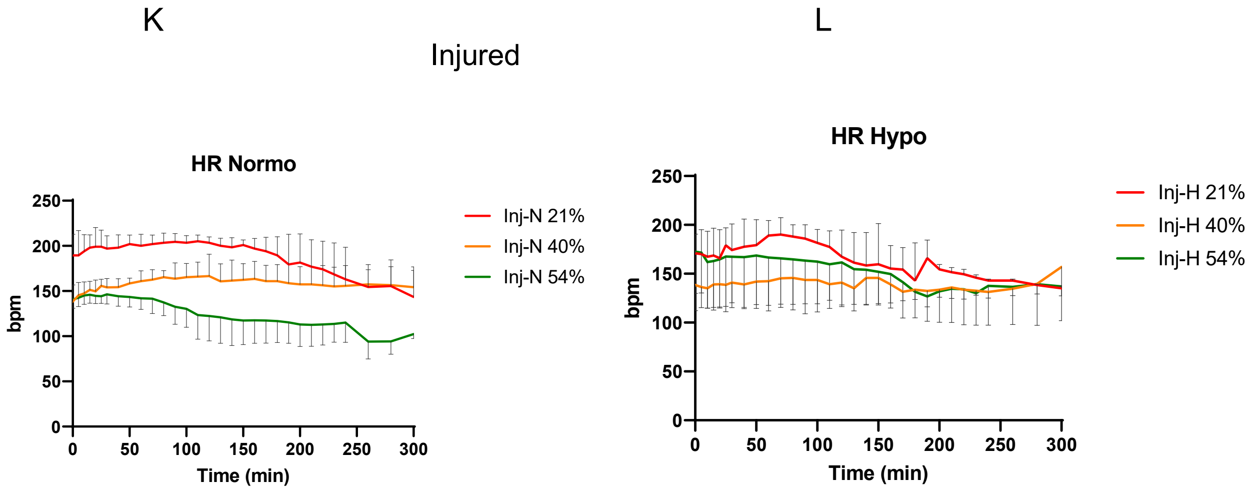


Figure 12: Hemodynamics changes during the entire observation period for Sham and Injured under 4 FiO_2 conditions. A-D: Mean arterial Pressure (MAP), E-K: Cardiac output (CO); and I-L: Heart rate (HR); Mean and Standard deviation.

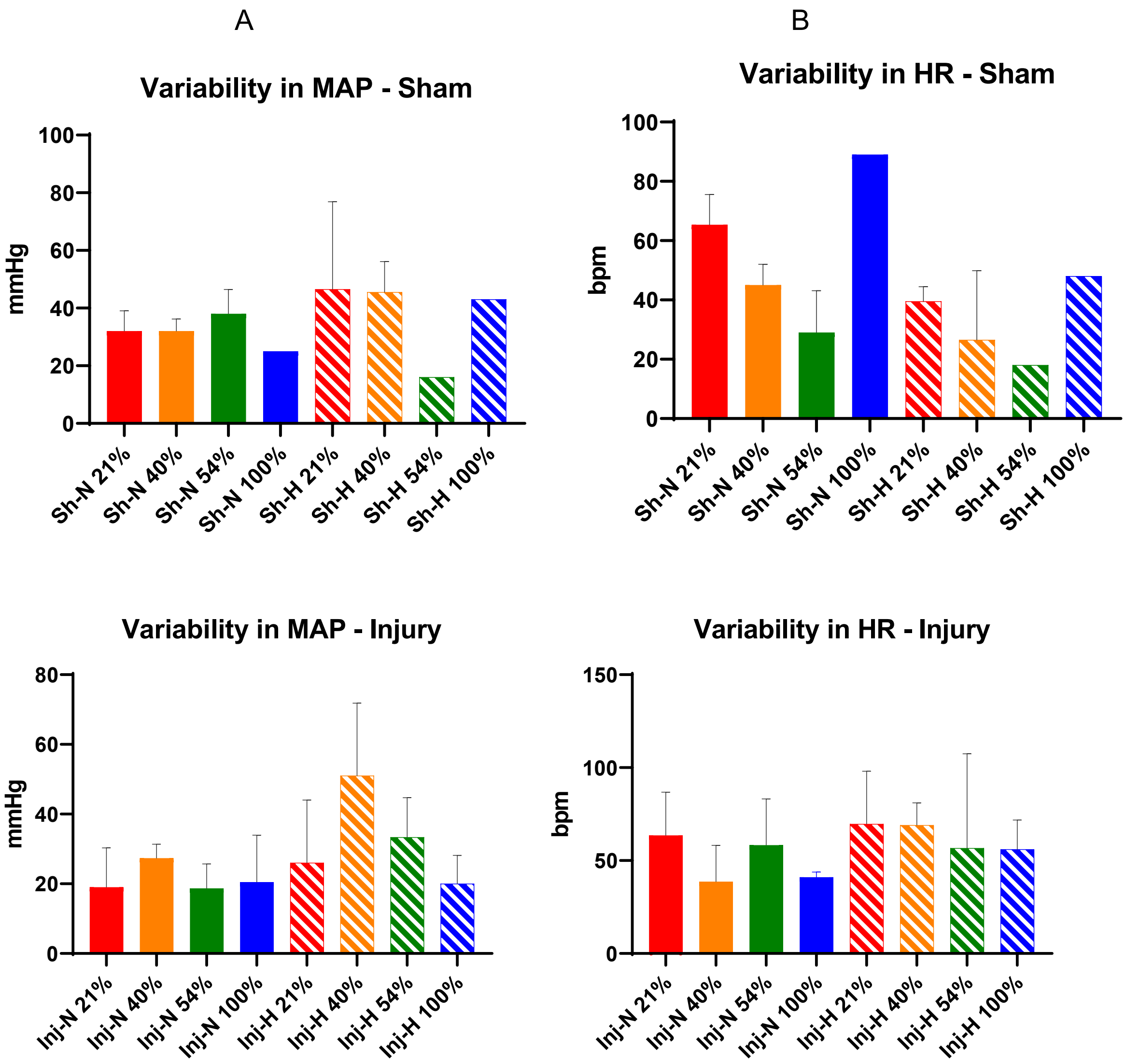


Figure 13: Variability over the course of the evacuation of A) MAP and B) HR for the Sham normo and hypo and the injured normo and hypo. The first bar represents the first half of the evacuation and the second bar the second half of the evacuation.

- Intracranial pressure and cerebral oxygen measurements

Intracranial pressure was measured directly with the Codman probe (Figure 14) was lower for the sham normo group compared to the sham hypo group ($p<0.01$); the reason for this is unclear. The various FiO2 levels did not impact ICP for the Sham normo or hypo or Injured normo or hypo over the 5 h evacuation (Fig 14). There was no difference over time for all FiO2 and ICP remained lower than 12 mmHg in average.). There was no statistical difference over time for any groups or conditions due to the level of FiO2. No brain hypertension was observed in any groups.

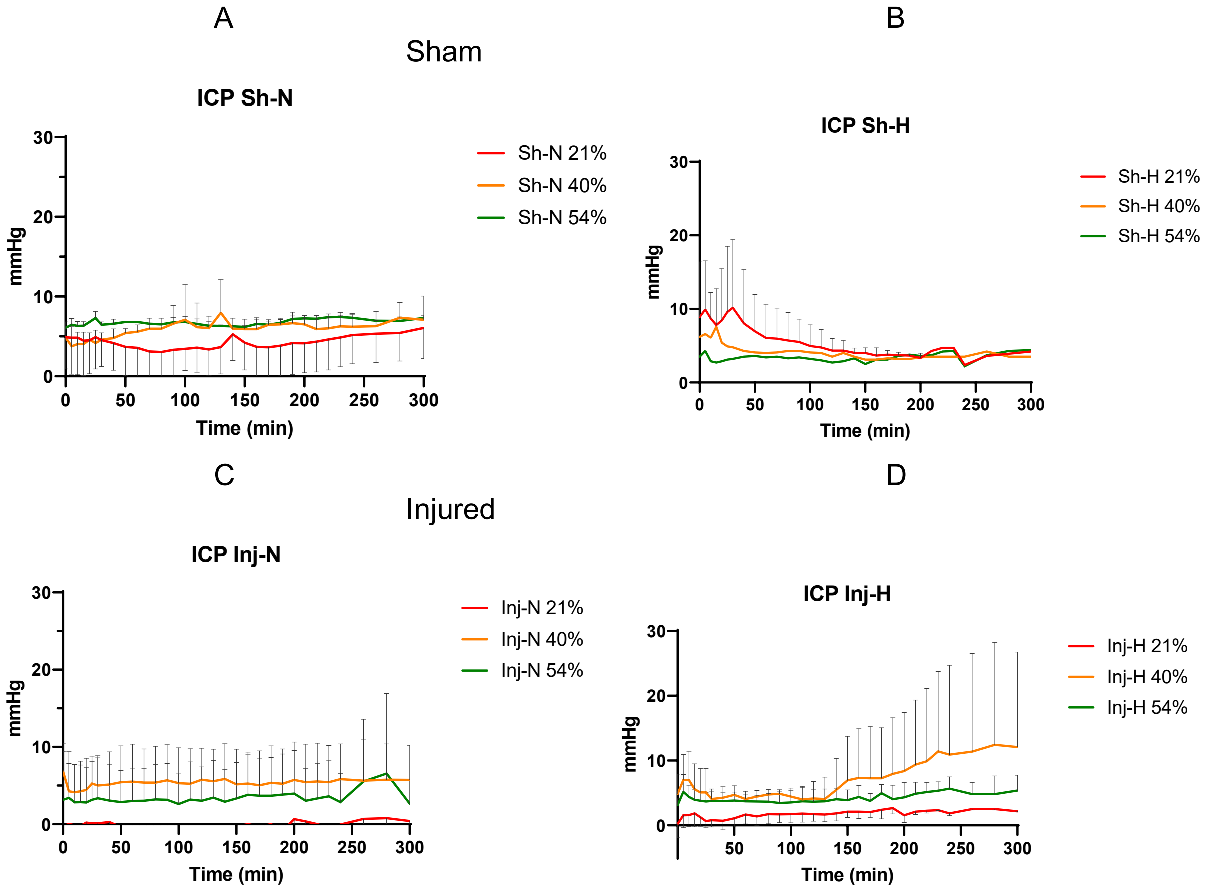
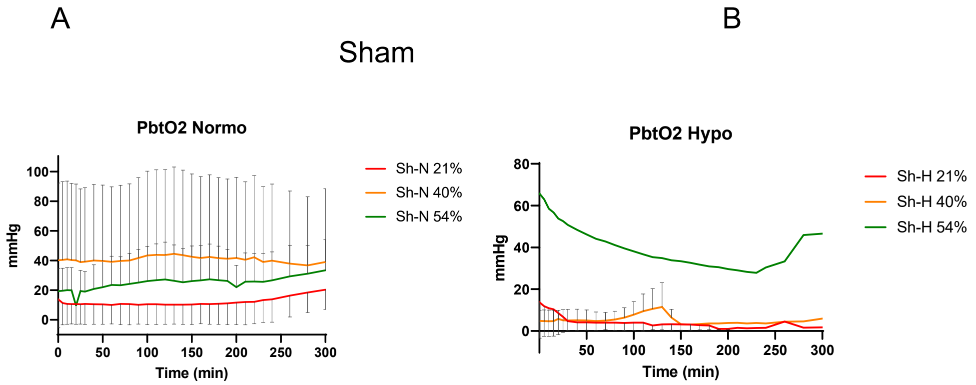


Figure 14: ICP for A) Sham normo and B) Sham hypo animals, C) Injured normo and) injured hypo animals under various FiO2 levels (21%,40% 54%, and 100%. Mean and SD.

Brain tissue Oxygenation that was measured with the Raumedic probe yielded variables and no analysis was performed (Fig 15). it was not possible to obtain a direct correlation between PbtO2 and LOI% for any of the FiO2 groups



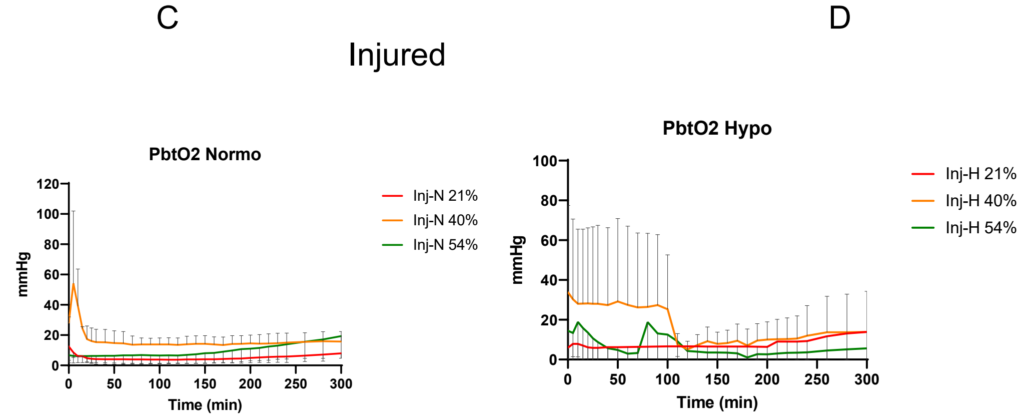
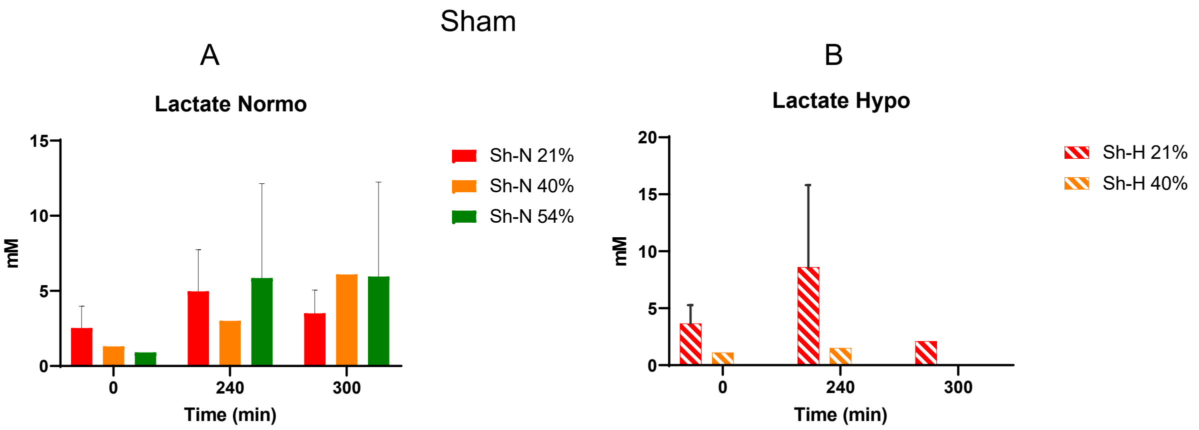


Figure 15: Brain Tissue oxygenation PbtO2) for A) Sham normo and B) Sham hypo animals, C) Injured normo and) injured hypo animals under various FiO2 levels. Mean and standard deviation.

- Laboratory Parameters.

Laboratory parameters including complete blood count (CBC), coagulation, and muscle, liver and kidney enzymes were also analyzed. There was an increase in serum lactate (Figure 16) primarily for injured hypo under 21% oxygen $p<0.05$). In the 21% group, there was an increase in aspartate aminotransferase (AST) at (insert time point or points) with (insert mean and standard deviation) under normo and hypo conditions; $p<0.05$ (Figure 17). The hematology and chemistry parameters were averaged during the 5 hr evacuation period as there was no difference over time for sham and injured animals. The average value of each parameter over the 5 hr is the average of combined Normo and Hypo conditions as there was no difference between these conditions. There were no observed differences in the other laboratory parameters measured (i.e. albumin, Lactate dehydrogenase, alanine transaminase and gamma-glutamyl transferase) when comparing groups by FiO2 levels. Table 5 summarizes the results.



Injured

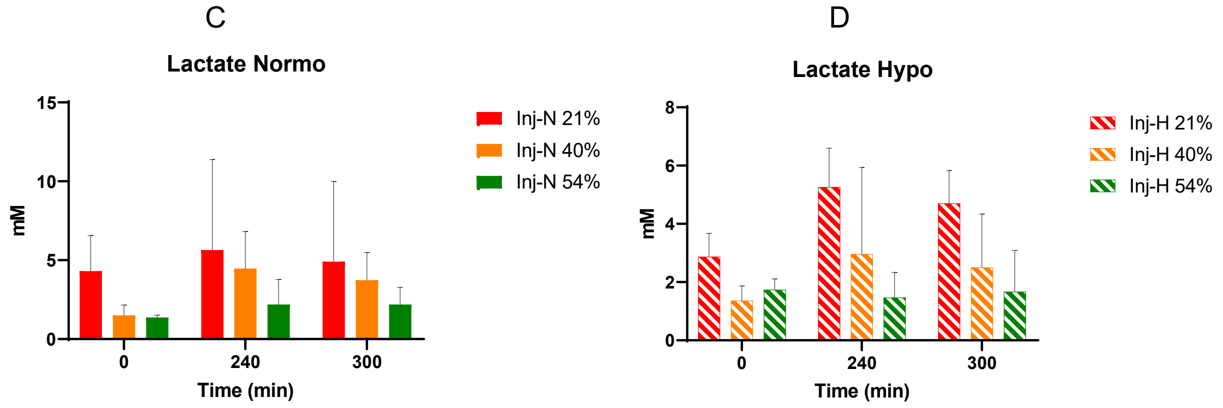
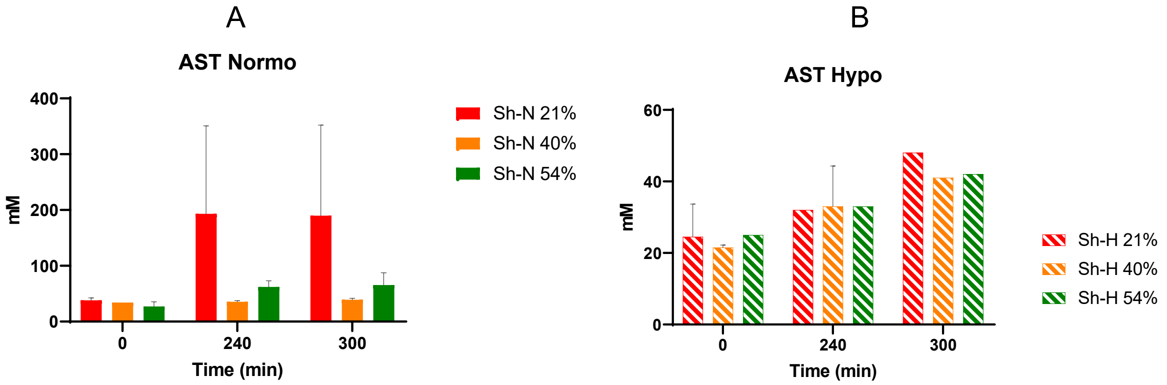


Figure 16: Lactate during surgery and the 5 hr evacuation period for:A) Sham Normo, B) Sham Hypo, C) Injured Normo, and D) Injured Hypo. *p<0.05. (Mean ± STD)

Sham



Injured

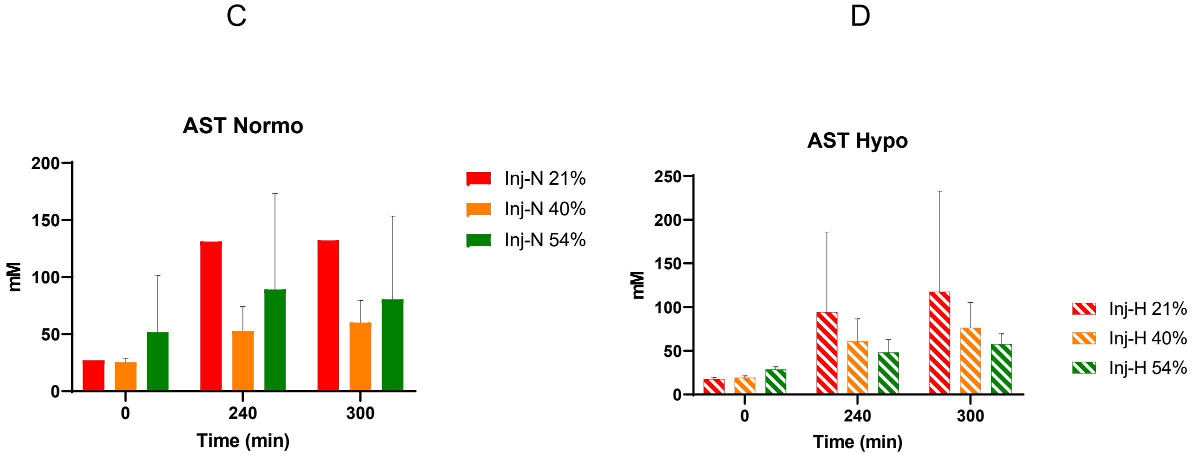


Figure 17: Aspartate aminotransferase (AST) during the 5 hr evacuation period for: for A) Sham normo and B) Sham hypo animals, C) Injured normo and) injured hypo animals

under various FiO2 levels. Mean and SD.There was an increase toward the end of the evacuation in the 21% group; p<0.01. (Mean ± STD)

Hematology and chemistry parameters during evacuation

	FiO$_2$	21% O$_2$	40% O$_2$	54% O$_2$
Hematology				
Hb (g/dl)	Sham	10.0 ± 0.8	10.2 ± 1.7	10.9 ± 1.1
	Injury	6.7 ± 0.6*	7.2 ± 1.3*	7.4 ± 1.0 *
WBC (10^6/ml)	Sham	21.4 ± 4.80	19.9 ± 8.8	24.1 ± 10.7
	Injury	20.2 ± 5.8*	24.5 ± 10.6	17.4 ± 4.1*
Neutrophils (%)	Sham	70.6 ± 8.1	55.9 ± 29.3	70.8 ± 5.4
	Injury	67.9 ± 5.9	69.8 ± 6.0	64.9 ± 11.6
Platelet (10^6/ml)	Sham	334 ± 60	280 ± 58	428 ± 132
	Injury	221 ± 82*	273 ± 59	230 ± 48*
CFT (sec)	Sham	151 ± 154	117 ± 53	78.7 ± 24.3
	Injury	112 ± 40	81.9 ± 24.6	110 ± 83
MCF (%)	Sham	68.1 ± 7.6	67.9 ± 3.5	68.8 ± 6.7
	Injury	63.8 ± 4.6	68.5 ± 3.78	63.3 ± 4.1
Aggregation (AUC)	Sham	15.4± 3.4	17.4 ± 4.9	21.4 ± 7.5
	Injury	13.6 ± 4.0	19.8 ± 1.9	14.7 ± 5.7
Prothrombin (sec)	Sham	14.4 ± 0.3	13.9 ± 0.3	13.7 ± 1.0
	Injury	17.8 ± 7.7*	14.3 ± 0.7	16.5 ± 1.6*
Glucose (mM)	Sham	4.81 ± 1.70	5.46 ± 1.31	5.52 ± 0.92
	Injury	5.74 ± 2.38	4.47 ± 2.22	4.73 ± 1.60
Chemistry				
CK (mM)	Sham	1929 ± 1544	1635 ± 935	2829 ± 2051
	Injury	988 ± 767*	1012 ± 518*	1786 ± 703
Creatinine (mM)	Sham	1.44 ± 0.36	1.38 ± 0.27	1.31 ± 0.21
	Injury	2.00 ± 0.23*	1.14 ± 0.25	1.62 ± 0.36*

Table 5: Summary of hematology and chemistry parameters averaged over the 3 samples taken during 5 hr evacuation period (collection at the beginning of the evacuation, after 4hrs and after 5hrs) as there was no difference over time for sham and injured animals. The average value of each parameter over the 5 hr is the average of combined Normo and Hypo conditions as there was no difference between these conditions. CFT: clot forming time, MCF (Maximum clot formation, CK: creatine kinase. (Mean ± STD). *p<0.05

- Histopathology

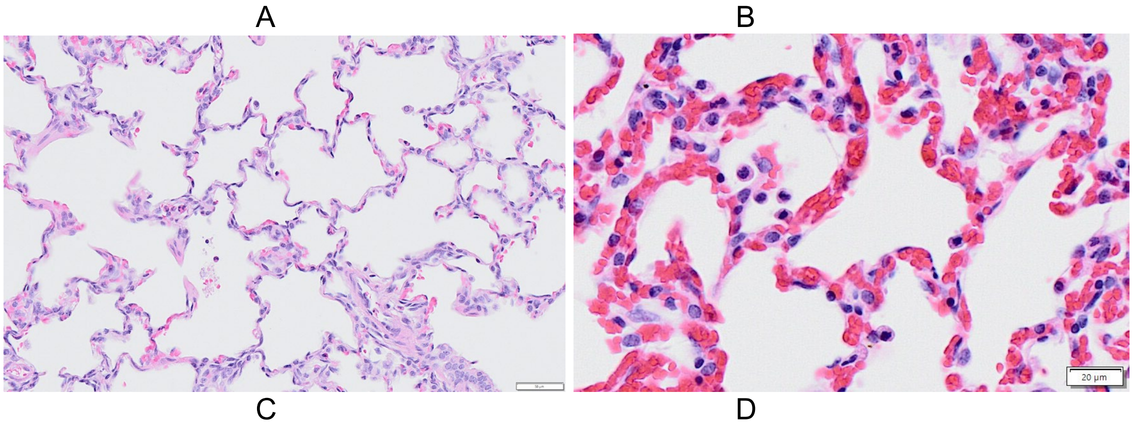
Particular histological emphasis was directed towards acute lung injury at the end of the experimentation period. Staining of tissue with hematoxylin and eosin was performed on lung tissue and a scoring system was applied as described in the methods section to make comparisons across groups of FiO2 supplementation. (Table 6, Figure 18)

The detection of neutrophilic and macrophages infiltration in the alveolar walls and alveolar spaces were the main focus to detect histologic markers of acute alveoli injury. The other makers of alveolar injury such as alveolar wall thickening, alveolar fibrin, and microthrombi were not identified in these animals. Presence of increased lymphoplasmacytic cellularity and bronchus-associated lymphoid tissue (BALT) was found but this may be more specifically related to the initial health status of the animal rather than the experimental outcome. In addition, the interlobular edema and tissue congestion were determined most likely to be related to tissue handling and processing. A summary of the relevant observations is reported in an incidence table (Table 6).

Score on incidence in both right and left lungs

	GROUPS	21 H Sh		21 H		40 N Sh		40 N		40 H	
	SCORE	1	2	1	2	1	2	1	2	1	2
Lesions	Infiltrate, neutrophilic, alveolar	0	0	0	0	0	0	2	0	1	0
	Infiltrate, neutrophilic, alveolar septal	0	0	0	0	0	0	1	0	2	0
	Macrophages, increased, alveoli	0	0	0	**3**	0	2	0	2	3	0
	Infiltrate, histiocytic, alveolar septal	0	1	2	2	0	2	2	0	1	**3**
	Infiltrate lymphoplasmacytic, alveolar	0	0	0	0	0	0	0	2	0	0
	Infiltrate lymphoplasmacytic, alveolar septal	0	0	2	2	0	0	1	0	0	1

Table 6: Incidence of the scores of histologic lesions observed in the lungs according to FiO2 groups 21 % and 40% in Sham normo, Sham hypo animals, Injured normo and injured hypo.



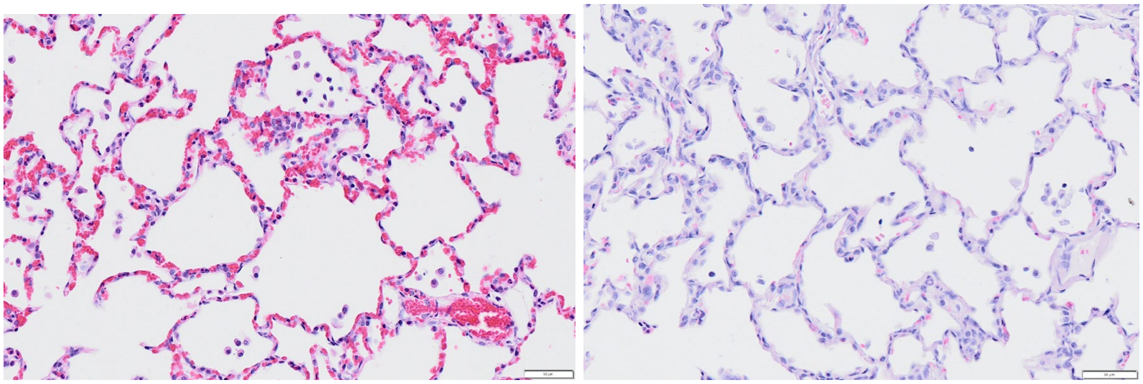


Figure 18: Illustration of representative (B) neutrophilic infiltrates as in the 40% injured hypo groups, (C) macrophages infiltrates as in the 40% injured hypo groups and (D) lymphoplasmacytic infiltrates as in the 21% injured Hypo groups compared to normal alveoli (A) as in 54% injured normo.

The number of pixels of counted for positive myeloperoxidase were standardized to the surface area (c/a) of the section calculated by Image J. Sections for injured animals were averaged as follows: 0.005 ± 0.002 c/a, 21% FiO_2, n=4; 0.005 ± 0.002 c/a, 40% FiO_2, n=6; 0.004 ± 0.007 c/a, 54% FiO_2, n=5; and 0.005 ± 0.009 c/a, 100% FiO_2, n=4. The results of staining as count/surface area did not indicate a significant difference among the 4 FiO_2 groups (Fig. 19).

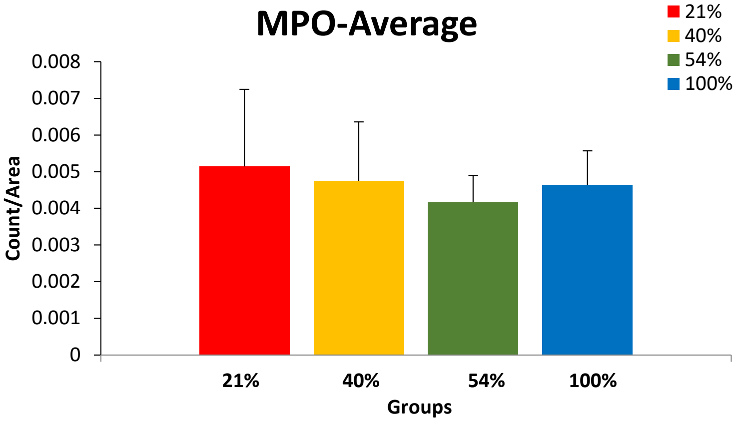


Figure 19: MPO results as average of count/surface area for 21%, 40% 54 % and 100% FiO_2.

Discussion

The objective of the study was to examine whether aeromedical evacuation closer to the onset of injury will result in worse histologic and inflammatory outcomes than delayed aeromedical evacuation. The swine projects proposed in this study were intended to be based on rat studies that would provide a preliminary insight on differences in organ specific damage related to the timing of aeromedical evacuation following polytrauma. Rats were exposed to blast overpressure (3 consecutive blasts at 110kPa on Day 1) and then delayed the simulated 6-hour flight (altitude at 8,000 ft cabin pressure) for 2, 7, or 14 days post blast. There were no significant injury or deficits in motor behavior among the animals exposed to blast and hypobaric flight conditions, nor plasma cytokine expression from several cohorts. Immunohistochemistry analysis of the brain using GFAP marker showed no remarkable injury between the different time groups. During the data review, there were no clearly observed differences between groups within the parameters examined. The team of investigators decided to suspend additional study of this aim until revisions to the experimental design could be examined in additional rat studies

Secondly, we sought to determine whether aeromedical evacuation at 4000 feet would result in decreased inflammatory outcomes in polytraumatically injured swine compared to aeromedical evacuation at 8000 ft. Due to factors described above, namely lack of pathologic findings noted at 8000 ft., this aim was cancelled.

Lastly, we sought to determine whether increasing oxygen supplementation at fixed levels would result in incrementally improved inflammatory outcomes on histologic evaluation. Investigation was performed for 21%, 40%, 54% oxygen supplementation in sham and polytraumatically injured animals with some pilot studies in a few animals who received 100% oxygen supplementation during SAE. The relevant findings were as follows: In this polytrauma model, the mortality rate (7/29) after injury resulted from a combination of injury severity, as well as inherent physiologic defects of animals which were discovered after autopsy. For the animals that entered the aero medical evacuation phase, their hemodynamics parameters remained unchanged during 5 hrs. Although the sample size for this study was not sufficient to generate significance in the oxygen supplantation data, it was noted that the outcomes of the animals in the 21% FiO_2 group revealed a low systemic (blood gases) and peripheral oxygenation (tissue oxygenation). We could not confirm our hypothesis that 100% FiO2 conferred any lung cytotoxicity to the animals shortly after evacuation. In performance of this aim of the study, there were some difficulties in obtaining reliable blood gas data, as well as invasive pulmonary artery catheter data for the 54% sham group. Furthermore, the 100% supplementation arm could not be fully evaluated due to expiration of several swine because of illness and/or physiologic abnormalities which were not detected until autopsy. Ultimately, we cannot really make any conclusive statements on this portion of the study due to low sample size / low statistical power for data interpretation.

In summary, we performed preliminary pre-clinical work to evaluate whether Oxygen supplementation would improve inflammatory and histologic outcomes. There were no significant observable trends regarding increased pathology between groups exposed to hypobaria vs. normobaria. This data can be used to inform further experimental design when determining how to study the effects of aeromedical evacuation on polytraumatized large-animal models.

What opportunities for training and professional development has the project provided?

This project provided opportunity for the implementation of a new device, the Profusa Lumee, to measure relevant outcomes in tissue oxygenation. Inclusion of this technology allowed for the development of new procedural techniques and data collection by the research team and provided a new platform for data analysis for the investigators. This also provided opportunity for direct collaboration with the manufacturer's scientific team adding value for all team participants. There were 2 medical students from USUHS who participated in this protocol. A surgical resident from WRNMMC participated in surgical procedures including Lumee implantation, craniotomy, and invasive line placement.

How were the results disseminated to communities of interest?

Various portions of the results from this experiment have been presented at national and international meetings as described below. Periodic reporting of the data was provided to update the sponsoring organization on significant results achieved during the duration of the award performance.

What do you plan to do during the next reporting period to accomplish the goals?

This report concludes the experimental data collection for this project. There are two manuscripts that are currently under preparation for publication as described below. The scientific team continues to work towards publication of this data.

4. IMPACT:

This project served as an initial investigation to examine the impact of various fixed levels of oxygen supplementation on a polytrauma model under normobaric and hypobaric conditions simulating evacuation. This project had insufficient data collection to make conclusive statements about the ideal levels of oxygen supplementation to prevent second hit injury from hypoxia. Additional focused work on this area should be performed to identify the threshold for beneficial oxygen supplementation as compared to hyperoxic conditions that lead to other detrimental physiologic outcomes.

What was the impact on the development of the principal discipline(s) of the project?

This project serves as initial preclinical investigation to determine optimal oxygen supplementation protocols to guide current practices in aeromedical evacuation. Additional preclinical work should be performed before conclusions can be made or changes in clinical practice can be considered.

What was the impact on other disciplines?

Initial investigation of the Profusa Lumee platform suggested that it may have a role in the recognition of early hemorrhagic shock in the prolonged field care setting. Specific data analysis on the role of this new technology will be reported at the conclusion of this study and ongoing device evaluation is warranted towards this specific aim.

What was the impact on technology transfer?

A new application of the Profusa Lumee was proposed and the results are being shared directly under the collaboration agreement shared between this for-profit entity and the researchers at NMRC.

What was the impact on society beyond science and technology?

Nothing to Report.

5. CHANGES/PROBLEMS:

Over the course of this project, there were substantial challenges to complete the project. To complete the proposed work, no-cost extensions were filed to compensate for time unavailable for non-essential large animal models during the COVID-19 pandemic.

Changes in approach and reasons for change

Early in the grant execution, the animal experiments were initiated and run primarily by the scientific team comprised of Dr. Francoise Arnaud with a team of research assistants in the En Route and Care Department at NMRC. Experimental studies were started in 2021, however, continued shortages in veterinarian staffing resulted in delaying experiments. In the course of 2021, there were changes in research assistant personnel and in the light of the pandemic, research priorities for the department were shifted.

Actual or anticipated problems or delays and actions or plans to resolve them

During 2021 to early 2022, a breakout infection occurred in our approved vendor's breeding colonies, animal purchases have been halted. This led to a safety stand down and investigation of the suppliers breeding practices and ultimately selection of a new breeder for ongoing swine work at our facility. This process took 4 months in entirety to come to an agreed upon breeder and supplier to allow for ongoing experimental work.

Changes that had a significant impact on expenditures

During the Covid-19 pandemic, delays in the ability to continue animal work prevented the use of purchased materials that ultimately expired (mainly medications), and salary support was continued for department employees without the ability to conduct additional animal trials. Purchases of animals from other vendors did generate additional cost.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report.

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

  - F Arnaud A. White J. Hubbell J. Duberstein A. Connor F. Yang M. Mehalick Y. Dayani C. Goforth Col D. Malone A. Scultetus. Oxygen supplementation of injured swine during simulated flight. MHSRS meeting, Kissimmee Aug 19-23, 2019

  - F Arnaud, S Dishman, D Malone, G Santiago, A Scultetus C Gosztyla. Comparison of aeromedical evacuation effects after Traumatic brain injury (TBI) with or without hemorrhagic shock (HS) in a porcine model. AsMA meeting May 22-26 May 2022, Reno,NV.

  - F Arnaud, M Lozano, N Coschigano, G Santiago, C Gosztyla. Continuous tissue oxygen monitoring during aero medical evacuation in a porcine model. International Conference of Aerospace Medicine September 22-24, 2022 Paris, France.

Journal publications.

- G Santiago, M Lozano, N Coschigano, F Arnaud, C Gosztyla. Continuous Tissue Oxygen Monitoring in Polytraumatized Swine using a Novel Phosphorescence Quenching Technology as a Means of Detecting Early Shock Physiology. In preparation
- G Santiago, F Arnaud, M Lozano, N Coschigano, D Malone, A Scultetus, C Gosztyla. Effect of various oxygen supplementation during aero-evacuation after polytrauma in a swine model. In preparation

Books or other non-periodical, one-time publications.

Other publications, conference papers and presentations.
Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript

Nothing to report.

- **Website(s) or other Internet site(s)**

Nothing to report.

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: LCDR Carolyn Gosztyla, MD
Project Role: Site PI
Nearest Person Month worked 4
Contribution to Project LCDR Gosztyla provides overall project management, including laboratory integration, data analysis and interpretation, publication strategy and final editorial responsibility for publications. LCDR Gosztyla is responsible for research program management with

responsibility to plan, coordinate, and execute swine experiments in hypobaric chamber; collect physiological data and histopathological analysis obtained from animal experiments and prepare reports; interpret data and make adequate changes for subsequent experimental design, preparation of data for publication in journals or for presentations to peer groups. Coordinate database management, scientific publications and technical writing.

Name: Dr. Francoise Arnaud
Project Role: Associate Investigator
Nearest Person Month worked 4
Contribution to Project: As an AI, Dr. Arnaud has provided scientific management and oversight of the project.

Name: Dr. Yaron Dayani
Project Role: Associate Investigator
Nearest Person Month worked 2
Contribution to Project: As an AI, Dr. Dayani has provided scientific management and oversight of the project.

Name: LCDR Gabriel Santiago
Project Role: Associate Investigator
Nearest Person Month worked 3
Contribution to Project: As an AI, LCDR Santiago has provided scientific management and oversight of the project.

Name: CDR Carl Goforth
Project Role: former principal Investigator
Nearest Person Month worked 1
Contribution to Project: As former PI from January 2018 to April 2020, CDR Carl Goforth has provided scientific management and oversight of the project. He contributed to the funding of this project.

Name: Dr. Anke Scultetus
Project Role: former principal Investigator
Nearest Person Month worked 1
Contribution to Project: As former PI from April 2020 to August 2020, Dr. Scultetus, has provided overall project management, planning for swine experiments in hypobaric chamber. She contributed to the funding of this project.

Name: Dr. Deb Malone
Project Role: Associate Investigator
Nearest Person Month worked 1
Contribution to Project: Dr. Malone has provided scientific management and oversight of the project.
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Name: Mr. Michael Hammett
Project Role: Laboratory assistant
Nearest Person Month worked 3
Contribution to Project: Mr. Hammett has provided technical assistance for the project.

Name: Mr. William Porter

Project Role: Engineer
Nearest Person Month worked 3
Contribution to Project: Mr. Porter has provided the maintenance of the hypobaric chamber.

Name: Mrs. Natalie Coschigano
Project Role: Surgical assistant
Nearest Person Month worked 4
Contribution to Project: Mrs. Coschigano has provided technical assistance for the project.

Name: Mrs. Jordan Hubbell
Project Role: Surgical assistant
Nearest Person Month worked 3
Contribution to Project: Mrs. Hubbell has provided technical assistance for the project.

Name: Mrs. Noemy Carballo
Project Role: Surgical assistant
Nearest Person Month worked 4
Contribution to Project: Mrs. Caballo has provided technical assistance for the project.
.
Name: Mr. Fang Yang
Project Role: Surgical assistant
Nearest Person Month worked 4
Contribution to Project: Mr. Yang has provided technical assistance for the project.

Name: LT. Sydney Dishman
Project Role: Associate Investigator
Nearest Person Month worked 2
Contribution to Project: LT. Sydney Dishman has provided surgical expertise and made suggestions to improve and simplify the vascular access for monitoring.

Personnel	**Role**	**Person month worked**
LCDR Carolyn Gosztyla	PI	4
Dr. Anke Scultetus	Former PI	1
Col Debra Malone	AI	1
Dr. Francoise Arnaud	Scientist: project management	4
Dr. Yaron Dayani	Scientist: data analysis	2
Michael Hammett	Research Assistant: hematology	2
William Porter	Chamber Operator	2
Natalie Coschigano	Research Assistant: animal data	4
Jordan Hubbell	Research Assistant: animal data	3
Noemy Carballo	Research Assistant:	4
Fang Yang	Research Assistant:	4
CDR Carl Goforth	Former PI	1

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Organization Name: PROFUSA, INC. (PROFUSA)
Location of Organization: (if foreign location list country) 45 Allerton Ave., South San Francisco, CA 94080
Partner's contribution to the project:

- *Financial support; None*
- *In-kind support:*
  Profusa will transfer the material(s) or equipment to NMRC consisting of a device to monitor tissue oxygenation (BEACON} and hydrogel oxygen sensors collectively referred to as (MATERIAL). Profusa owns, controls, or otherwise has all rights in MATERIAL.
- *Facilities: NMRC will use the MATERIAL solely for monitoring tissue oxygen level in skin, muscle, brain and liver in animal injury models (PURPOSE), under the direction and control of LCDR Carolyn Gosztyla; and will follow the United States Federal statutes, rules and regulations controlling the handling and use of research equipment and/or materials of the type described as the MATERIAL, as applicable. The PURPOSE is consistent with the mission of NMRC.*
- *Collaboration: NMRC partners LCDR Carolyn Gosztyla, LCDR Gabriel Santiago, Francoise Arnaud, Natalie Coschigano.*
- *Profusa Partners: Monica Lozano, Sean Givens, founder and former CEO: Natalie Wisniewski*

SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

Evaluation of the Timing of Aeromedical Evacuation in Rat and Swine Models of TBI and Polytrauma

Joint En Route Care Award – Intramural
Log Number DM167040 -Project 1
PI: LCDR Carolyn Gosztyla, Dr. Stephen T. Ahlers **Org:** NMRC/USUHS **Award Amount:** $1,176,000

Study/Product Aim(s)

- This proposal aims to clarify appropriate timing for altitude transport based on whole animal physiology, regional organ perfusion, inflammatory markers and tissue damage.
- We hypothesize that long range aeromedical transport of trauma victimseffects specific organ blood flow, inflammation and histological markers of tissue damage and that these endpoints can be modified by the timing of altitude transport.

Approach

We propose to investigate the relationship between standard versus delayed aeromedical evacuation and possible influences on patient outcome in a realistic combat casualty care, evacuation and definitive care study in rats and swine. Rats will receive one 110 kPa blast; swine will receive TBI or ARDS. Animals will undergo aeromedical evacuation on day 3 after injury (standard), or they will be on a delayed transport schedule of 7,10 or 14 days.

Rapid evacuation of combat casualties to CONUS is current standard. Our group has demonstrated that hypobaria reduces brain tissue oxygenation in TBI swine. This study will evaluate the impact of the timing of evacuation.

Timeline and Cost

Activities FY	17	18	19	20	21	22	23
IACUC/ACURO approval	🟩						
Rat blast/AE timing experiments		🟩	🟩				
Swine TBI/polytrauma AE timing experiments				🟥 cancelled	🟥		
Data analysis, final report, rat blast manuscript preparation					🟩	🟩	
Estimated Budget ($K)							

Updated: JAN2023

Goals/Milestones

FY17 Goals
☑ IACUC/ACURO protocol written, submitted and approved
FY18 Goals
☑ Begin rat blast experiments
☑ Complete rat blast experiments
FY19 Goals
☑ Initiate swine experiments
☑ Data analysis rat study
FY20 Goals
☑ IACUC/ACURO protocol written, submitted and approved
FY21 Goals
☑ Finalize rat study
FY22 Goals
☑ Manuscript preparation and Final study report

Comments/Challenges/Issues/Concerns: swine timing aim was removed

Budget Expenditure to Date: $1,176,000

The Effects of Oxygen Supplementation During Aeromedical Evacuation on Brain Oxygenation in Swine with Fluid-Percussion (FP) -Traumatic Brain Injury (TBI)

Joint En Route Care Award – Intramural
Log Number DM167040 -Project 2
PI: LCDR Carolyn Gosztyla **Site-PI:** Dr. Françoise Arnaud, Dr. Richard Maho **Org:** NMRC/USUHS **Award Amount:** $577,610

Study/Product Aim(s)

- We hypothesize that hypobaria during simulated long range aero-medical evacuation has adverse effects on brain blood flow, lung function and tissue oxygenation in neurotrauma and polytrauma patients.
- In a swine model, we plan to test the hypothesis that adapted supplementation with oxygen will be beneficial to the wounded during hypobaric aeromedical evacuation.

Approach

In a polytrauma swine model combining traumatic brain injury (TBI) and hemorrhage (HS), animal physiology, and metabolic, immunologic and histologic markers of injury will be evaluated at three supplemental oxygen levels (FiO2 of 30, 50 and 100%) during simulated altitude transport at 8,000ft, 2 hours after injury. In flight conditions will be reproduced in a hypobaric chamber at NMRC. A total of 62 swine are needed to conduct this research.

Severely wounded are often aero evacuated with 100% oxygen supplementation. The benefit of this strategy to brain and organ function is unknown. This study evaluates 3 levels of oxygen supplementation (30, 50 and 100%) particularly on tissue oxygenation using a pre-clinical polytrauma swine model.

Timeline and Cost

Activities FY	17	18	19	20	21	22	23
IACUC/ACURO approval	🟩			🟩			
Swine supplemental O2 /AE experiments		🟩	🟩	🟩	🟩	🟩	
Data analysis, final report, manuscript preparation					🟩	🟩	
Estimated Budget ($K)							

Updated: JAN2023

Goals/Milestones
FY17 Goals
☑ IACUC/ACURO protocol written, submitted and approved
☑ Initiate supplemental O2/Aero-Evacuation experiments
FY18 Goals
☑ Finalize Normo and Hypo settings
☑ Collect physiology and laboratory data
FY19/20 Goal
FY21/22 Goals
☑ Complete experiments
☑ Data analysis
☑ Write report
☑ Submit manuscript
Comments/Challenges/Issues/Concerns: Revised animal number
Budget Expenditure to Date: $577,610

Physiological Consequences of 4,000 and 8,000 ft. Altitude Aeromedical Evacuation on Swine with Traumatic Brain Injury and Hemorrhagic Shock

Joint En Route Care Award –Intramural

Log Number DM167040 -Project 3

PI: LCDR Carolyn Gosztyla **Site-PI:** Col Debra Malone, MC, USAF, Dr. Françoise Arnaud **Org:** NMRC/USUHS Award Amount: $919,589

Study/Product Aim(s)

- With aeromedical evacuation as a critical part of combat casualty care, this study aims to determine if there are differences in the neurologic, cardiac, and pulmonary effects of a 4 h transport at 4,000 ft. vs. 8,000 ft. on casualties with TBI or TBI + hemorrhagic shock (HS).
- Additionally, we seek to determine if the type and severity of the injury (TBI or TBI + HS) is affected by altitude.

Approach

- Animals will undergo TBI, TBI + HS, or Sham (no injury) and, after a 90 min stabilization period, will be exposed to one of three simulated transport altitudes (0, 4,000 or 8,000 ft.) for 4 h using a hypobaric chamber.
- TBI will be a fluid percussion injury of moderate severity (3.5 atm.) to allow comparison with previous studies; and HS will be induced by loss of 40% of blood volume.

US Navy combat nurse Lt. Cdr. Eric Gryntends to a critically injured civilian en route to hospital. The hypobaric chamber at the Center for Hypobaric Experimentation, Simulation and Testing (CHEST) will simulate such transport in swine.

Timeline and Cost

Activities FY	17	18	19	20	21	22	23
IACUC/ACURO approval				🟩🟪			
Swine TBI/polytrauma AE altitude experiments					🟩🟪🟥	🟥 cancelled	
Data analysis, final report, manuscript preparation						🟩🟪	
Estimated Budget ($K)							

Updated: JAN2023

Goals/Milestones

FY19 Goals

FY20 Goals

☑ IACUC/ACURO protocol written, submitted and approved

FY21 Goals

☑ Pilot animals (N = 5) for technique and system verification

☑ Begin in vivo experiments

☑ **FY21/22Goals**

☑ Complete in vivo experiments (N = 16)

☑ Batchedbiosampleanalysis, histopathology

☑ Final database

☑ Statistical Analysis

FY22 Goal

☑ Manuscript preparation and submission

Comments/Challenges/Issues/Concerns: Revised animal number

Budget Expenditure to Date: $919,589

5. APPENDICES:

N/A