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14. ABSTRACT

There is a dearth of knowledge about the effects of long range aero-medical evacuation on injured organs, as well as an emerging published database suggesting clinically significant adverse effects of hypobaria on even healthy tissues. Cabin pressure is equivalent to an altitude around 8,000ft. at which inspired oxygen is sufficient to maintain blood oxygen saturation above 90% in a healthy individual. In combat casualties with multiple injuries this could however compromise oxygen delivery and result in hypoxemia. Additionally, increase in altitude with concomitant decrease in atmospheric pressure allows gas expansion in body cavities. The volume of trapped gas expands by approximately 35% from sea level to an altitude of 8,000 feet. This can expose already vulnerable patients to severe complications. In light of this, a thorough investigation of the effects of hypobaria in clinical settings simulating the most important injury patterns encountered by combat casualties is necessary to optimize treatment efficacy and safety.

This study demonstrated potentially deleterious effects of altitude transport on wounded warfighters and requires additional investigations to optimize transport conditions. For example, the impact of timing of AE (early vs. delayed transport time from injury), transport treatments (e.g. oxygen therapy) and maintaining cabin pressures closer to sea level (e.g. 4000 ft.) may be optimized to ameliorate secondary brain injury and improve long-term functional outcomes. Our laboratory secured funding through other mechanisms and is currently investigating the aforementioned research gaps identified as a result of this grant.

15. SUBJECT TERMS
Aeromedical evacuation, en-route care, hypobaric conditions, hypobaric chamber, swine model

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1. INTRODUCTION

Current medical practice based on evidence derived from recent military conflicts has greatly diminished morbidity and mortality among combat casualties. Damage control resuscitation (1), delayed wound closure (2), tourniquets (3), and rapid evacuation for damage control surgery are examples of those observations contributing to improved care. In a study of 390 laparotomy casualties in Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF), survival was improved with damage control resuscitation (1). To maximize benefits for those treatment options, rapid evacuation of combat casualties to definitive care in the United States is paramount (4). However, not much is known about the effects of long range aero-medical evacuation in hypobaric environments on the physiology and organ function of injured warfighters. Fixed-wing transport aircraft generally have a cruising altitudes of 30 000 to 35 000 feet (ft), while keeping cabin pressure equivalent to an altitude of 5000 to 8000 ft (5). At 8000 ft, the partial pressure of inspired oxygen is approximately 108 mmHg. This is sufficient to maintain the blood oxygen saturation above 90% in a healthy individual (6). In combat casualties with multiple injuries this could however compromise oxygen delivery and result in hypoxemia. These polytraumatized patients, even if seemingly 'resuscitated' and stabilized by the time of aero-medical evacuation, have often endured severe insults due to hemorrhagic shock and resuscitation. Traumatic brain injury (TBI) patients are of particular concern, since small changes in ambient conditions such as cabin pressure and temperature could potentially have detrimental effects on the already vulnerable brain. The majority of TBI patients require ventilator support, with the risk of developing ventilator-associated Acute Respiratory Distress Syndrome (ARDS). Even more so, polytrauma patients with combined TBI and hemorrhagic shock, even if 'stabilized', are particularly tenuous to the negative interactive effects of low blood pressure on TBI outcome and of TBI homeostasis on shock outcome (7). These are additional groups of patients that could potentially have severe complications due to hypobaria and for whom optimization of all potential aero-medical evacuation effectors might significantly improve outcome.

For example, in a recent publication, it was found that acute lung injury was reduced in rats preconditioned with hyperbaric oxygen before high altitude flight (8). Another study demonstrated attenuation of high-altitude exposure (HAE)-induced acute lung injury and edema upon up regulation of the expression of Heat Shock Protein (HSP) 70 in lungs prior to the onset of HAE (9). Additionally, there is evidence that hypobaria as well as in-flight cabin pressure fluctuations can induce neurological symptoms in otherwise healthy persons due to altitude decompression sickness (10-14). These studies suggest that high altitude hypobaric conditions can have detrimental effects on pulmonary and neurologic outcome and that aero-medical conditions and/or therapeutics can be optimized to attenuate such adverse effects.

Another patient risk associated with an increase in altitude with concomitant decrease in atmospheric pressure is gas expansion in body cavities (15). The volume of trapped gas expands by approximately 35% from sea level to an altitude of 8000 feet. This can expose already vulnerable patients to complications such as pneumothorax, wound dehiscence or intracranial hemorrhage (16).

In light of the dearth of knowledge about the effects of long range aero-medical evacuation on injured organs (and non-injured organs—prevention of 'secondary brain injury'), as well as the emerging published database suggesting clinically significant adverse effects of hypobaria on even healthy tissues, a thorough investigation of the effects of hypobaria in clinical settings simulating the most important injury patterns encountered by combat casualties is necessary to optimize treatment efficacy and safety. In the study proposed herein, we plan to investigate the

effects of aero-medical evacuation on neurophysiology and lung function in swine models of TBI with and without hemorrhagic shock (HS) and/or ARDS (polytrauma).

Our hypothesis was that hypobaria during simulated long-range aeromedical evacuation (AE) has adverse effects on brain blood flow and tissue oxygenation, as well as lung function in swine models of neurotrauma and polytrauma. We investigated the effects of AE on neurophysiology and lung function in swine models of TBI with and without hemorrhagic shock (HS) and/or ARDS (polytrauma). Data from this study directly addressed the knowledge gap for the influence on hypobaria on neurotrauma and polytrauma casualties and has the potential to offer revised evacuation guidelines.

2. BODY

The objective of this study was to confirm our hypothesis related to the adverse effects of hypobaria on patients with TBI alone or in combination with HS and ARDS. Data from this study could potentially aid in the improvement of safety recommendations for enroute care and AE of combat casualties. Experiments were conducted under normobaric (ground) conditions and hypobaric (enroute AE) conditions using an altitude chamber specifically designed for large animal research. Hypobaric conditions were defined as atmospheric pressure equivalent to an altitude of 8000 ft, which is 75.3 kilo Pascal (kPa). Normobaric control conditions were ambient laboratory atmospheric pressure.

The following tasks were completed during the performance period of this grant in alignment with the statement of work:

Task 1. Build Hypobaric chamber (months 1-14):

Subtask 1. Complete engineering plans for construction (months 1-2).
Subtask 2. Acquire materials (months 2-4).
Subtask 3. Construction of chamber in machine shop at NMRC (months 5-14).

An integral part for the work to be accomplished for this grant was the design and construction of a chamber that can be de-pressurized to simulate long-range flight cabin pressures equivalent to 8,000 feet for the use of experiments in large animals. This is a unique capability within the entire military and civilian research community. Engineers at the Naval Medical Research Center experienced with the construction and maintenance of hyperbaric chambers for swine experiments to simulate deep sea dives in the Undersea Medicine Department have designed and completed construction of the hypobaric chamber for the experiments planned on this project within the first year. The NMRC Center for Hypobaric Experimentation, Simulation and Testing (CHEST) provides a unique platform for evaluation of long-range evacuation effects on physiology and therapeutic interventions in military relevant large animal combat injury models, which may contribute to optimization of combat casualty care and evacuation guidelines.

To our knowledge, this is the first hypobaric chamber built for trauma research and extensive data collection in swine. As a result, there were several unanticipated challenges associated with pressure differentials inside and outside the chamber, all of which have been resolved. The first pilot experiment has been completed within the first year of this grant, with a successful “flight” of an anesthetized, 30 kilogram Yorkshire swine.

Task 2. IACUC and ACURO approval for animal study (months 1-4):

Subtask 1. Write IACUC protocol (month 1).
Subtask 2. IACUC review and approval (months 1-2).

Subtask 3. ACURO review and approval (months 3-4).

IACUC and ACURO animal use protocol approval (WRAIR/NMRC Protocol # 13-OUMD-02LS and 16-OUMD-15LS

Task 3. Animal experiments during normobaric conditions (months 5-28):

Subtask 1. Complete 72 animal experiments in Sham, TBI alone, TBI+HS, ARDS alone, TBI+ARDS and TBI+HS+ARDS groups. Animals will be randomized (months 5-28)
Subtask 2. Hematologic and hematologic analysis of blood samples (months 5-28).
Subtask 3. Necropsy, gross pathology, histopathologic analysis (months 5-28).

Task 4. Animal experiments during hypobaric conditions (months 15-39):

Subtask 1. Complete 10 pilot animals to test hypobaric chamber and animal set up for monitoring within the chamber (months15-16).
Subtask 2. Complete 72 animal experiments in Sham, TBI alone, TBI+HS, ARDS alone, TBI+ARDS and TBI+HS+ARDS groups. Animals will be randomized (months 17-39)
Subtask 3. Hematologic and hematologic analysis of blood samples (months 17-39).
Subtask 4. Necropsy, gross pathology, histopathologic analysis (months 17-39).

After surgical instrumentation and stabilization, animals underwent one of the following injuries: TBI alone, TBI + HS, ARDS alone, TBI + ARDS, TBI + HS + ARDS. TBI was induced via fluid percussion injury, ARDS via broncho-alveolar lavage (In polytrauma studies) or oleic acid (in ARDS alone), and HS via 30% estimated blood volume controlled hemorrhage. Animals underwent injury-specific pre-hospital care and resuscitation. Once the animal was stable, a 4 hour AE was simulated in a hypobaric chamber with atmospheric pressure equivalent to an altitude of 8000 ft. , similar to an overseas flight from Central Asia to Landstuhl Regional Medical Center (LRMC, Germany) in a hypobaric chamber or during normobaric (ground transport) control. Control animals were kept at normobaric (ground transport) conditions. At 6 hours, animals were euthanized and a full necropsy was performed. During the experiment, systemic (vital signs, pulmonary artery catheter) and neurophysiology (intracranial and cerebral perfusion pressure) data were collected. Brain tissue oxygenation and brain blood flow were measured via Licox® Oxylite/Oxyflo as well as more globally via measurement of sagittal sinus blood oxygen saturation and lactate levels. Organ blood flow in brain, lungs, kidneys and liver was measured via microsphere injection. Lung function physiology was assessed with the ventilator and blood gas analysis. Blood was analyzed for electrolytes, organ function assays, coagulation and platelet function. Tissues harvested were paraffin embedded and analyzed for histopathology and immunohistochemistry.

Task 5. Data analysis and publication (months 40-48):

Subtask 1. Quality control of databases and lock databases (months 40-42)
Subtask 2. Statistical analysis (month 43-44).
Subtask 3. Final Study report preparation (months 45-47).
Subtask 4. Manuscript preparation and submission to peer-reviewed journal (months 46-48).

Please note that due to unforeseen animal health issue we had to halt experiments before the end of this grant. We received a one year no cost extension to complete the animal work.

3. KEY RESEARCH ACCOMPLISHMENTS

The study's aims were addressed by systematically exploring neurophysiological and systemic physiological changes in animals during normobaric and hypobaric conditions in several injury models in anesthetized swine. We also investigated hematologic and histologic changes. All experiments included sham controls, where the animals were instrumented and monitored, but received no injury.

During this reporting period, the following research goals have been accomplished:

- IACUC and ACURO animal use protocol approval (Protocol # 13-OUMD-02LS and 16-OUMD-15LS)
- Design and construction of a hypobaric chamber that can be de-pressurized to atmospheric pressure equivalent to 8,000 ft, and that can collect physiologic and neurophysiologic data in anesthetized large animals.
- Completion of pilot experiments to test chamber in a live, anesthetized animal.
- Animal experiments with TBI, TBI+HS, ARDS, TBI+ARDS and TBI+HS+ARDS have been completed.
- Histopathological analyses have been completed.
- Manuscripts for peer-reviewed publication have been accepted and published or are under journal review or in preparation.

The research results were distributed to the research communities of interest through oral and poster presentations at peer-reviewed conferences, in peer-reviewed manuscript publications and through personal interactions with potential collaborators.

This project provided training and professional development opportunities for several surgical residents and medical students from the local military community. One WRNMMC/USU, Department of Surgery Resident (Capt Steve Chun, MC, USA), spent a one year research rotation in our laboratory working on this project. USU medical students LT Bradley Yingst, USN, MC, LT Brian Vaught, USN, MC, ENS Davis Frease, USN, MC, ENS William Parker, USN, MC, each spent 1-3 months on this project. Additionally, Col Debra Malone, MC, USAF, Chief Trauma Research WRNMMC, was a guest researcher on this project and continues to be involved in other en route care projects in the NeuroTrauma Department as a Co-PI.

4. REPORTABLE OUTCOMES

Anke Scultetus, Richard Mahon, William Porter, William Hickman, Ashraful Haque, Noemy Carballo, Charles Auker, David Regis: "The Large Animal Medevac Simulation Suite (LAMSS) at the Naval Medical Research Center". Military Health System Research Symposium, Ft. Lauderdale, FL, August 2014.

R. Mahon, M. Bodo, M. Harssema, A. Scultetus "The Use of Pressure Reactivity Index (PRx) as a Measure of Cerebral Autoregulation during Flight in a Swine Model of Aeromedical Evacuation". USUHS Neuroscience Open House, Bethesda, MD, October 2015.

Ashraful Haque, Biswajit Saha, Brittany Hazzard, Noemy Carballo, Martin Harssema, Richard Mahon, Charles Auker, Debra Malone, Richard McCarron, Anke Scultetus: "Evaluation of physiology and lung function in hypobaric conditions during aeromedical evacuation in swine with mild ARDS", Annual Meeting of the American Society of Anesthesiologists, San Diego, CA, October 2015.

Anke Scultetus, Ashraful Haque, Steve Chun, Richard Mahon, Martin Harssema, Charles Auker, Debra Malone, Richard McCarron: "Hypobaria during aeromedical evacuation reduced

arterial oxygen pressure and increased pulmonary shunt fraction in swine with ARDS". Military Health System Research Symposium, Ft. Lauderdale, FL, August 2015.

Steve Chun, MD; Ashraful Haque, MD; Brittany Hazzard, BS; Saha Biswajit, MD, Martin Harssema, MD, Charles Auker, MD, PhD2, Debra Malone, MD, Richard McCarron, PhD; Anke Scultetus, MD: "Brain hypoxia is exacerbated in hypobaric conditions during aeromedical evacuation in swine with traumatic brain injury". Eastern Association for the Surgery of Trauma Annual Meeting, San Antonio, TX, January 2016.

Sudhamsh Tippireddy, Lam Thuy Vi Tran Ho, Anke Scultetus: "Hypobaria During Aeromedical Evacuation has Adverse Effects on Physiology and Neurophysiology in a Swine Polytrauma Model". SEAP/NREIP Student Presentations, Silver Spring, MD, August 2016.

Anke Scultetus MD; Ashraful Haque, MD; Brittany Hazzard, BS; Saha Biswajit, MD; Steve Chun, MD; Martin Harssema, MD; Charles Auker, MD, PhD; Debra Malone, MD; Richard McCarron, PhD: "Effects of long-range aeromedical evacuation on TBI in a swine model". Military Health System Research Symposium, Ft. Lauderdale, FL, August 2016.

Scultetus AH, Haque A, Chun SJ, Hazzard B, Mahon RT, Harssema MJ, Auker CR, Moon-Massat P, Malone DL, McCarron RM: Brain hypoxia is exacerbated in hypobaria during aeromedical evacuation in swine with traumatic brain injury. J Trauma Acute Care Surg. 2016 Jul;81(1):101-7.

Steve Chun, Ashraful Haque, Brittany Hazzard, Biswajit Saha, Martin Harrsema, Charles Auker, Debra Malone, Richard McCarron, Anke Scultetus, "Effects of Long-Range Aeromedical Evacuation on TBI in a Swine Model". Grand Rounds, Joint Base Andrews (JBA, MD), February 2017

Kirk Blackmoore, Ashraful Haque, Biswajit Saha, Noemy Carballo, Lam Thuy Vi Tran Ho, Neda Ilieva, Brittany Hazzard, Charles Auker, Richard McCarron, Anke Scultetus: "Brain Tissue Oxygenation was Reduced During Aeromedical Evacuation in a Swine Model of TBI and Hemorrhagic Shock" National Capital Area TBI Research Symposium, Bethesda, MD, March 2017.

Neda Ilieva, Ashraful Haque, Biswajit Saha, Noemy Carballo, Lam Thuy Vi Tran Ho, Kirk Blackmoore, Brittany Hazzard, Charles Auker, Debra Malone, Richard McCarron, Anke Scultetus: "Brain Tissue Oxygenation Decreased During Aeromedical Evacuation in a Swine Model of Traumatic Brain and Lung Injury". National Capital Area TBI Research Symposium, Bethesda, MD, March 2017.

Debra L. Malone, Richard M. McCarron, Anke H. Scultetus: "Effects of long-range aeromedical evacuation on TBI in a swine model." SHORESH Meeting, Rockville, MD, March 2017.

Anke Scultetus, Ashraful Haque, Steve Chun, Brittany Hazzard, Lam Thuy Vi Tran Ho, Saha Biswajit, Neda Ilieva, Martin Harssema, Charles Auker, MD, Richard McCarron, Debra Malone, "Brain tissue oxygenation was reduced in polytrauma swine during simulated aeromedical evacuation" (Panel presentation). Aerospace Medical Association Annual Meeting, Denver, CO May 2017.

Anke Scultetus, Ashraful Haque, Steve Chun, Brittany Hazzard, Lam Thuy Vi Tran Ho, Saha Biswajit, Neda Ilieva, Martin Harssema, Charles Auker, Richard McCarron, Debra Malone: "Hypobaria during aeromedical evacuation reduced brain tissue oxygenation in a swine polytrauma model". Military Health System Research Symposium, Ft. Lauderdale, FL, August 2017.

Col Debra Malone, MC, USAF; Ashraful Haque, MD; MAJ Michelle Jefferson, DVM, VC, USA; Lam Thuy Vi Tran Ho; Saha Biswajit, MD; Brittany Hazzard, BS; Neda Ilieva, BS; Capt Steve

Chun, MC, USA; LtCol Martin Harssema, MC, USAF; Charles Auker, MD, PhD; Richard McCarron, PhD; Anke Scultetus, MD: "Simulated Aeromedical Evacuation Significantly Increased Histopathological Evidence of Lung and Kidney Injury in a Swine Model of Acute Lung Injury". Excelsior Society Annual Meeting at the Clinical Congress of the American College of Surgeons, San Diego, CA, October 2017.

Col Debra Malone, MC, USAF; Ashraful Haque, MD; MAJ Michelle Jefferson, DVM, VC, USA; Lam Thuy Vi Tran Ho, BS; Biswajit Saha, MD; Capt Steve Chun, MC,USA; Kirk Blackmoore, BS; Neda Ilieva, BS; Charles Auker, MD, PhD; Richard McCarron, PhD; Anke Scultetus, MD: "Hypobaria during long range flight resulted in significantly increased histopathological evidence of lung and brain damage in a swine model". Eastern Association for the Surgery of Trauma (EAST) Annual Symposium, Lake Buena Vista, FL, January 2018.

Meghan Patterson, Jordan Hubbell, Andrea White, Biswajit Saha, Ashraful Haque, Noemy Carballo, Richard McCarron, Debra Malone, Anke Scultetus: "Histopathological Evidence of Brain and Lung Damage After Long-Range Flight in a Swine Model". National Capital Area TBI Research Symposium, Bethesda, MD, March 2018.

Jordan Hubbell, Meghan Patterson, Andrea White, Biswajit Saha, Ashraful Haque, Noemy Carballo, Richard McCarron, Debra Malone, Anke Scultetus: "Neurophysiological Changes During Aeromedical Evacuation in Swine with TBI and Polytrauma". National Capital Area TBI Research Symposium, Bethesda, MD, March 2018.

Andrea White, Jordan Hubbell, Meghan Patterson, Biswajit Saha, Ashraful Haque, Noemy Carballo, Richard McCarron, Debra Malone, Anke Scultetus: "Hypobaria in aeromedical evacuation exacerbates brain hypoxia and pulmonary hypertension in swine with concurrent traumatic brain injury and hemorrhagic shock". National Capital Area TBI Research Symposium, Bethesda, MD, March 2018.

Col Debra Malone, CDR Carl Goforth, LtCol Martin Harssema, Richard McCarron, Anke Scultetus: "Simulated aeromedical evacuation in swine increased lung and kidney damage with and without acute lung injury". Ramstein Aerospace Medicine Summit and NATO STO Technical Course 2018, Ramstein, Germany. March 2018.

Anke Scultetus, CDR Carl Goforth, LtCol Martin Harssema, Richard McCarron, Col Debra Malone: "Recovery was slowed and brain tissue oxygenation was reduced during simulated aeromedical evacuation in swine models of traumatic brain injury". Ramstein Aerospace Medicine Summit and NATO STO Technical Course 2018, Ramstein, Germany. March 2018.

Col Debra Malone, Anke Scultetus: "Lung and Kidney Injury is Exacerbated after Simulated Aeromedical Evacuation in Swine with and without Induced Acute Lung Injury". Aerospace Medical Association (AsMA) Annual Meeting, Dallas, TX, May 2018. – Accepted abstract

Anke Scultetus: "Recovery is Slowed after Simulated Aeromedical Evacuation from Battlefield to CONUS in Swine with Traumatic Brain Injury". Aerospace Medical Association (AsMA) Annual Meeting, Dallas, TX, May 2018. – Accepted abstract

Anke Scultetus, MAJ Michelle Jefferson, Francoise Arnaud, Andrea White, Meghan Patterson, Jordan Hubbell, Paula Moon-Massat, CDR Carl Goforth, Richard McCarron, Col Debra Malone: "Histopathological evidence of multiple organ damage after simulated long-range flight in a swine model". Military Health System Research Symposium, Kissimmee, FL, August 2018. – Submitted abstract

Anke H. Scultetus; MAJ Michelle A. Jefferson, Ashraful Haque; Lam Thuy Vi Tran Ho; Brittany Hazzard; Biswajit K. Saha; Capt Steve J. Chun; Charles R. Auker; Paula F. Moon-Massat; Richard M. McCarron; Col Debra L. Malone. Hypobaria during long range flight resulted in

significantly increased histopathological evidence of lung and brain damage in a swine model. J Trauma Acute Care Surg. *In press.*

Anke H. Scultetus; Ashraful Haque; Saha Biswajit; Brittany Hazzard; Lam Thuy Vi Tran Ho; Andrea N. White; Jordan N. Hubbell; Meghan A. Patterson ; LtCol Martin J. Harssema; Paula Moon-Massat; Francoise G. Arnaud, PhD; Richard M. McCarron, PhD; Col Debra L. Malone. Prolonged aeromedical evacuation resulted in physiological instablity in swine with traumatic brain injury and hemorrhage. J Trauma Acute Care Surg. *Under Review.*

Anke H. Scultetus; Paula F. Moon-Massat; Ashraful Haque; Brittany Hazzard; Steve J. Chun; Richard M. McCarron; Debra L. Malone. Simulated Aeromedical Evacuation Worsens Acute Lung and Kidney Injury in Swine. *In preparation.*

Anke H. Scultetus; Paula F. Moon-Massat; Ashraful Haque; Brittany Hazzard; Steve J. Chun; Richard M. McCarron; Debra L. Malone. The effects of simulated aeromedical evacuation in swine on polytrauma injuries. *In preparation.*

5. CONCLUSIONS

The hypobaric chamber for simulating swine experiment during long-range AE flights built for this grant provides a unique platform for the evaluation of possible effects of AE on physiology and therapeutic interventions in military relevant large animal combat injury models. The creation of the NMRC Center for Hypobaric Experimentation, Simulation and Testing (CHEST) may contribute to optimization of combat casualty care and evacuation guidelines. It allows for extensive and invasive physiological and neurophysiological monitoring and data collection during the entire simulated flight duration and is a unique capability with the military and civilian research community.

This grant funded the first study ever to extensively evaluate possible effects of AE on injured swine which were fully instrumented to allow for collection of data elements not possible in the human AE setting currently employed for rapid evacuation of wounded warriors with Critical Care Air Transport Teams (CCATT) to the Continental United States (CONUS). Several observations and conclusions drawn from the data collected provide reason for concern about possible effects of AE on the injured and need to be further investigated in follow on studies.

Our data showed that a 4 h simulated aeromedical evacuation flight at cabin pressures equivalent to 8,000 ft. reduced brain tissue oxygenation (Figure 1), cerebral blood flow (Figure 2) and cerebral perfusion pressure in swine with TBI when exposed to transport within two hours of injury. We presume that these clinical decrements may potentiate secondary neurological insults following TBI. These data suggest that in this fluid percussion model of TBI, the duration of altitude exposure and time of aeromedical evacuation from injury results in a patient that is less stable hemodynamically and neurologically than a similar patient at normobaric conditions. Brain tissue oxygenation in the normobaric swine indicated that these swine began to recover from their injury during transport, with brain oxygenation rising 25 – 50% above injury levels. This cerebral hypoxemia in the HYPO animals is most likely due to their lower Mean Arterial Pressure (MAP) (*vs*. NORMO swine) which resulted in significantly reduced cerebral perfusion pressure for the entire duration of the simulated aeromedical evacuation. This prolonged reduction in cerebral perfusion pressure not only reduced brain oxygenation for an equivalent duration but eventually also caused a reduction in cerebral blood flow, as directly measured by microspheres. There were no clinically relevant differences between the two groups in other laboratory measurements (i.e., electrolytes, glucose, base excess, bicarbonate, or complete blood count; data not shown). A limitation of this study is that this was not a survival study, so the long term sequelae of these changes on cerebral structure

(histopathology) and function (behavioral studies) could not be determined. Other limitations include the use of anesthetics and aeromedical evacuation within two hours of injury. In currently ongoing studies in our lab these potential pitfalls have been addressed by making the study a 14 day survival study and by simulating aeromedical evacuation flights on Day 3 and Day 6 after injury.

The clinical evidence on whether or not altitude-induced hypoxemia occurs in our wounded warriors during aeromedical evacuation is conflicting. In a study of 63 combat casualties with severe isolated TBI who were transported by the U.S. Air Force CCATT from 2003 through 2006, there was a relatively low incidence in the occurrence of in-flight altitude-induced hypoxia and this was attributed to successful supplementation of in-flight oxygen (17). On the other hand, data from our study supports a more recent study that reported the occurrence of hypoxemia (oxygen saturation [SpO_2] < 90%) in a high percentage (90%) of transported casualties. In this study, the hypoxemic events lasted on average 44 minutes. Furthermore, 56% of patients exhibited a SpO_2 < 85% for ~12 minutes. This latter study suggests hypoxemia may be a common event during aero-medical evacuations (18). This same study also showed that this mode of evacuation resulted in increases in intracranial pressure (ICP) from external stimuli (vibration, noise) and acceleration and deceleration forces; all potential additional contributors to the development of secondary brain injury.

In the sham group, we evaluated one transport condition, altitude (hypobaria), on acute physiological parameters and the histopathology of selected organs from anesthetized, mechanically ventilated swine (Figure 3 and 4). We performed these experiments in order to obtain a baseline understanding of the clinical impact of hypobaria so that we can assess the efficacy and safety of hypobaric transport for wounded warriors. Given the data demonstrating that healthy individuals flying at cabin pressures equivalent to altitudes of 1,526 to 2,441 m (5,000 to 8,000 ft.) can compensate for the lower partial pressure of inspired oxygen (~108 mm Hg) (6) and given the lack of critical symptoms in flight crew personnel, it was not surprising that there were minimal to no immediately life threatening clinical changes (hemodynamic, neurologic, oxygen transport, blood gas, chemistry, or complete blood count data) in these healthy swine. However, the animals exposed to hypobaric conditions had significantly more histopathological changes in their lungs compared to normobaric control pigs. The overall Lung Injury Score was significantly worse in the HYPO group and this increased score was largely due to an increased number of histopathological categories (Figure 5). Specifically, in the HYPO group, there was more edema/fibrin/hemorrhage, more congestion, evidence of microatelectasis, and increased neutrophilic and histiocytic inflammation (Figure 6).

While there is no direct evidence to link the results of our data to the occupational hazards of aircrew, an association can be drawn and should not be disregarded. As an example, the prevalence of medical symptoms from 323 Israeli Air Force personnel was recently reviewed and respiratory symptoms were the second most frequent symptom exhibited (behind spinal pain); with a frequency of one in five aviators (22%) (19). More precisely, these respiratory symptoms included rhinorrhea (17%), cough (10%) and dyspnea (1%). The symptoms associated with the respiratory pathology found in our study are unknown since these pigs were anesthetized but coughing and dyspnea are conceivable. Furthermore, the pathological changes in the hypobaric pigs developed acutely, were mild and, most likely, were reversible as were the symptoms in this clinical report. In addition, short exposure to high altitude has been shown to increase pulmonary edema in healthy, awake, spontaneously breathing humans after only one day at 4,559 m (14,957 ft.), supporting the possibility of alveolar-capillary damage (20) with additional evidence that pulmonary edema (21) and pulmonary oxidative stress (22) are triggered by altitude alone.

On the other hand, an earlier assessment of these Israeli Air Force aviators found no correlation between any of their symptoms and the hours of flying or type of plane, leading the authors to conclude that flight itself might not be a trigger for their symptoms (23). Altitude was not directly investigated in this early Israeli study. In contrast, the only difference between the hypobaric transport group and the normobaric control group in our study was, indeed, flight or, more precisely, being at 2,441 m (8,000 ft.) altitude for four hours. Thus, it may not be the hours in a plane or the type of plane but the exposure to certain altitudes for unspecified amounts of time.

The potentially confounding effects of anesthesia, mechanical ventilation, and breathing dry inspired gases (vs. humidified gases when awake) occurred in this swine study and could be additive or synergistic with any effects of altitude alone. A short duration of anesthesia and controlled ventilation is known to cause alveolar damage, atelectasis, and impaired gas exchange (24-26). Awake animal models are needed to more appropriately mimic air crew scenarios and determine the underlying mechanisms of the occupational medical health hazards they face. Nonetheless, even if the effects of anesthesia and controlled ventilation make the results in this study incomparable to flight crews, the results demonstrate that transport at altitude may have detrimental effects on wounded warriors being aeromedically evacuated. These wounded warriors may receive in-flight sedation and respiratory support (e.g., a sedated soldier requiring mechanical ventilation due to a severe head injury). When one then adds on the impact of transporting a wounded warrior with lung injury, the combined effects of hypobaria, anesthesia and controlled ventilation may be significant enough to affect morbidity and mortality.

One puzzling result of this study was the increased renal inflammation in the normobaric group compared to the hypobaric group. The scoring between the two groups was similar (scores ranging from 0 to 1) and all pigs had low scores (minimal inflammation), with no outlier in either group. There is no clear explanation for this outcome and renal pathology should continue to be evaluated in future studies.

It is clear that our study raises more questions than it answers. It is unknown when the adverse pulmonary pathological changes demonstrated in this study began to develop, although there was a visual trend of some parameters changing after 2 h of hypobaric transport. It is unknown if the pathological changes become more severe with longer flights or higher altitude, if it is transient and spontaneously resolves, or if it progresses to a more severe condition.

Military and civilian aircraft are pressurized in a similar fashion to a cabin pressure equivalent to 8,000 ft. At the discretion of the medical officer in charge of the aeromedical evacuation, the aircraft can be further pressurized to 4,000 ft or even lower if the condition of a specific patient necessitates; but this is not routinely done. Research in this area is just emerging and future studies are planned to assess the effects of different transport altitudes on injuries. While helicopters are not pressurized, their altitude at flight for aeromedical transport in theatre is usually 3,000 - 4,000 ft. However, this can vary depending on the starting altitude at ground level. For instance, while the ground level in Iraq is at sea level, the ground level in Afghanistan varies widely with base altitudes of up to 10,000 ft. It is therefore not unusual for helicopters to fly as high as 14,000 ft with unpressurized cabins. While these altitudes are reached for much shorter duration, their effect on aeromedical transport is certainly worthy of future investigations.

The effect of superimposing four hours of simulated aeromedical evacuation to 8,000 ft. (hypobaric conditions) on this subclinical lung injury model was three-fold. First, the underlying pulmonary disease, while it was subclinical prior to transport, progressed and manifested itself clinically as a reduction in arterial partial pressure of oxygen (P_aO_2) during the latter portion of

the flight and also progressed histopathologically, with worse injury scores compared to the ground transport group . Secondly, most of the other organs (the brain being the only exception) were also adversely affected by the combination of exposure to both the acid and the hypobaric conditions, although this was only apparent histologically with higher organ injury scores. Thirdly, the hypobaric group became hemodynamically more “unstable” in the final hours of transport compared to the normobaric group; a finding similar to previous studies using identical transport conditions (27). It is important to note that these effects were solely due to hypobaric conditions and not due to an altitude-induced reduction in inspired oxygen; both groups were administered 40% oxygen throughout the transport to specifically evaluate the hypobaric condition.

The lung injury, itself, resulted in classical morphological changes from oleic acid exposure, including congestion and interstitial and intra-alveolar edema (Figures 7). Oleic acid often produces a heterogeneous pattern of lung injury with areas of both normal and abnormal regions. However, despite the potential for this heterogeneity to make comparative analysis difficult, there was clear evidence that the four hours of hypobaria promoted further damage to the lungs (Figure 8-10). The kidneys, another organ that showed evidence of injury from the oleic acid, were also adversely affected by hypobaric conditions based on histopathology. Furthermore, hypobaria induced measureable pathology in adrenals, liver and pancreas, organs previously unaffected by the oleic acid. The brain appeared uniquely protected, with no histopathological differences between the groups (Table 1 and 2).

In our study, addition of high altitude “air transport” made this oleic acid-induced renal injury more severe which should have been predicted, given that the lungs were also more severely affected by hypobaric conditions. However, it was unexpected given that our previous paper showed there was more renal damage in the normobaric, not hypobaric, group when we investigated the effects of hypobaria on healthy swine. An explanation for these conflicting results is not readily apparent. A post hoc analysis of the injury scores from the four groups (two groups from each of the two studies) suggests that the previous finding in the normobaric healthy swine study to be the outlier, but this assumption cannot be conclusively proven without a more detailed study focusing on the kidneys. Current kidney results highlight that future studies should focus on renal disease to better understand how such long-distance flight may affect the kidneys and should include specific measurements of renal function (i.e., glomerular filtration rate, creatinine).

Of note, nearly every subcategory of injury type for both the lungs and kidneys showed significantly worse scores when animals were exposed to hypobaric conditions; there was no single over-riding subcategory classification that may have “skewed” the total injury scores for either organ. Hyaline membrane formation in the lungs was not different between the two groups but this may be a reflection of the lack of severity in degree of lung damage rather than the lack of effect of transport conditions. Renal inflammation scores were not different either although there was a trend for this score to be worse in the hypobaric group, suggesting a larger number of animals may have found this to be significant as well.

Despite the histopathology scores of all organs (except the brain) being worse in the hypobaric group, there were only a few, minor physiological differences observed in the hypobaric group during the transport which supports the clinical view that most patients can endure AE. However, during simulated air transport, P_aO_2 began to decline and some hemodynamic parameters showed more fluctuations and less stability than those in the simulated ground transport group. While the lower P_aO_2 did not decrease to hypoxemic levels, under different clinical conditions, this deterioration during transport could have more dire consequences. The onset of this “physiological instability” was after approximately two hours of simulated flight; exactly the same time when similar changes were observed in our previous

studies monitoring healthy swine, swine with TBI, and swine with polytrauma. Collectively, there is growing evidence that duration of flight, at this altitude, may affect injured troops in subtle ways, requiring vigilant medical attention even when such troops appear stable early in the flight.

This report of an adverse effect of hypobaria on renal morphology also cannot be directly compared to earlier studies. Nearly all other studies of non-pulmonary altitude-induced organ injury (21) focus on the effect of altitude-induced hypoxia which was, by design, not a factor in the current study. Thus, this study extends knowledge beyond these previous studies by showing that hypobaria, by itself and without concurrent altitude-induced hypoxia, can have an impact when there is an underlying injury (oleic acid as a stimulant for oxidative stress in all organs) and that both pulmonary and non-pulmonary organs can be adversely affected by high-altitude in a setting that mimics aeromedical transport (four hours at 8,000 ft.).

A study limitation is that many standard measurements of pulmonary function (i.e., shunt fractions, alveolar to arterial (A-a) gradients, pulmonary compliance, lung volumes, peak inspiratory pressures) were not collected and thus the physiological impact of these findings is restricted; the mechanism behind the P_aO_2 decrease cannot be determined. These parameters were deliberately excluded from analysis due to uncertainty in the precision of the measurements in the hypobaric chamber. For example, determination of dynamic lung compliance required correcting for the volume of the non-patient part of the respiratory circuit (e.g., compressible hoses) and although this correction factor was known during normobaric conditions, the effect of hypobaria was unknown. Further, our swine were anesthetized and laboratory personnel are not currently permitted into the hypobaric chamber at this institution so spirometric variables (i.e., forced vital capacity) and other variables that can be collected in humans during flight could not be collected in this setting.

This study supports the conclusion that hypobaria may be detrimental to those with subclinical lung and renal disease even when the inspired oxygen concentration prevents hypoxemia. Extending these findings to the clinical scenario, hypobaric conditions may be particularly detrimental to those with preexisting poor gas exchange due to pulmonary injuries as any resulting hypoxemia could affect a multitude of organ systems. Furthermore, all of our injured swine hypobaric transport studies have shown, regardless of being isolated TBI, TBI plus hemorrhage, or mild lung injury, that conditions change during transport causing previously “stabile” appearing animals to begin to become “unstable” after two hours of 8,000 ft. altitude. The lungs and kidneys were particularly sensitive organs to hypobaric injury in the current study but other organs may also become sensitized to hypobaria if injured through other mechanisms (i.e., traumatic brain injury). The possibility of differential organ sensitivity to hypobaric conditions may be useful to medical responders such as Critical Care Air Transport Teams (CCATTs) or Acute Lung Rescue Teams (ALRTs) such that special attention should be given to those with underlying pulmonary or renal disorders.

In the TBI+HS polytrauma study, data showed that detrimental neurological and hemodynamic effects of the simulated 4-h flight (8,000 ft.) were observed, particularly in the final 2 hours of the “flight.” (Figure 11-13). Our previous study, using an isolated PF-TBI model, showed that an identical 4 hour “flight” reduced cerebral blood flow, cerebral perfusion pressure (CPP) and showed a larger decrease in brain tissue oxygenation ($P_{bt}O_2$) than “ground transport” (normobaric) animals with no difference in ICP (27). Thus, the current study provides the additional information that concurrent blood loss superimposed on TBI results in hemodynamic decline despite allowing 2 hours for medical treatments and stabilization before transport.

While it seems intuitive that a patient with multiple injuries may be less stable than a patient with an isolated injury, it was not intuitive that transport conditions of 8,000 ft. would expose the

vulnerability of the former patient compared to the latter patient; especially when both groups of patients underwent an “ER/ICU” phase designed to produce stable hemodynamics (e.g., treatment with fluids, including blood transfusions) and management algorithms to minimize neurological complications (e.g., mannitol for elevations in ICP).

In this study, the percent change in $P_{bt}O_2$ from baseline was worse during hypobaric (aeromedical evacuation) conditions compared to normobaric (ground) conditions and this was similar to our previous aeromedical evacuation study of isolated TBI in swine (27). In the polytrauma study, the ICP elevation was observed soon after the beginning of the hypobaric transport and continued to progress for the duration of the transport while a similarly detrimental, but delayed effect was observed for CPP. However, this was not statistically different to similar trends observed in the normobaric group. Similarly, these neurological parameters were not different between the two transport conditions in the earlier isolated TBI swine model. Of note, however, close visual observation of the CPP from the isolated TBI study suggested there was a non-significant tendency for CPP to also decrease in the latter half of hypobaric transport. These data provide evidence that the animals in the TBI+HS model were neurologically more sensitive to the effect of altitude, even though their blood loss was treated with appropriate fluids prior to transport and also that both studies showed some evidence that the prolonged effects of altitude can result in some degree of physiological decompensation.

Similarly, hemodynamic effects of altitude were more readily apparent in the polytrauma model than in the isolated TBI model. In this polytrauma study, hemodynamic differences (MAP, HR, CI, and MPAP) due to transport conditions became apparent during the last two hours of transport while there were no hemodynamic differences due to transport conditions in the isolated TBI study (27). That is, MAP, MPAP, CVP and CI tended to be higher in the HYPO group than in the NORMO group in the current study in the final phase of transport. A mechanism for these differences, based on altitude, is unknown and warrants further investigation in additional studies.

Although research in the field of aeromedical evacuation is mounting, most preclinical studies have been performed on small laboratory species (mice and rats) (28, 29) making comparisons to our swine studies difficult. In addition, nearly all of those studies used an isolated TBI model, not polytrauma, although all studies consistently showed the effects of hypobaria to be deleterious. The effect of hypobaria on isolated blood loss has been described once, using a hemorrhagic shock mouse model, and those investigators concluded that no adverse effects occurred when a 5 hour simulated aeromedical evacuation was compared to normobaric conditions (28). This, in part, supports our findings that the detrimental effects of hypobaria were focused on neurologic, not hemodynamic, parameters but our finding further suggest that blood loss may set the stage for a patient to be more sensitive to the neurologic effects of hypobaria.

Despite the paucity of polytrauma models to which to compare, the present study supports the observation of clinicians who care for wounded warfighters under prolonged aeromedical transport conditions. During CCATT aeromedical evacuations during recent conflicts, one collaborator (LtCol M.H., Critical Care Air Transport Team 779th Medical Group, Joint Base Andrews, MD) has observed that patients often seemed stable during the early portion of flight but became increasingly in need of additional treatments to maintain a stable condition later in these flights. There are currently no published clinical data to support this observation of generalized downward trends after 2 hour of flight, although our current study is in full agreement with the clinical impressions shared with the authors. Clearly, hypobaric conditions are an additional stressor to wounded troops and the duration of hypobaric transport may result in more unstable or “fragile” patients. Importantly, this study is a laboratory model of pure blood loss with fluid percussion TBI while the clinical scenarios include many diverse types of trauma

(i.e., blast or crush injury, pulmonary disorders, etc.), rarely in isolation, and in combination with concomitant environmental situations (temperature extremes, vibration, etc.). Thus, the detrimental effects of hypobaric transport in the current study are likely intensified in the clinical arena.

An important aspect of our study design, compared to other preclinical studies (28) is that these swine were not breathing reduced amounts oxygen as normally endured when at high altitude. These swine were inspiring 40% oxygen; the type of oxygen supplementation commonly provided to wounded warfighters during transport. Although altitude-induced hypoxemia is counteracted by troops breathing enriched oxygen mixtures, there is conflicting evidence on whether or not hypoxemia is prevented during flight. In one study of combat casualties with severe isolated TBI, there was a relatively low incidence of in-flight hypoxia and this was attributed to successful supplementation of in-flight oxygen (17). On the other hand, another study reported hypoxemia occurred in a high percentage (90%) of transported casualties (18). We purposefully chose a study design to eliminate altitude-induced hypoxemia as an additional cause of any affects observed in our study.

A limitation of this study is that this was not a survival study, so the long-term sequelae of these changes on cerebral structure (histopathology) and function (behavioral studies) could not be determined. Other limitations include the use of anesthetics, mechanical ventilation and studying aeromedical evacuation shortly after the injury (2 hours). While anesthetics could not be avoided without causing inhumane treatment in this acute study, our laboratory is addressing these other potential pitfalls by developing a 14-day survival study as well as evaluating simulated aeromedical evacuation on Day 3 and Day 6 after injury. We do not consider the use of mechanical ventilation a critical limitation of this study, given a recent chart review of injured troops transported out of Afghanistan that reported 62% of the casualties were mechanically ventilated during transport (30).

In summary, the potentially deleterious effects of altitude transport on wounded warfighters require additional investigations to optimize transport conditions. For example, the impact of timing of AE (early *vs.* delayed transport time from injury), transport treatments (e.g. oxygen therapy) and maintaining cabin pressures closer to sea level (e.g. 4000 ft.) may be optimized to ameliorate secondary brain injury and improve long-term functional outcomes. Our laboratory secured funding through other mechanisms and is currently investigating the aforementioned research gaps identified as a result of this grant.

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7. SUPPORTING DATA

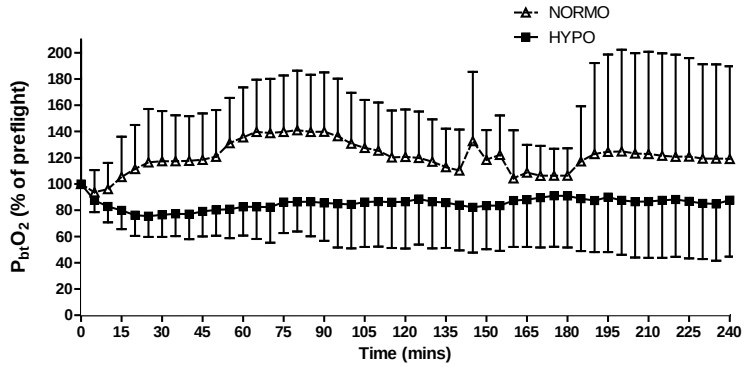

Fig 1. Change in brain tissue oxygen concentration ($P_{bt}O_2$, mean ± SEM) during 4 h simulated transport of swine with TBI at 8,000 ft. altitude (HYPO) or at sea level, 0 ft. (NORMO). Time 0 = BL before transport (sea level).

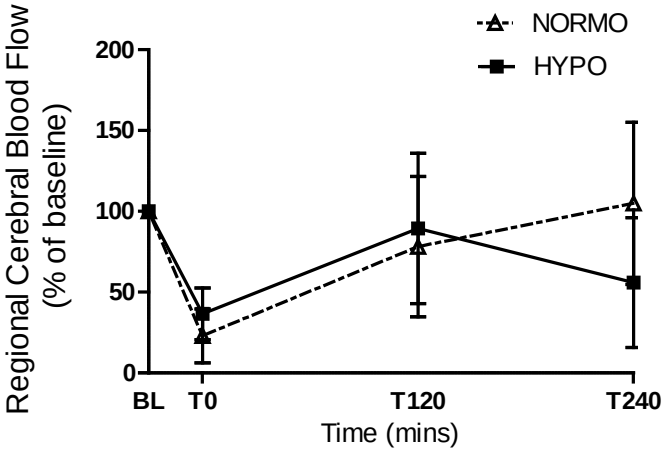

Fig 2. Change in regional cerebral blood flow (rCBF, mean ± SEM) of swine with TBI at 8,000 ft. altitude (HYPO) or at sea level, 0 ft. (NORMO). BL = before injury. T0 = BL before transport (sea level). T120 and T240 = 2 and 4 hours into transport.

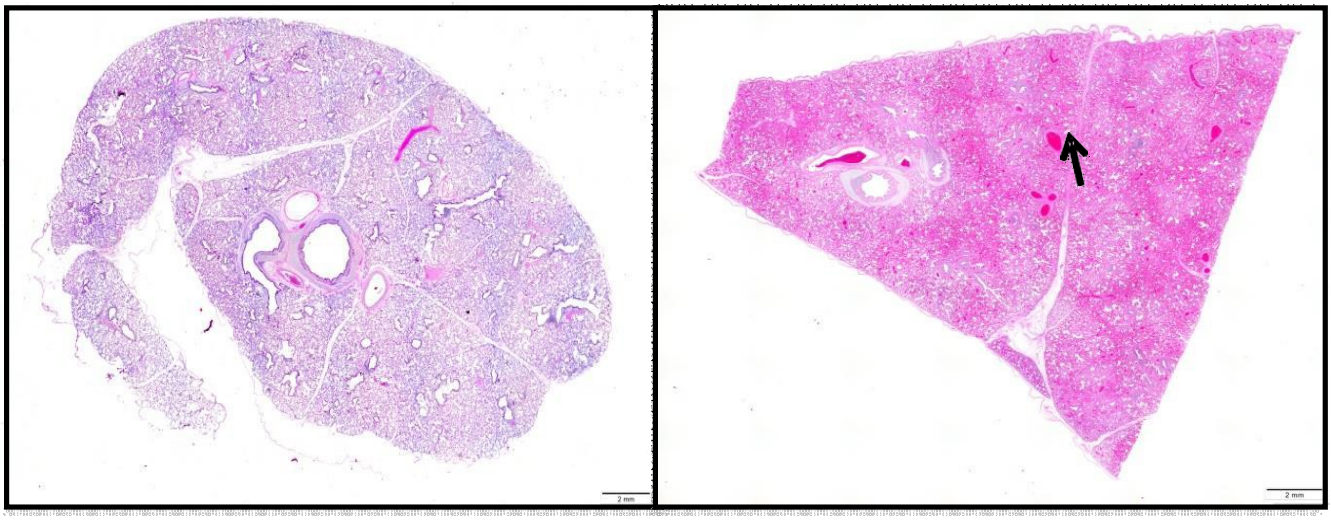

Figure 3. A.) Sham animals, normobaric conditions. Lung, H&E, 4x. B) Hypobaric conditions. Lung, H&E, 4x. In B, there is increased cellularity with moderate evidence of congestion, edema and hemorrhage (arrow) and microatelectasis when compared to A.

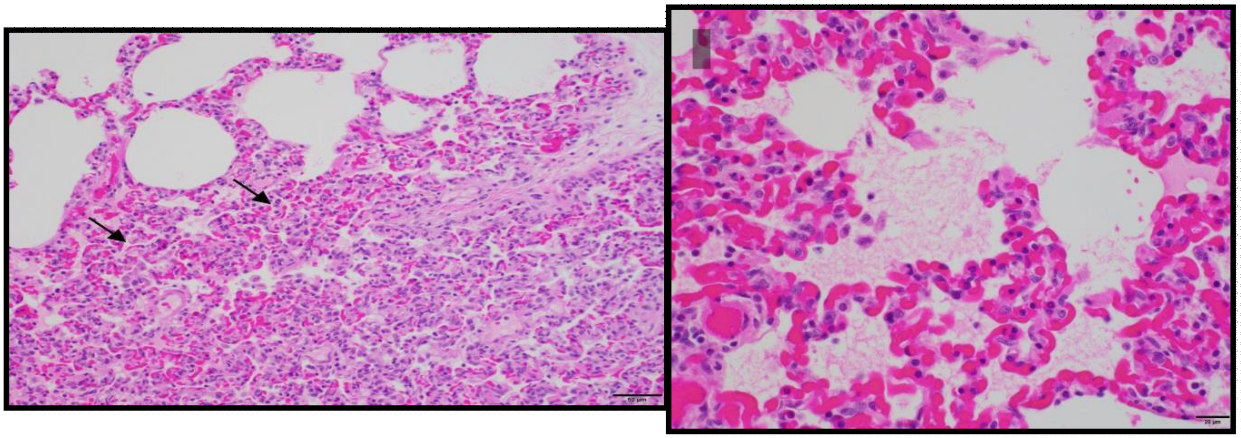

Figure 4. A.) Sham animals, hypobaric conditions. Lung, H&E, 20x. Microatelectasis with scattered inflammation in the alveolar lumen and septa (arrows). B) Higher magnification of A, 40x showing marked congestion, alveolar lumen fibrin and edema.

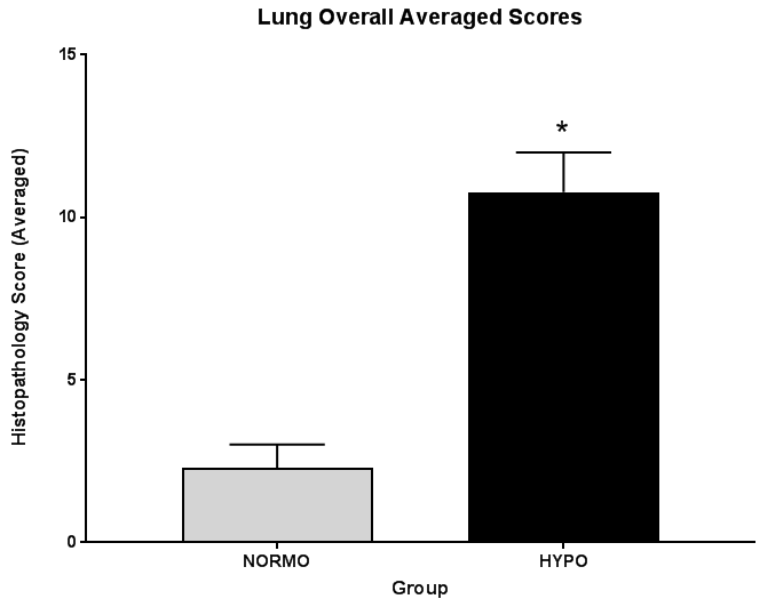

Figure 5: Sham animals: Overall lung scores.

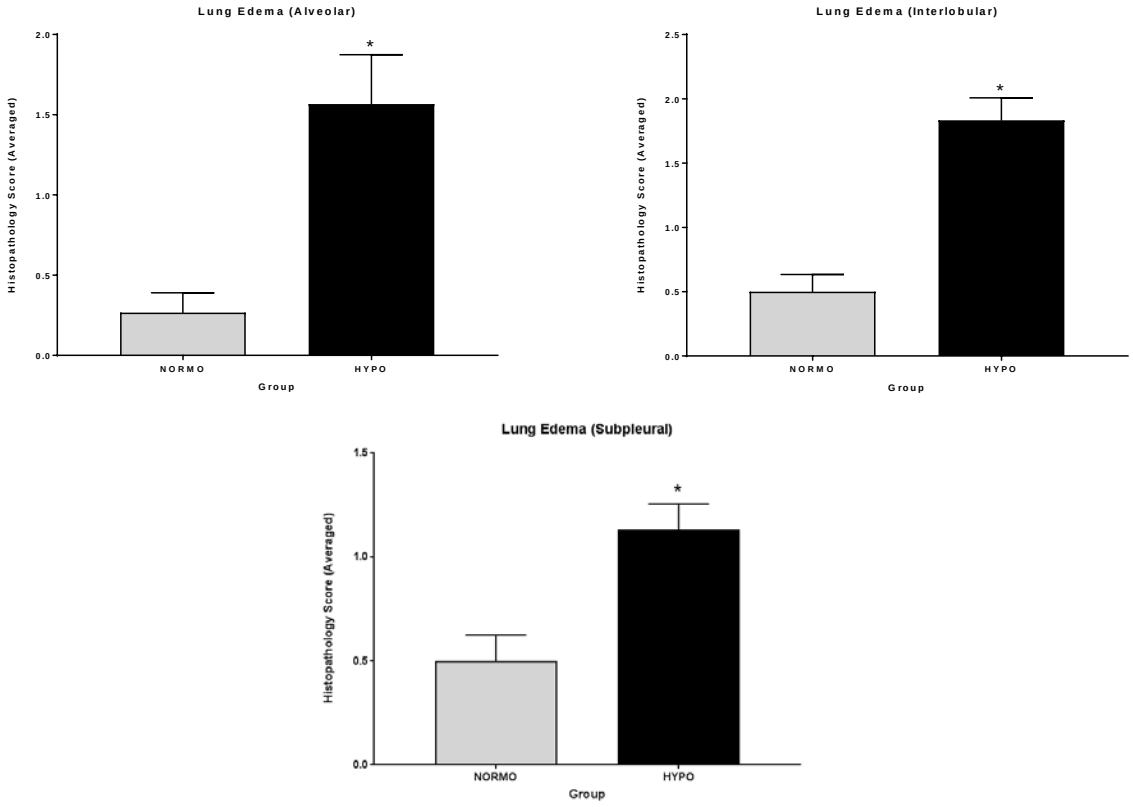

Figure 6: Sham animals: edema in various regions of the lung.

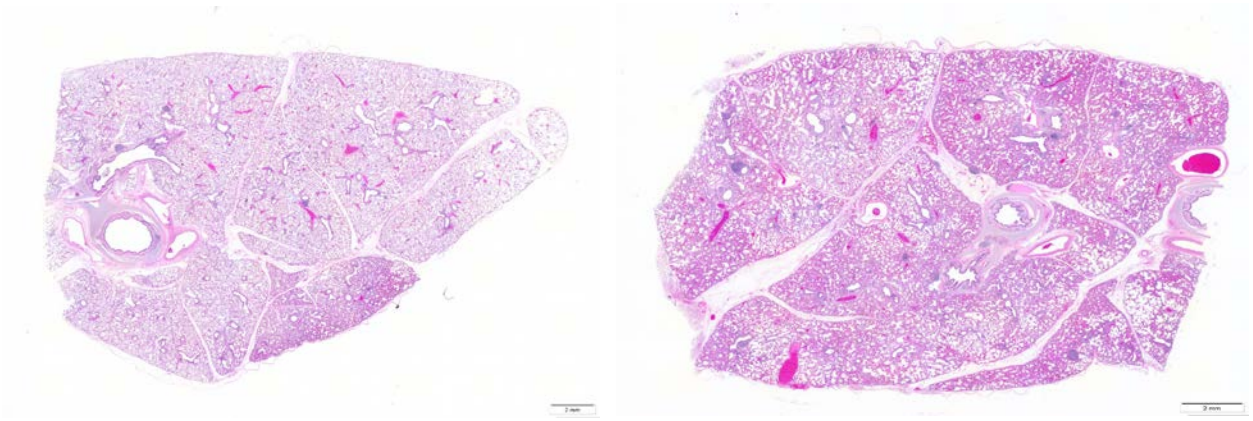

Figure 7: NORMO (left) and HYPO (right) lung tissue in lung injury groups: moderate histiocytosis, mild neutrophil infiltration, moderate hemorrhage, fibrin and edema in a HYPO animal.

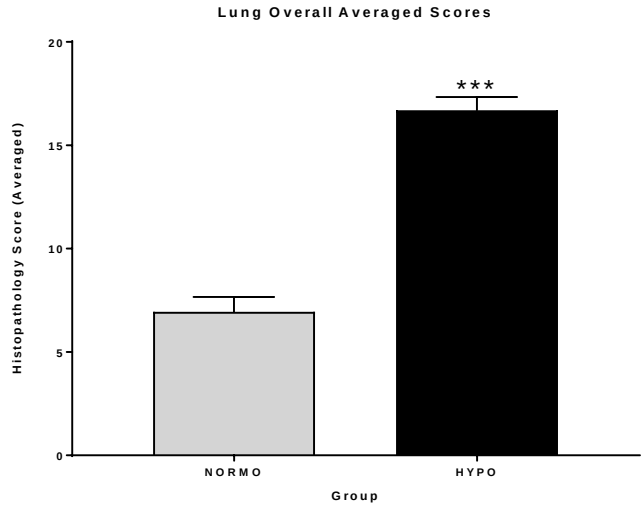

Figure 8: Lung injury groups overall injury score.

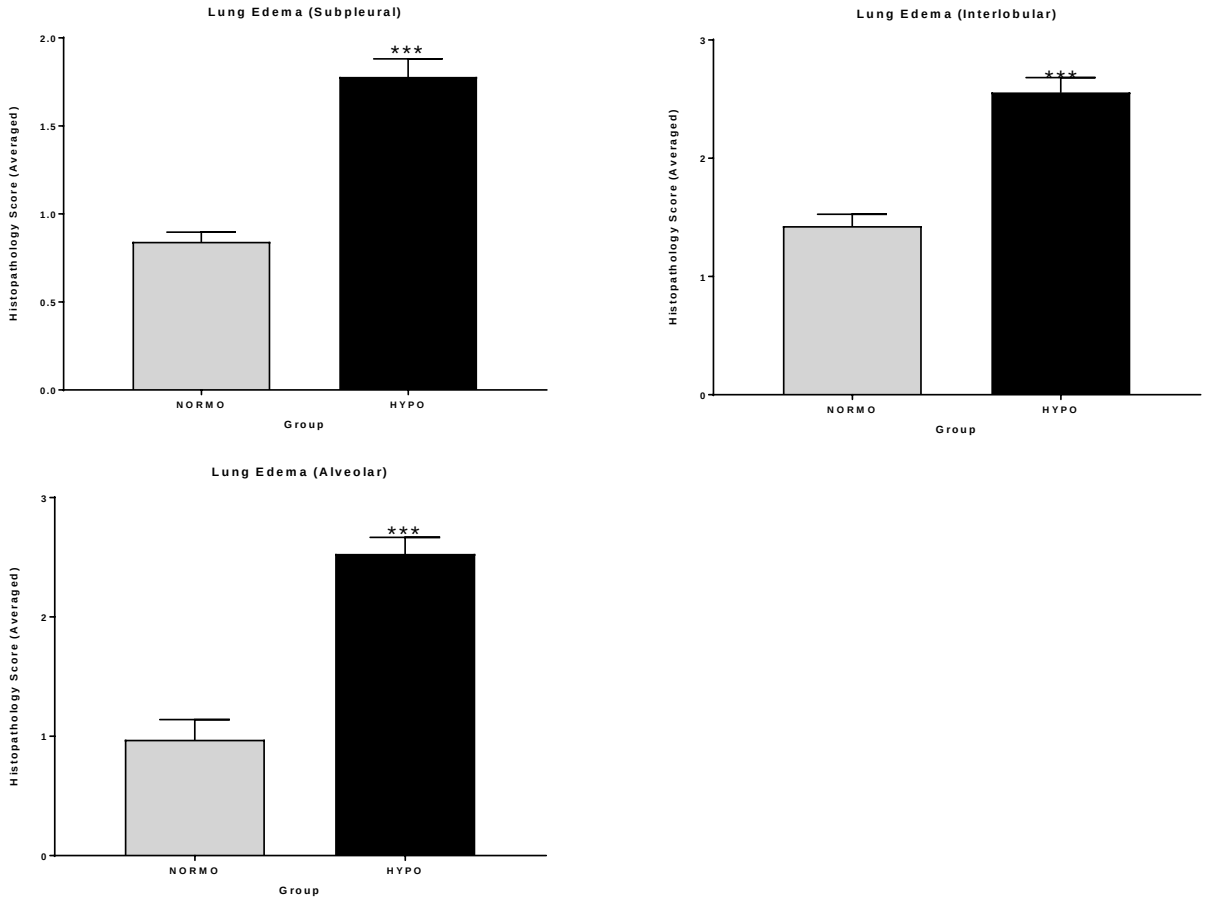

Figure 9: Edema scores in several lung regions.

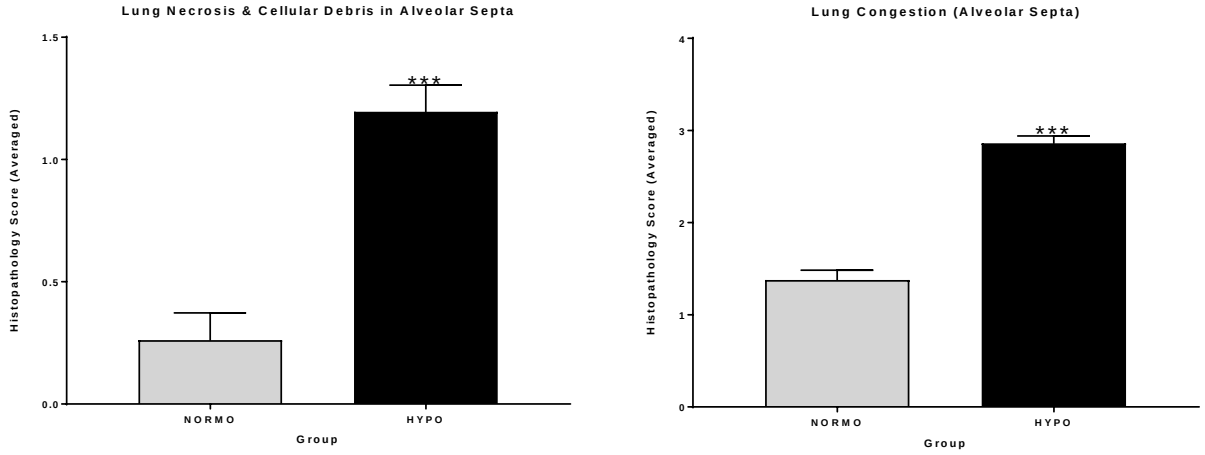

Figure 10: Lung injury groups lung necrosis and congestion scores.

Organ	Group	Injury Scores
Lung	HYPO	16.7 ± 0.6*
	NORMO	6.9 ± 0.7
Kidney	HYPO	5.9 ± 0.2*
	NORMO	3.0 ± 0.6
Liver	HYPO	5.5 ± 1.0*
	NORMO	1.5 ± 0.8
Adrenal Gland	HYPO	3.0 ± 0.5*
	NORMO	1.2 ± 0.4
Pancreas	HYPO	0.8 ± 0.2*
	NORMO	0.0 ± 0.0
Brain	HYPO	5.1 ± 0.3
	NORMO	5.0 ± 0.2

Table 1: Organ injury scores (mean ± SEM) of oleic acid-injured swine exposed to four hours of simulated “transport” under normobaric (NORMO) or hypobaric (HYPO) conditions *Significantly different ($p \leq 0.05$) between groups.

	Lung Injury Scores	
Subcategory	HYPO	NORMO
Edema/fibrin/±hemorrhage		
Subpleural	1.8 ± 0.1*	0.8 ± 0.1
Alveolar	2.5 ± 0.1*	1.0 ± 0.2
Interlobular	2.6 ± 0.1*	1.4 ± 0.1
Inflammation (alveolar septum/lumen)		
Neutrophilic	2.2 ± 0.1*	1.0 ± 0.1
Histiocytic	2.4 ± 0.1*	1.3 ± 0.1
Necrosis/ cellular debris (alveolar septa)	1.2 ± 0.1*	0.3 ± 0.1
Congestion (alveolar septa)	2.9 ± 0.1*	1.4 ± 0.1
Microatelectasis	1.1 ± 0.2*	0.6 ± 0.1
Hyaline membranes	0.0 ± 0.0	0.0 ± 0.0

Kidney Injury Scores		
Subcategory	HYPO	NORMO
Hemorrhage	0.5 ± 0.1*	0.0 ± 0.0
Edema (glomerular tufts)	2.1 ± 0.1*	1.0 ± 0.3
Congestion	1.8 ± 0.2*	1.1 ± 0.2
Inflammation	1.5 ± 0.1	1.0 ± 0.3

Table 2. Injury scores by subcategory (mean ± SEM) for lungs (all lobes) and kidneys (both combined) for oleic acid-injured swine exposed to four hours of simulated “transport” under normobaric (NORMO) or hypobaric (HYPO) conditions. *Significantly different ($p \leq 0.05$) between groups.

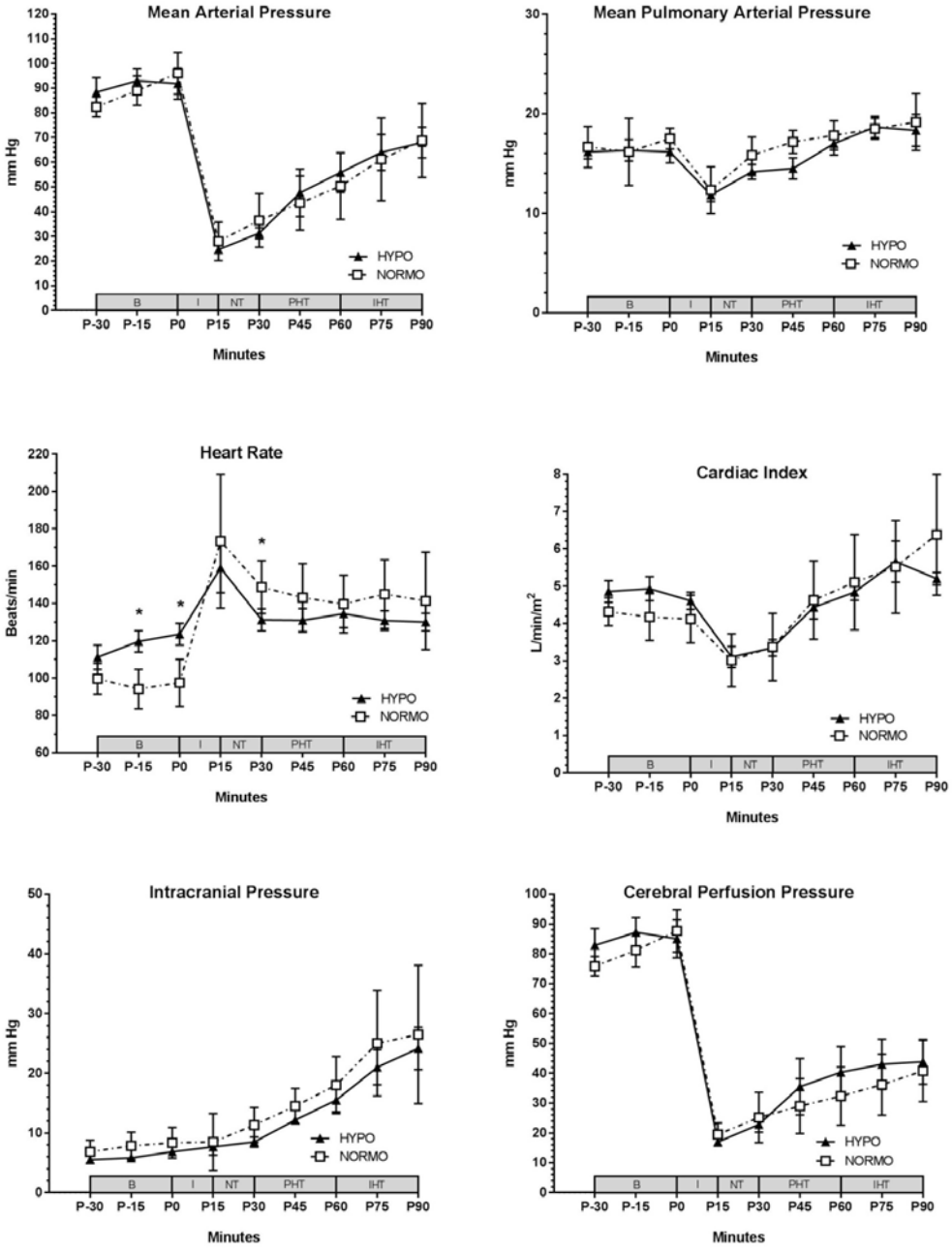

Figure 11: Pre-flight Parameters. B=Baseline, I=Injury (TBI and HEM), NT = No Treatment, PHT= Pre-Hospital Treatment, IHT= In-Hospital Treatment. * = significantly different (P ≤ 0.05) betweengroups

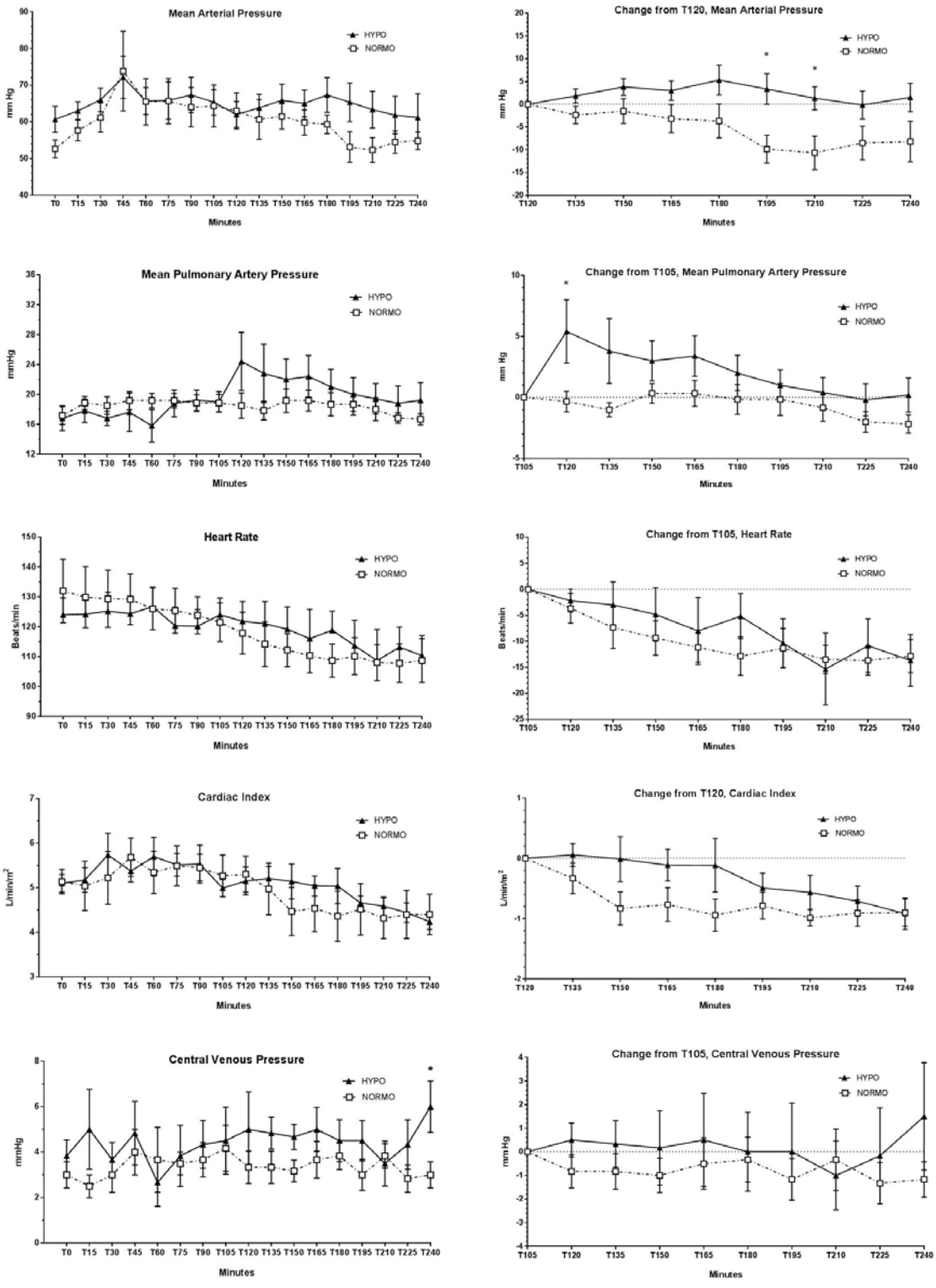

Figure 12: Flight Hemodynamics (Mean ± SEM). Absolute values (left panels) and change (right panels) from T105 (for MPAP, HR, CVP) or T120 (for MAP and CI); * = significantly different ($P \leq 0.05$) between groups.

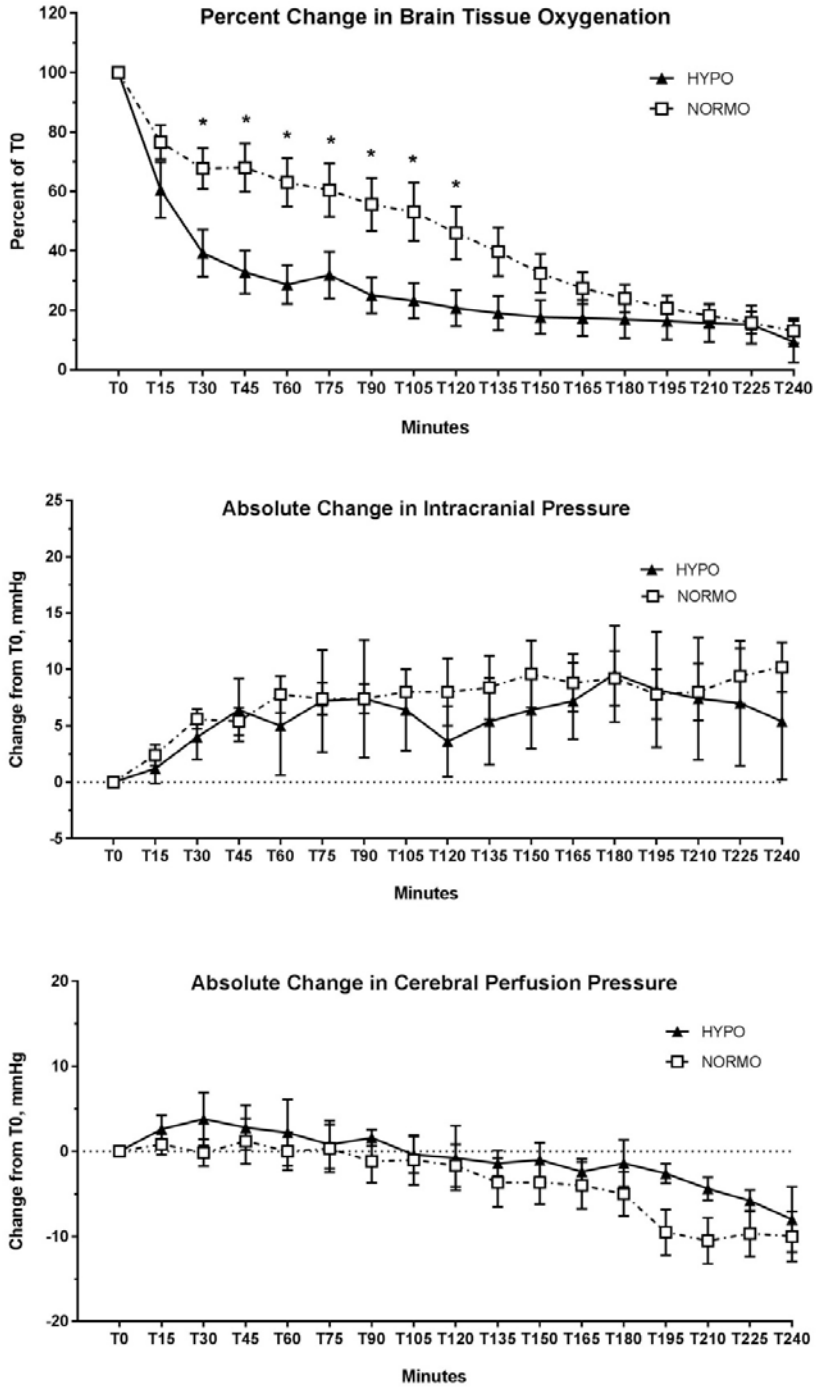

Figure 13: Flight neurophysiological parameters change from T0. * = significantly different (P ≤ 0.05) between groups.

8. APPENDICES

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Brain hypoxia is exacerbated in hypobaria during aeromedical evacuation in swine with traumatic brain injury

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BACKGROUND: There is inadequate information on the physiologic effects of aeromedical evacuation on wounded war fighters with traumatic brain injury (TBI). At altitudes of 8,000 ft, the inspired oxygen is lower than standard sea level values. In troops experiencing TBI, this reduced oxygen may worsen or cause secondary brain injury. We tested the hypothesis that the effects of prolonged aeromedical evacuation on critical neurophysiologic parameters (i.e., brain oxygenation [$P_{bt}O_2$]) of swine with a fluid percussion injury/TBI would be detrimental compared with ground (normobaric) transport.

METHODS: Yorkshire swine underwent fluid percussion injury/TBI with pretransport stabilization before being randomized to a 4-hour aeromedical transport at simulated flight altitude of 8,000 ft (HYPO, n = 8) or normobaric ground transport (NORMO, n = 8). Physiologic measurements (i.e., $P_{bt}O_2$, cerebral perfusion pressure, intracranial pressure, regional cerebral blood flow, mean arterial blood pressure, and oxygen transport variables) were analyzed.

RESULTS: Survival was equivalent between groups. Measurements were similar in both groups at all phases up to and including onset of flight. During the flight, $P_{bt}O_2$, cerebral perfusion pressure, and mean arterial blood pressure were significantly lower in the HYPO than in the NORMO group. At the end of flight, regional cerebral blood flow was lower in the HYPO than in the NORMO group. Other parameters such as intracranial pressure, cardiac output, and mean pulmonary artery pressure were not significantly different between the two groups.

CONCLUSION: A 4-hour aeromedical evacuation at a simulated flight altitude of 8,000 ft caused a notable reduction in neurophysiologic parameters compared with normobaric conditions in this TBI swine model. Results suggest that hypobaric conditions exacerbate cerebral hypoxia and may worsen TBI in casualties already in critical condition. (*J Trauma Acute Care Surg.* 2016;81: 101–107. Copyright © 2016 Wolters Kluwer Health, Inc. All rights reserved.)

KEY WORDS: Aeromedical evacuation; altitude; hypobaria; cerebral oxygenation; traumatic brain injury; swine.

Current medical practice based on evidence derived from recent military conflicts has greatly diminished morbidity and mortality among combat casualties. Damage-control resuscitation,[1] delayed wound closure,[2] tourniquets,[3] and rapid evacuation for damage-control surgery are examples of those interventions contributing to improved care. In a study of 390 laparotomy casualties in Operation Iraqi Freedom and Operation Enduring Freedom, survival was improved with damage-control resuscitation.[1] To maximize benefits for these treatment options, rapid evacuation of combat casualties to definitive care is paramount.[4]

However, not much is known about the physiology and organ function of injured war fighters when they are subjected to the effects of long-range aeromedical evacuation (i.e., hypobaric environments with lower fraction of inspired oxygen [FIO_2]). As emphasized in the textbook *Aeromedical Evacuation*,[5] rapid evacuation of severely injured patients is necessary because "it is impossible to provide adequate fixed or mobile medical facilities to allow for full patient recovery and convalescence in the geographic area where their injury occurred." The conundrum is that the patients are "stabilized, rather than stable," and thus are more susceptible to the adverse effects of altitude than are healthy passengers. The transport time from Central Asia to Landstuhl Regional Medical Center, Germany, is approximately 4 hours, while the time from Landstuhl Regional Medical Center to the United States is approximately 6 hours.

Fixed-wing transport aircraft generally have cruising altitudes of 30,000 f. to 35,000 ft, while keeping cabin pressure equivalent to an altitude of 5,000 f. to 8,000 ft.[5] At sea level (0 ft), the partial pressure of oxygen is 149 mm Hg. However, at 8,000 ft, the partial pressure of inspired oxygen decreases to approximately 108 mm Hg; this amount is still sufficient to

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maintain the arterial oxygen saturation of greater than 90% in a healthy individual,[6] but the reduced oxygen supply could compromise oxygen delivery and result in hypoxemia in critically injured combat casualties. Polytraumatized patients, even if seemingly resuscitated by the time of aeromedical evacuation, have often endured severe insults because of hemorrhagic shock and resuscitation. Traumatic brain injury (TBI) patients are of particular concern because small changes in ambient conditions such as cabin pressure and temperature could potentially have detrimental effects on the already vulnerable brain. The majority of TBI patients require ventilator support, with the risk of developing ventilator-associated pneumonia and/or adult respiratory distress syndrome. Even more so, polytrauma patients with combined TBI and hemorrhagic shock, even if "stabilized," are particularly vulnerable to the negative interactive effects of low blood pressure on TBI outcome and of TBI homeostasis on shock outcome.[7] These are groups of patients that could potentially have severe complications due to hypobaria and for whom optimization of all potential aeromedical evacuation effectors might significantly improve outcome. For example, there is evidence that hypobaria as well as in-flight cabin pressure fluctuations can induce neurologic symptoms in otherwise healthy persons because of altitude decompression sickness.[8–12] These studies suggest that high-altitude hypobaric conditions can have detrimental effects on the neurologic outcome and that aeromedical conditions and/or therapeutics can be optimized to attenuate such adverse effects.

In light of these concerns, we investigated the effects of aeromedical evacuation on neurophysiology in a swine model of TBI in the study reported herein. Our hypothesis was that hypobaria during simulated long-range aeromedical evacuation has adverse effects on neurophysiologic parameters, namely, cerebral blood flow (CBF), brain tissue oxygenation ($P_{bt}O_2$), intracranial pressure (ICP), and cerebral perfusion pressure (CPP), as well as overall survival. Data from this study could aid in the improvement of safety recommendations for en route care and aeromedical evacuation of combat casualties.

MATERIALS AND METHODS

The study protocol was reviewed and approved by the Walter Reed Army Institute of Research/Naval Medical Research Center Institutional Animal Care and Use Committee in compliance with all applicable federal regulations governing the protection of animals in research.

Sixteen farm-bred male and female immature Yorkshire swine (Animal Biotech Industries, Danboro, PA) weighing 32.3 ± 2.9 kg were fasted for approximately 12 hours before the surgical procedure with water provided ad libitum. Induction of anesthesia was initiated with an intramuscular (IM) injection of ketamine hydrochloride (33 mg/kg IM) and atropine sulfate (0.05 mg/kg IM), followed by mask induction using isoflurane (1–5%) in 100% oxygen. After intubation, the animals were kept under isoflurane anesthesia adjusted between 2.0% and 2.5% to maintain a surgical plane of general anesthesia with an FIO_2 of 0.4. Animals were placed in dorsal recumbency and mechanically ventilated (Apollo, Draeger Medical Inc., Telford, PA) in synchronized intermittent mechanical ventilation mode. Positive end-expiratory pressure was set to 5 cm H_2O. Tidal volume and respiration rate were adjusted to maintain end-tidal carbon dioxide partial pressure between 35 mm Hg and 40 mm Hg as determined by continuous capnography or arterial blood gas measurements. All animals received an intravenous (IV) maintenance fluid infusion of normal saline at 3 mL/kg per hour to balance fluid loss from anesthesia.

Before surgical procedures, buprenorphine (0.01 mg/kg IM) was administered for preemptive analgesia. A 9 Fr introducer sheath (Insitu Technologies Inc., St. Paul, MN) was placed in the right external jugular vein, through which a 7.5 Fr Swan-Ganz CCOmbo V pulmonary artery catheter (Edwards Lifesciences, Irvine, CA) was inserted and advanced until proper wave forms indicated measurements of mean pulmonary artery pressures (MPAPs) and pulmonary capillary wedge pressures. This catheter also permitted measurement of cardiac output and collection of mixed venous blood. A 5 Fr pigtail catheter (Cordis Corporation, Fremont, CA) was inserted in the left ventricle for microsphere injection. A 14-gauge catheter was introduced into the femoral vein for infusion of IV anesthetics and maintenance fluids. A 5 Fr introducer sheath (Insitu Technologies Inc.) was placed in the femoral artery for blood pressure monitoring and blood sampling. A Foley catheter was placed into the bladder via the urethra (female) or directly inserted into the bladder via cut down (male) to monitor urine production. After a femoral venous access line was established, anesthesia was switched from isoflurane to total IV anesthesia using propofol and fentanyl.

Brain Instrumentation

After other instrumentation, pigs were turned to a prone position, and a 16-mm diameter craniotomy was performed in the right parietal region adjacent to the sagittal suture and anterior to the coronal suture. A T-shaped bolt, connected to the fluid percussion injury device, was screwed into the craniotomy so it abutted the intact dura. A second craniotomy was performed in the left anterior parietal region and a Codman Microsensor intraparenchymal catheter was inserted into the superficial brain tissue. The other end of the catheter was connected to the Codman Express (Codman Neuro, Raynham, MA) for ICP monitoring. A third craniotomy was performed in the left posterior parietal region adjacent to the sagittal suture and anterior to the coronal suture. A Licox PMO probe was inserted through the craniotomy into cerebral white matter and connected to a Licox CMP Monitor (Integra Neuroscience, Plainsboro, NJ) for $P_{bt}O_2$ and temperature monitoring. A moderate fluid percussion injury to the right frontoparietal area of the brain was inflicted.

Study Design

Experiments were conducted under normobaric (ground) conditions and hypobaric (aeromedical evacuation) conditions using an altitude chamber that was specifically designed and built for large animal research at the Naval Medical Research Center in Silver Spring, Maryland. Normobaric control conditions were defined as ambient laboratory atmospheric pressure in Silver Spring (397 ft, 14.5 psi). Hypobaric conditions were defined as an atmospheric pressure equivalent to an altitude of 8,000 ft (10.9 psi). Before simulated transport to definitive care, animals were randomly assigned to normobaric (NORMO, n = 8) or hypobaric (HYPO, n = 8) conditions.

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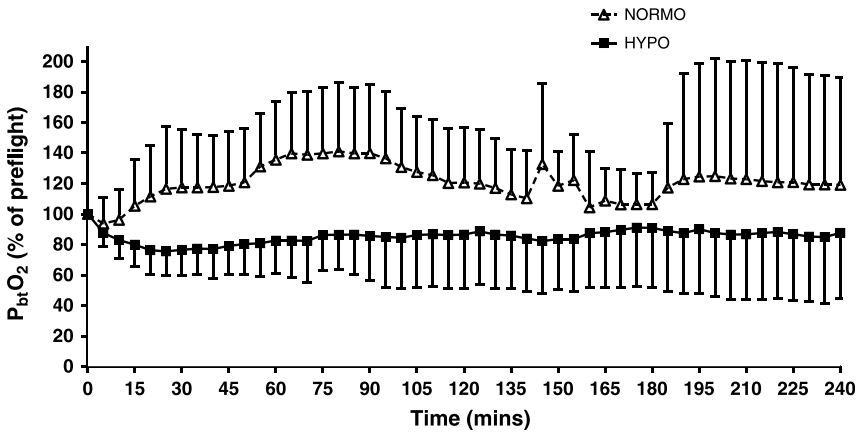

Figure 1. Change in brain tissue oxygen partial pressure ($P_{bt}O_2$, mean ± SEM) during 4-hour simulated transport of swine with TBI at 8,000 ft altitude (HYPO) or at sea level at 0 ft (NORMO). Time 0 = baseline (BL) before transport (sea level).

Following injury (T0), there was a 15-minute delay before the beginning of resuscitation to simulate the time from the battlefield or civilian point of injury to the arrival of corpsman, medic, or paramedic and initiation of prehospital resuscitation at T15. "Hospital arrival" was at T30, and animals were stabilized for 90 minutes (from T30 to T120) before simulated transportation to definitive hospital care. During this simulated emergency department/intensive care unit period, an animal could undergo multiple treatments, based on the assessment of physical signs, blood, and/or ICPs and laboratory blood results. These treatments were based on a predetermined algorithm. In general, pulmonary stabilization included adjustment of minute ventilation and FIO_2 to maintain normocapnia and prevent hypoxemia (defined as oxygen saturation ≤ 92%). Mannitol was provided if ICP was greater than 20 mm Hg unless mean arterial pressure (MAP) was lower than 40 mm Hg. A maximum of two doses of mannitol could be administered as a 1-g/kg IV bolus over 5 minutes.

At 2 hours after injury (T120), the animals were moved to the hypobaric chamber, and a 4-hour transport was simulated at either hypobaric or normobaric (ground transport control) conditions. During this time, to simulate transport conditions, fluids were continued, and ventilator parameters were adjusted, but no additional treatment was allowed. In the chamber, animals were ventilated using an iVent 201 transport ventilator (GE HealthCare, Chicago, IL), which is also in the chamber under hypobaric conditions. At the end of the 4-hour flight (T240), animals were euthanized.

Measurements

Data were collected at predetermined time points for routine hemodynamic, pulmonary, and neurophysiologic measurements, and blood samples were collected for blood gas analysis, electrolytes, and hematology parameters. Physiologic parameters (heart rate [HR], MAP, MPAP, central venous pressure, cardiac index [CI], oxygen saturation, respiration rate, and end-tidal carbon dioxide) and neurologic parameters (ICP, $P_{bt}O_2$, regional CBF [rCBF], and brain temperature) were collected every 5 minutes for the first 120 minutes and every 15 minutes thereafter. Urine output and body temperature were measured every 15 minutes. Arterial and mixed venous blood samples for blood gas and electrolyte analysis were collected every 30 minutes for the determination of hemoglobin (Hb), oxygen content, oxygen delivery, and oxygen extraction (using standard formulae) as well as indirect assessment of tissue oxygenation (lactate, pH, base excess, and HCO_3^-). Complete blood count was performed using a Pentra $60C^+$ cell counter (Horiba ABX Diagnostics, Irvine, CA). $P_{bt}O_2$ was measured via the Licox Oxylite/Oxyflo probes. rCBF was measured at T0, T120, T240, and T360 via a fluorescent microsphere technique.[13]

Statistical Analysis

The HYPO and NORMO groups were analyzed for within-group changes over time and to each other. An omnibus 2 (group) × 17 (observation period) mixed-model analysis of variance was used to compare physiologic parameters and blood data in the HYPO and NORMO groups. Specifically, this test was used to determine whether the differences between the HYPO and NORMO animals occurred as a function of the group and observation period. Following this analysis, one-way repeated-measures analyses of variance were performed on the experimental groups across the observation periods. These tests were performed to determine whether the various parameters changed over time. Post hoc comparisons using

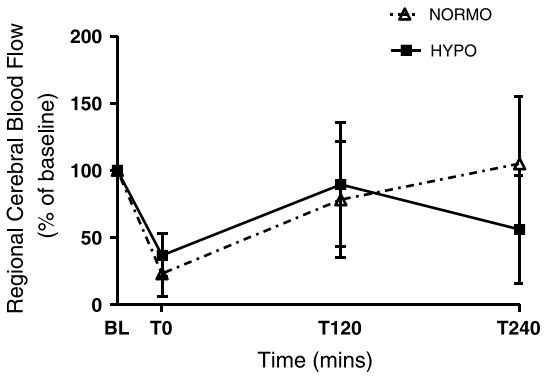

Figure 2. Change in rCBF (mean ± SEM) of swine with TBI at 8,000 ft altitude (HYPO) or at sea level at 0 ft (NORMO). BL = before injury. T0 = BL before transport (sea level). T120 and T240 = 2 and 4 hours into transport.

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least significance difference were performed to find the within-group effect of hypobaria or normobaria over time. To assess differences between groups at certain time points, an independent sample *t* test was performed. Body weights were analyzed using an independent sample *t* test only. For all parameters, a $p \leq 0.05$ was considered statistically significant. Results are expressed as mean ± SEM.

RESULTS

All swine survived the study protocol. There were both statistically significant differences and nonstatistically significant but clinically relevant trends in the data that suggest critical effects on patients with TBI exposed to a 4-hour aeromedical evacuation at a simulated flight altitude of 8,000 ft.

Specific to neurophysiologic parameters, $P_{bt}O_2$ percent change from baseline at T0 before flight was significantly lower in the HYPO group compared with the NORMO group ($p = 0.024$, Fig. 1). Specifically, $P_{bt}O_2$ percent change from baseline before flight (BL) was significantly lower from T30 to T105 in the HYPO group compared with the NORMO group (*t* test, $p \leq 0.05$). The HYPO animals displayed a steady trend toward reduced $P_{bt}O_2$ percent change from BL, although this did not reach statistical significance. rCBF tended to be higher in the NORMO animals

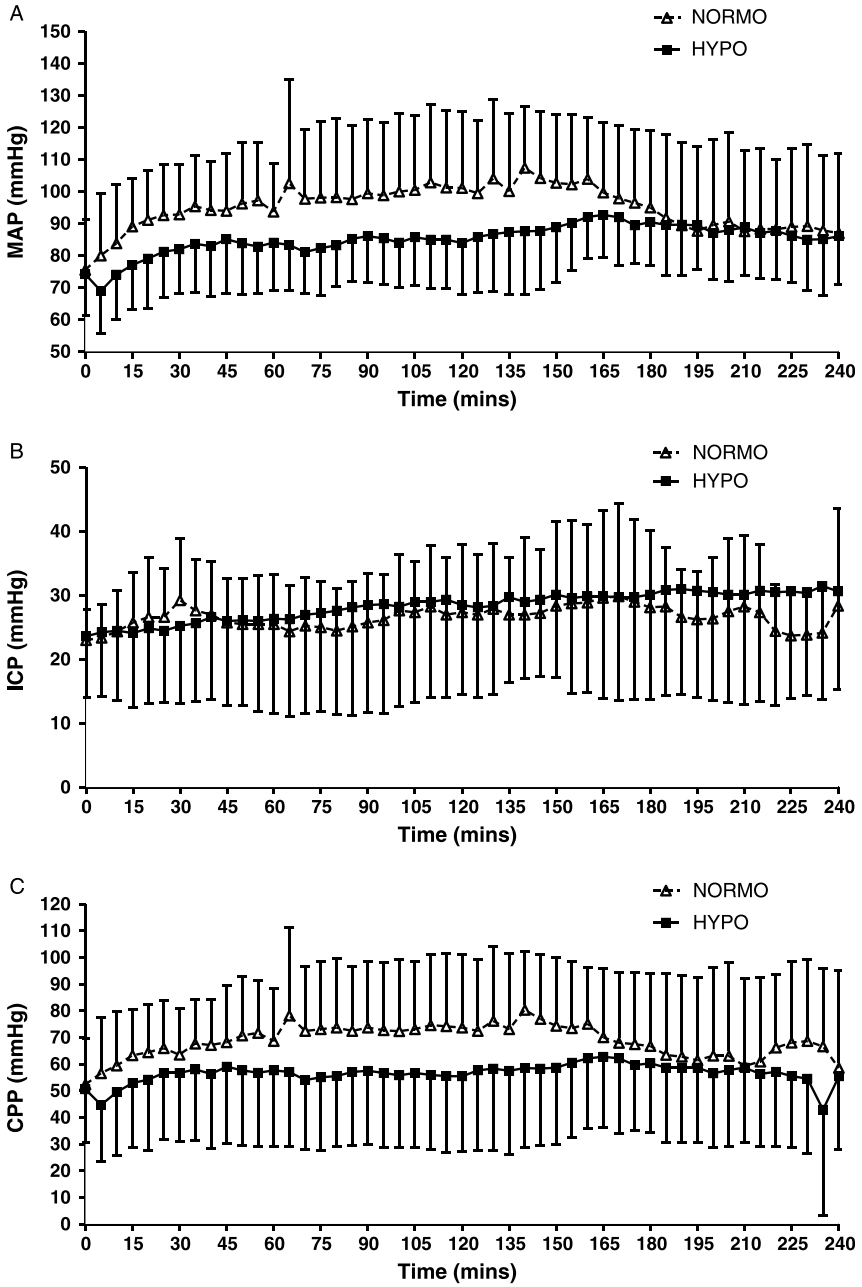

Figure 3. MAP (*A*), ICP (*B*), and CPP (*C*) during 4-hour simulated transport of swine with TBI at 8,000 ft altitude (HYPO) or at sea level at 0 ft (NORMO). Mean ± SEM. Time 0, BL before transport (sea level).

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TABLE 1. Selected Hemodynamic Measurements (Mean ± SEM) of Swine With TBI During 4-Hour Simulated Transport at 8,000-ft Altitude (TBI HYPO) or at Sea Level at 0 ft (TBI NORMO)

	HR, beats/min		MPAP, mm Hg		CI, L/min/m^2		SVRI, mm Hg/L/min/m^2	
Time	HYPO	NORMO	HYPO	NORMO	HYPO	NORMO	HYPO	NORMO
0	126 ± 5	129 ± 6	19.3 ± 0.9	19.3 ± 1.2	6.9 ± 0.5	7.0 ± 0.7	9.8 ± 1.2	11.0 ± 1.4
30	125 ± 8	126 ± 9	18.1 ± 1.0	21.8 ± 1.4	6.7 ± 0.4	6.9 ± 0.5	11.5 ± 0.9	13.3 ± 1.6*
60	124 ± 9	124 ± 7	16.9 ± 0.7	20.9 ± 1.4	6.8 ± 0.5	6.1 ± 0.5	12.1 ± 1.2	14.2 ± 1.8*
90	123 ± 10	125 ± 6	17.5 ± 1.1	20.6 ± 1.3	6.8 ± 0.5	6.9 ± 0.4	12.6 ± 1.1	14.0 ± 1.5
120	123 ± 8	124 ± 8	17.2 ± 1.0	21.7 ± 1.5	6.8 ± 0.5	6.9 ± 0.5	12.5 ± 1.4	14.7 ± 1.5*
150	122 ± 8	128 ± 11	18.9 ± 1.0	20.8 ± 1.5	6.4 ± 0.4	6.4 ± 0.5	13.2 ± 1.3	15.9 ± 1.2*
180	120 ± 9	129 ± 11	17.9 ± 1.8	19.4 ± 1.4	6.5 ± 0.5	6.6 ± 0.3	14.0 ± 1.4	14.0 ± 1.1
210	119 ± 8	128 ± 10	16.8 ± 1.9	18.8 ± 1.2	6.5 ± 0.4	6.6 ± 0.5	13.8 ± 1.3	13.4 ± 1.5*
240	118 ± 7	134 ± 11	17.2 ± 1.5	18.6 ± 1.3	6.2 ± 0.3	6.7 ± 0.4	12.8 ± 1.3	12.8 ± 1.2*

*A significant difference within the NORMO group from T0 ($p \leq 0.05$).
Time 0, BL before transport (sea level).
SVRI, systemic vascular resistance index.

compared with the HYPO animals at the end of the flight, but this difference did not reach statistical significance (Fig. 2). ICP was similar in both groups (Fig. 3*B*). There was a significant interaction between group and time for CPP ($p = 0.032$) and MAP ($p = 0.004$, Fig 3*A* and *C*, respectively), indicating that the differences between the groups in both CPP and MAP increased over time during the flight. CPP and MAP were also significantly lower from T0 over time in the HYPO animals ($p = 0.037$ and $p < 0.0001$, respectively).

Aside from MAP, there were no significant differences in hemodynamic variables (MPAP, HR, CI, and systemic vascular resistance index) between the two treatment groups (Table 1).

Hb and oxygen transport data are provided in Table 2. As expected, PaO_2 was lower in the HYPO group than in the NORMO group, but because animals were breathing an enriched oxygen supply, the absolute PaO_2 in the HYPO group was still higher than levels considered hypoxemic ($PaO_2 \leq 60$ mm Hg). There was a significant ($p \leq 0.05$) interaction between group and time for both PaO_2 and CaO_2, indicating that the difference between the groups in both parameters increased over time during the flight. For all other oxygen transport variables (i.e., arterial oxygen content, oxygen delivery, oxygen consumption, oxygen extraction ratio), there were no differences between the two groups although arterial oxygen content was transiently reduced during the first half of the flight compared with preflight values in the HYPO group (Table 2).

DISCUSSION

Our data showed that a 4-hour simulated aeromedical evacuation flight at cabin pressures equivalent to 8,000 ft reduced $P_{bt}O_2$, CBF, and CPP in swine with TBI when exposed to transport within 2 hours of injury. We presume that these clinical decrements may potentiate secondary neurologic insults following TBI. These data suggest that in this fluid percussion model of TBI, the duration of altitude exposure and time of aeromedical evacuation from injury result in a patient who is less stable hemodynamically and neurologically than a similar patient at normobaric conditions.

TABLE 2. Selected Oxygen Transport Measurements (mean ± SEM) of Swine With TBI During 4-Hour Simulated Transport at 8,000-ft Altitude (TBI HYPO) or at Sea Level at 0 ft (TBI NORMO)

	Hb, g/dL		PaO_2, mm Hg		CaO_2, mL/dL		DO_2, mL/min		VO_2, mL/min		O_2ER	
Time	HYPO*	NORMO*	HYPO*	NORMO*	HYPO*	NORMO*	HYPO	NORMO	HYPO	NORMO	HYPO	NORMO
0	7.7 ± 0.1	7.5 ± 0.2	202.7 ± 7	183.8 ± 9	11.0 ± 0.1	10.6 ± 0.2	668 ± 54	667 ± 55	200 ± 16	224 ± 17	30.3 ± 1.8	33.8 ± 1.4
30	6.7 ± 0.3**	7.6 ± 0.2	195.3 ± 3	175.0 ± 7	9.5 ± 0.4**	10.6 ± 0.2	556 ± 60	648 ± 50	162 ± 17	211 ± 15	29.5 ± 1.2	33.0 ± 1.8
60	7.1 ± 0.2	7.4 ± 0.2	190.1 ± 5	183.2 ± 7	10.1 ± 0.3**	10.5 ± 0.3	595 ± 52	626 ± 41	179 ± 12	203 ± 10	30.6 ± 1.3	33.19 ± 1.9
90	7.4 ± 0.1	7.6 ± 0.3	183.0 ± 6**	178.7 ± 8	10.5 ± 0.2**	10.6 ± 0.4	630 ± 68	661 ± 34	192 ± 12	222 ± 13	31.6 ± 1.8	33.8 ± 1.5
120	7.5 ± 0.1	7.7 ± 0.2	178.0 ± 5**	182.1 ± 9	10.6 ± 0.2	10.8 ± 0.3	631 ± 48	659 ± 39	197 ± 15	218 ± 14	31.5 ± 1.7	33.5 ± 2.1
150	7.3 ± 0.1	7.7 ± 0.2	177.1 ± 6**	183.6 ± 9	10.3 ± 0.2**	10.8 ± 0.4	587 ± 57	638 ± 27	180 ± 16	223 ± 10	31.1 ± 1.6	35.2 ± 1.7
180	7.5 ± .1	7.8 ± 0.2	176.5 ± 6**	177.7 ± 8	10.6 ± 0.1	10.9 ± 0.3	605 ± 59	647 ± 28	195 ± 16	230 ± 14	33.9 ± 1.5	35.6 ± 1.7
210	7.6 ± 0.1	7.5 ± 0.2	176.0 ± 6**	185.5 ± 9	10.7 ± 0.1	10.5 ± 0.2	589 ± 48	610 ± 41	190 ± 8	207 ± 16	33.0 ± 1.5	34.3 ± 2.6
240	7.6 ± 0.1	7.7 ± 0.2	177.1 ± 6**	180.6 ± 7	10.7 ± 0.2	10.8 ± 0.3	589 ± 46	656 ± 40	191 ± 14	221 ± 19	32.9 ± 1.8	34.1 ± 2.6

*Significant interaction between time and groups ($p \leq 0.05$).
**Significant difference within Hypo group from T0 ($p \leq 0.05$).
Time 0, BL before transport (sea level).
CaO_2, arterial oxygen content; O_2ER, oxygen extraction ratio; VO_2, oxygen consumption.

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The reduction in brain oxygenation was not unexpected since ascent to altitude should result in a lowering of PaO_2 (due to the lower partial pressure of oxygen), which might then result in lowered arterial oxygen content, and this is what was observed. However, what was initially surprising was that these expected changes caused no differences between the two groups in overall oxygen delivery, consumption, or extraction ratio and, early in flight, no change in CBF. The likely explanation for the lack of significant differences in these parameters is that, although PaO_2 was lower in hypobaric animals during flight (vs. preflight) and lower than in the normobaric pigs, it remained high enough to permit normal oxygen saturation. Brain tissue oxygenation in the normobaric swine indicated that these swine began to recover from their injury during transport, with brain oxygenation rising to 25% to 50% above injury levels. This cerebral hypoxemia in the HYPO animals is most likely caused by their lower MAP (vs. NORMO swine), which resulted in a significantly reduced CPP for the entire duration of the simulated aeromedical evacuation. This prolonged reduction in CPP not only reduced brain oxygenation for an equivalent duration but also eventually caused a reduction in CBF, as directly measured by microspheres. There were no clinically relevant differences between the two groups in other laboratory measurements (i.e., electrolytes, glucose, base excess, bicarbonate, or complete blood count; data not shown). A limitation of this study is that this was not a survival study, so the long-term sequelae of these changes on cerebral structure (histopathology) and function (behavioral studies) could not be determined. Other limitations include the use of anesthetics and aeromedical evacuation within 2 hours of injury. In currently ongoing studies in our laboratory, these potential pitfalls have been addressed by making the study a 14-day survival study and by simulating aeromedical evacuation flights on Days 3 and 5 after injury.

Most other animal studies evaluating the physiologic effects of aeromedical evacuation have been performed on smaller species such as rats, which makes a direct comparison of our data challenging. Nonetheless, they collectively show evidence of detrimental neurologic effects of altitude. Rats with a penetrating TBI (nail gunshot) exposed to a simulated altitude of 12,000 ft[14] and rats with a blunt trauma TBI[15] both had reductions in $P_{bt}O_2$ and rCBF, similar to our pig data. Those investigators also found evidence of cerebral edema (measured by brain water content) and blood-brain barrier disruption (measured by Evans blue content), higher mortality within 72 hours after injury, more severe histopathologic changes in the rats at high altitude,[14] and a detrimental effect of altitude on neurologic function and behavior.[15] In mice with blunt TBI, exposure to 5 hours of hypobaric (8,800 ft) conditions 3 hours after the injury caused increases in the neuroinflammatory response to injury (cerebral levels of IL-6, MIP-1α, and serum NSE) and in the severity of secondary brain injury.[16] This mouse study also showed functional changes as assessed by a prolonged righting reflex response compared with sham injury after flight conditions.

The clinical evidence on whether altitude-induced hypoxemia occurs in our wounded warriors during aeromedical evacuation is conflicting. In a study of 63 combat casualties with severe isolated TBI who were transported by the US Air Force Critical Care Air Transport Teams from 2003 through 2006, there was a relatively low incidence in the occurrence of in-flight altitude-induced hypoxia, and this was attributed to successful supplementation of in-flight oxygen.[17] On the other hand, data from our study support a more recent study that reported the occurrence of hypoxemia (oxygen saturation < 90%) in a high percentage (90%) of transported casualties. In this study, the hypoxemic events lasted on average for 44 minutes. Furthermore, 56% of the patients exhibited an oxygen saturation of less than 85% for approximately 12 minutes. This latter study suggests that hypoxemia may be a common event during aeromedical evacuations.[18] This same study also showed that this mode of evacuation resulted in increases in ICP from external stimuli (vibration, noise) as well as acceleration and deceleration forces; all potential additional contributors to the development of secondary brain injury.

In summary, prolonged exposure to hypobaria during simulated aeromedical evacuation significantly impacted the neurophysiology of swine with TBI. Our data showed that a 4-hour flight at a cabin pressure equivalent to 8,000 ft reduces $P_{bt}O_2$, CBF, and CPP and may potentiate secondary neurologic insults following TBI in anesthetized, ventilated swine breathing 40% oxygen. The potential deleterious effects of altitude transport on wounded war fighters with TBI require additional investigations to optimize transport conditions. For example, the impact of timing of aeromedical evacuation (early vs. delayed transport time from injury),[19] transport treatments (e.g., oxygen therapy), and maintaining cabin pressures closer to sea level (e.g., 4000 ft) may be optimized to ameliorate secondary brain injury and improve long-term functional outcomes.

AUTHORSHIP

A.H.S., M.J.H., D.L.M., and R.M.M. designed this study. A.H. performed the literature search. A.H.S., A.H., S.J.C., and B.H. contributed to data collection. A.H.S., A.H., B.H., and R.T.M. contributed to data analysis. A.H.S., A.H., C.R.A., P.M-M., and D.L.M. performed data interpretation. A.H. and P.M-M. contributed to writing. A.H.S., D.L.M., and R.M.M. participated in critical revision.

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DISCLOSURE

For all authors, no conflicts are declared. This work was funded by CDMRP award W81XWH-13-2-0022.

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EDITORIAL CRITIQUE

In this notable study, Dr. Scultetus and colleagues examined a simulated 4-hour aeromedical evacuation and its role on neurophysiologic parameters in a model of traumatic brain injury (TBI). Although an interesting and timely study, it also highlights the challenges in developing a model that accurately represents the environmental milieu of combat casualty aeromedical evacuation.

While it is established that hypoxia contributes to secondary injury in models of TBI, the independent role of hypobaria has yet to be elucidated. The animals in this model did not experience hypoxemia because they were given supplemental oxygen via mechanical ventilation and yet the investigators measured a decrease in brain tissue oxygenation. The authors opined the most likely cause of decreased brain tissue oxygenation was a decrease in the mean arterial pressure and thus cerebral perfusion pressure (CPP). Currently, patients with severe TBI are transported by the providers of a Critical Care Air Transport team (CCAT). These providers receive extensive training regarding the management of TBI during transport. Their focus is to mitigate the effects of noise, vibration, light, acceleration and deceleration forces as they all contribute to variations in ICP. Further, they provide ongoing resuscitation and interventions to maintain CPP. Consideration should be given in future TBI models to ensuring that the state of resuscitation and other physiologic variables that contribute to cerebral oxygen delivery are accounted for in order to assure that the resultant observations are truly related to exposure to hypobaria.

Lastly, military aeromedical evacuation times can vary greatly depending on location. While the last 15 years have largely involved a 6–8 hour flight time from Afghanistan or Iraq to Germany and a 7–9 hour flight time from Germany to the United States, future requirements may involve longer evacuation times. Incorporating the contribution of evacuation times to risk will also warrant consideration.

While challenges remain in optimizing the care of combat casualties during aeromedical evacuation we commend the authors on their efforts and encourage them to continue to investigate a very important topic.

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HYPOBARIA DURING LONG RANGE FLIGHT RESULTED IN SIGNIFICANTLY INCREASED HISTOPATHOLOGICAL EVIDENCE OF LUNG AND BRAIN DAMAGE IN A SWINE MODEL

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January 5, 2017

Ernest E. Moore, M.D.
Editor
Journal of Trauma and Acute Care Surgery

RE: JT-D-17-07543

Dear Dr. Moore,

We are submitting the revisions to our manuscript #JT-D-15-04471 titled **"Hypobaria during long range flight resulted in significantly increased histopathological evidence of lung and brain damage in a swine model"** after your peer-review.

Please find below our responses to the reviewers' questions and comments that required modification of the manuscript. Changes in the resubmitted manuscript as a result of the reviewers' comments have been highlighted in track changes. Please note that we also made some minor stylistic and grammatical corrections, which have not been highlighted.

We thank the Journal of Trauma and Acute Care Surgery for the opportunity to send our revised manuscript and look forward to hearing from you.

Sincerely,

Anke H. Scultetus

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Response to Reviewer Comments:

Reviewer #1:

This is a very interesting study that examined the role of decreased pressure on subclinical inflammation and lung injury. The interesting part of the study is as much what the authors did demonstrate as what they did not. They did demonstrate improvement in kidney inflammation in the hypobaric group, without much of an attempt to explain this finding. Additionally, they demonstrated differences in histologic lung inflammation and damage on pathologic examination that did not manifest clinically.

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This is more interesting in light of aeromedical evacuation transport in wounded troops from combat operations, less so as an indicator of risk for air crew. However, I still have a few questions:

1) was the scoring for organ specific injury scored by the pathologist as a nominal variable, or an ordinal variable. It seems as if it is analyzed as an ordinal variable (mean +/- SEM or SD), but if the pathologist never, for example, awarded a specimen a 4.5 or a 3.2, etc., as an actual score, then the results should be calculated and presented as nominal variables? To further explain, if the criteria for grading lung injury as a 3, just as an example, were 50 neutrophils/ 10 hpf, and the patient had only a max of 48, at any one time, why then the patient is a 2, and not a 2.9. In this sense I wonder if the inflammation differences are durable when calculated as categorical (nominal) variables. Please address this in the discussion, and if in fact these are nominal variables, change the calculations to reflect so.

Author's Response: A multiparametric, semi-quantitative histopathological scoring system was used by the pathologist in accordance with accepted methods (Gibson-Corley KN, Olivier AK, Meyerholz DK. Principles for valid histopathologic scoring in research. Veterinary pathology. 2013;50(6):1007-15). The ordinal variables of this scoring system were analyzed using an independent sample T-Test. We re-analyzed our data using a nominal category and the STD and SEM produced the same outputs. The differences in inflammation, etc. were the same with the different analysis methods.

2) What is the protocol for transport of critically injured personnel? Are the craft pressurized, as, for example, commercial airliners are? If so, at what altitude are they pressurized.

Author's Response: Military and civilian aircraft are pressurized in a similar fashion to a cabin pressure equivalent to 8,000ft. At the discretion of the medical officer in charge of the aeromedical evacuation, the aircraft can be further pressurized to 4,000 ft or even lower if the condition of a specific patient necessitates; but this is not routinely done. Research in this area is just emerging and future studies are planned to assess the effects of different transport altitudes on injuries. A sentence to this effect has been added to the manuscript on page 11.

3) I am aware that helicopter transport is not pressurized, but they are typically at lower altitudes and for shorter duration. In light of your findings and the interest it holds for aeromedical evacuation, I found myself questioning the similarities and differences between long term aeromedical evac (at high altitudes, for example), and helicopter transport. I would heartily appreciate some comparison, even though I know that this was directed at longer flights. Surely, it would help readers extrapolate possible risks (or lack thereof, perhaps more importantly) coincident with helicopter transport.

Author's Response: We thank the reviewer for this excellent comment. While helicopters are not pressurized, their altitude at flight for aeromedical transport in theatre is usually 3,000-4,000ft. However, this can vary depending on the starting altitude at ground level. For instance, while the ground level in Iraq is at sea level, the ground level

in Afghanistan varies widely with base altitudes of up to 10,000 ft. It is therefore not unusual for helicopters to fly as high as 14,000 ft with unpressurized cabins. While these altitudes are reached for much shorter duration, their effect on aeromedical transport is certainly worthy of future investigations. A comment about this has been added to the manuscript on page 11.

We thank the reviewer for his/her insightful comments and suggestions that helped improve our manuscript.

Reviewer #2:

Why did the NORMO group have significantly higher blood lactate level at baseline?

Author's Response: The HYPO group had a lactate of 0.8±0.1mmol/L at TO, compared to 1.2±0.2 mmol/L in the NORMO group. While there was a difference, this was not statistically or clinically significant. We therefore believe this is within an acceptable range for baseline variables.

Is it possible that hypobaric conditions could be nephroprotective?

Author's Response: Based on our data, this is a valuable thought. This is, however, the first study of this kind, and further studies are indicated to confirm this trend. In the absence of any supporting data we were uncomfortable speculating on this issue in the text.

This is an optimally well planned, well conducted, well statistically evaluated, and well written paper. There is not anything substantial to criticize on this paper and its conclusions are perplexing and interesting.

Author's Response: We thank the reviewer for his/her comments on our work.

There is only one objection I have and I've debated whether to mention it but I feel I should. Despite having done years of animal research at the beginning of my career, I now believe it to be unethical.

Author's Response: We thank the reviewer for his/her personal comment.

We thank the reviewer for his/her insightful comments and suggestions that contributed to the improvement of our manuscript.

Abstract

Background: Aeromedical evacuation to definitive care is standard in current military conflicts. However, there is minimal knowledge on the effects of hypobaria on either the flight crew or patients. The effects of hypobaria was investigated using healthy swine.

Methods: Anesthetized Yorkshire swine underwent a simulated 4 h "transport" to an altitude of 2,441 m (8,000 ft.; HYPO, N = 6) or at normobaric conditions (NORMO, N = 6). Physiological and biochemical data were collected. Organ damage was assessed for hemorrhage, inflammation, edema, necrosis and, for lungs only, microatelectasis.

Results: All parameters were similar prior to and after "transport" with no significant effects of hypobaria on hemodynamic, neurologic, or oxygen transport parameters, nor on blood gas, chemistry, or complete blood count data. However, the overall Lung Injury Score was significantly worse in the HYPO than the NORMO group (10.78 ±1.22 vs. 2.31 ± 0.71, respectively) with more edema/fibrin/hemorrhage in the subpleural, interlobular and alveolar space, more congestion in alveolar septa, and evidence of microatelectasis (vs. no microatelectasis in the NORMO group). There was also increased severity of pulmonary neutrophilic (1.69 ±0.20 vs. 0.19 ±0.13) and histiocytic inflammation (1.83 ±0.23 vs. 0.47 ±0.17) for HYPO vs. NORMO, respectively. On the other hand, there was increased renal inflammation in NORMO compared to HYPO (1.00 ±0.13 vs. 0.33 ±0.17, respectively). There were no histopathological differences in brain (whole or individual regions), liver, pancreas or adrenals.

Conclusion: Hypobaria, itself, may have an adverse effect on the respiratory system, even in healthy individuals and this may be superimposed on combat casualties where there may be pre-existing lung injury. The additional effects of anesthesia and controlled ventilation on these results are unknown and further studies are indicated using awake models to better characterize the mechanisms for this pathology and the factors that influence its severity.

Level of Evidence: Level II, Therapeutic/Care Management

Key Words: aeromedical evacuation, hypobaria, lung injury, swine

HYPOBARIA DURING LONG RANGE FLIGHT RESULTED IN SIGNIFICANTLY INCREASED HISTOPATHOLOGICAL EVIDENCE OF LUNG AND BRAIN DAMAGE IN A SWINE MODEL

Short title: Effects of altitude on lungs in swine

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Meetings: This manuscript will be presented (D. Malone) as an oral quick shot at the 31st Annual Scientific Assembly for the Eastern Association for the Surgery of Trauma (EAST) held in Lake Buena Vista, FL from January 9-13, 2018.

BACKGROUND

Recent military conflicts have provided evidence that rapid evacuation of combat casualties to definitive care is of paramount importance to achieve diminished morbidity and mortality (1). However, despite growing interest in determining the potential damage of long-range flight on the wounded warrior(2), it has been assumed that the flight crew can adequately compensate for the physiological changes that occur during such flights. Nonetheless, commonly reported medical symptoms for military aircrew include headache, decreased cognitive function(3, 4), fatigue(5), barodontalgia (6), sensorineural hearing loss(7) and, most commonly, spinal pain and respiratory symptoms (5, 8-13). The etiologies of these occupational health concerns are multifactorial and may include altitude-induced hypoxia, pressure changes, vibrations, noise, and air-quality as well as characteristics related to the mission (i.e., time and length of travel).

The overall goal of our laboratory is to identify which factors associated with aeromedical transport conditions can be attenuated to improve the safety of injured patients. We previously provided evidence that hypobaric conditions exacerbate cerebral hypoxia and worsen traumatic brain injury (TBI)(2). Evaluation of how such transport conditions affect the pathophysiology of healthy individuals is an equally important step in assessing hypobaric transport conditions. The study reported herein uses a healthy swine model and a realistic military timeline to simulate hypobaric transport conditions (i.e., from Afghanistan to Germany). The study aim was to investigate if there were acute cardiopulmonary, neurological or biochemical changes and/or histological evidence of a detrimental effect of hypobaria on healthy swine. Results will not only improve our understanding of studies using injury models or

clinical trauma data but also expand our knowledge of how such transport may affect the aircrew exposed to these conditions.

METHODS

The study protocol was reviewed and approved by the Walter Reed Army Institute of Research/Naval Medical Research Center Institutional Animal Care and Use Committee in compliance with all applicable Federal regulations governing the protection of animals in research. All animals used in the protocol were maintained under the surveillance of a veterinarian.

Yorkshire swine (31.8 ± 1.6 kg [mean ± SEM]; both genders; Animal Biotech Industries, Danboro, PA) were sedated (33 mg/kg IM ketamine and 0.05 mg/kg IM atropine) prior to mask induction with isoflurane to facilitate endotracheal intubation. Maintenance anesthesia was initially with isoflurane but was changed to total intravenous anesthesia using propofol (250-350 µg/kg/min IV) and fentanyl (5—10 µg/kg/h IV) once venous access was established. Swine were ventilated using synchronized intermittent mechanical ventilation mode (Apollo®, Draeger Medical Inc. Telford, PA) with an FiO_2 of 0.4 and a PEEP of 5 cm H_2O. Ventilator settings were adjusted to maintain end-tidal carbon dioxide ($ETCO_2$) between 35-40 mm Hg. Animals received 3 mL/kg/h IV 0.9% saline as a maintenance fluid to make up insensible fluid loss.

Before surgical procedures, buprenorphine (0.01mg/kg IM) was administered for preemptive analgesia. Catheters were placed to permit invasive hemodynamic monitoring. A 7.5 Fr Swan-Ganz CCOmbo V pulmonary artery catheter (Edwards Lifesciences LLC, Irvine, CA) was advanced down a jugular vein until proper waveforms were observed for measurements of mean pulmonary artery pressures (MPAP), central venous pressure (CVP), and cardiac output (CO), as well as provided access for collection of mixed venous blood and a thermistor for

monitoring body temperature. Femoral arterial and venous catheters were placed for blood pressure monitoring, blood sample collection, and infusion of maintenance fluids. A Foley catheter was placed into the bladder via the urethra (female) or directly inserted into the bladder via cut down (male) to monitor urine production. Non-invasive monitoring included ECG and pulse oximeter measurements. Body temperature was supported by supplemental heat *via* a warming blanket.

To obtain intracranial pressure (ICP) measurements, pigs were turned to a prone position and a 5-mm diameter craniotomy was performed in the left anterior parietal region and a Codman Microsensor™ Intraparenchymal catheter was inserted into the superficial brain tissue. The other end of the catheter was connected to the Codman Express™ (Codman Neuro, Raynham, MA) for ICP monitoring.

Study design. Experiments were conducted under normobaric (ground) conditions and hypobaric (aeromedical evacuation) conditions using an altitude chamber that was specifically designed and built for large animal research. Normobaric control conditions were defined as ambient laboratory atmospheric pressure in Silver Spring, MD 121 m (397 ft, 14.5 psi). Hypobaric conditions were defined as an atmospheric pressure equivalent to an altitude of 2,441 m (8,000 ft., 10.9 psi). Prior to simulated transport, animals were randomly assigned to Normobaric (NORMO, N = 6) or Hypobaric conditions (HYPO, N = 6). After instrumentation animals were observed in the OR for 1 hour and then moved to hypobaric chamber. Following baseline measurements at normobaric conditions in both groups (T0), the HYPO group "ascended" to 2,441 m (8,000 ft.) over a 15-min time span, after which pigs remained at "transport" altitude for the duration of the experiment. Once altitude was reached, all pressure transducers were recalibrated. All equipment, including the ventilator, was located within the

hypobaric chamber. During this time, to simulate transport conditions, fluids were continued, and ventilator parameters were adjusted, but no additional treatment was allowed. In the chamber, animals were ventilated using an iVent 201 transport ventilator (GE HealthCare,Chicago, IL). After the last measurement at T240, HYPO pigs were brought to normobaric conditions prior to euthanasia. Actual normobaric and hypobaric pressures were measured and converted to altitude using an avionic digital pressure gauge (CPA 2501, Mensor Corp., San Marcos, TX).

Measurements. Physiological (heart rate, MAP, MPAP, CVP, CO, oxygen saturation, respiration rate, $ETCO_2$) and neurological (ICP) data were collected every 5 minutes for the entire study. Arterial and mixed venous blood samples were collected every 30 minutes for determination of hemoglobin (Hb), pH and blood gases, oxygen saturation, electrolytes, and indirect assessment of tissue oxygenation (lactate, pH, base excess and HCO_3^-). Calculations from these data included Cerebral Perfusion Pressure (CPP), Cardiac Index (CI), SVRI, and oxygen transport variables (oxygen contents, oxygen delivery [DO_2], oxygen consumption [VO_2] and oxygen extraction [O_2ER]) using standard formulae. A blood sample for a Complete Blood Count (CBC) was collected at T0, T120 and T240 and analyzed using a Pentra $60C^+$ cell counter (Horiba ABX Diagnostics, Irvine, CA).

Following euthanasia at the end of T240, a complete gross necropsy was performed and tissue samples from various organs were paraffin-embedded for later staining with standard H&E stain and batch analysis by a board-certified pathologist blinded to the identification of the study groups. The specific organs and type of pathology assessment for each organ are provided in Table I but, in general, tissues were evaluated for hemorrhage, inflammation, edema, and necrosis. Tissue sections were graded on a scale of 0 –5 for the severity or degree of histologic

findings present in the examined tissue: 0 (not present), 1 (minimal), 2 (mild), 3 (moderate), 4 (marked), and 5 (severe).

Statistical Analysis. Time averaging (15 min increments; e.g., T5, T10, T15 inclusive were averaged for Ta15) was performed on the parameters HR, MAP, PAP CVP, CI, ICP, CPP, and SVRI. Time points are: T 0, Ta 15, Ta 30, Ta 45, Ta 60, Ta 75, Ta 90, Ta 105, Ta 120, Ta 135, Ta 150, Ta 165, Ta 180, Ta 195, Ta 210, Ta 225, and Ta 240. Calculated parameters were determined prior to any time averaging (i.e., CPP and CI). For calculation of oxygen transport measurements, raw data (not time averaged) was used for the same time point in which the blood gas and hemoglobin measurements were collected. All results are reported as mean ± SEM.

Data analyses were performed using IBM SPSS Statistics 23.0 (IBM Corporation, Armonk, NY). The data was assessed graphically for normality prior to the inferential statistical analysis. Similar to previous studies(2) an omnibus 2 (Group) x 17 (observation period) mixed-model ANOVA was used to compare HR, blood pressures, blood gases, and lactate data in NORMO and HYPO groups from T0 to Ta240. Specifically, this test was used to determine whether the differences between the NORMO and HYPO differed as a function of group and observation period. Then independent sample T-Test was used for the above-mentioned variables. Following this analysis, a one-way repeated measure ANOVA was performed on HYPO across the observation period. This test was performed to determine whether measurements of variables changed over time in HYPO. Finally, post hoc comparisons using Least Significant Difference (LSD) were performed to find the effect of hypobaria over time. As there was a trend in certain variables to be different between the groups after T165, a percent change from T165 was calculated for those and then analyzed using the same mixed model ANOVA described above. CBC results were analyzed between the two groups using

independent sample T-Test and, within a group, to compare results over the sample periods. Histological scoring was analyzed between the two groups using independent sample T-Test. For all parameters, a p-value ≤ 0.05 was considered statistically significant.

RESULTS

All swine survived the study with no clinically apparent side effects. Pre-flight altitude was similar for HYPO and NORMO (16.2 ± 16.5 m [53 ± 54 ft.] vs. 5.5 ± 0.3 m [18 ± 1 ft.], respectively). It took ≤15 min for HYPO pigs to reach the target transport altitude of 2,438.4 m (8,000 ft.). During the experiment, the measured transport altitude was 2,364.6 ± 3.0 m (8,086 ± 10 ft.) for the HYPO group while the NORMO group remained at 5.8 ± 0 m (19 ± 0 ft.). There were no statistical or clinically relevant differences in HYPO vs. NORMO in any parameter at baseline (T0, prior to "transport").

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all data was within normal ranges, and there were no significant differences between the groups at baseline or by the end of the experiment (data not shown).

Physiology results. There were no significant differences in HR, CI, CPP, CVP, MPAP, and SVRI between the two groups (Table III). Only MAP and ICP showed significant changes during the study. Specifically, there were no differences between the two groups in MAP and ICP but both parameters increased significantly across time in both groups, (**FIGURE 1**). The MAP of the NORMO group was elevated early in simulated ground transport (T0 vs. T45 to T120) while the MAP of the HYPO group was significantly higher than T0 later in the simulated flight (T0 vs. T120 to T240).

Histopathology results. There were no significant differences in the injury scores for the brain (total or individual regions), liver, adrenal gland, or pancreas between HYPO and NORMO groups (Table IV). However, there were significant differences between the groups for the lungs and kidneys (Table IV).

The overall lung injury score was significantly worse in the HYPO group than the NORMO group (Table IV). The HYPO group exhibited significantly worse edema/fibrin ± hemorrhage in the subpleural, interlobular and alveolar space compared the NORMO group (1.06 ±0.08 vs 0.44 ±0.12, 1.83 ±0.12 vs. 0.44 ±0.13 and 1.61 ±0.29 vs. 0.31 ±0.15, respectively; **Figure 2**) as well as significantly more evidence of alveolar septa congestion (1.89 ±0.33 vs. 0.44 ±0.19, respectively). The HYPO group also exhibited significantly increased severity of neutrophilic and histiocytic inflammation compared to the NORMO group (neutrophilic inflammation scores of 1.69 ±0.20 vs. 0.19 ±0.13 and histiocytic inflammation scores of 1.83 ±0.23 vs. 0.47 ±0.17, for HYPO and NORMO, respectively; **Figure 3**). Neither group exhibited

evidence of alveolar septal necrosis/cellular debris or hyaline membranes while only the HYPO group had evidence of microatelectasis (0.86 ±0.31, $p \leq 0.05$ vs. NORMO; **Figure 2**).

Surprisingly, the overall injury scores for the kidney were reversed from the lung; with a higher score in the NORMO group than the HYPO group (Table IV). This difference between the groups was due to a significantly increased renal inflammation score in the NORMO group compared to the HYPO group (1.00 ±0.18 vs. 0.33 ± 0.17, respectively) as there were no differences between them for renal hemorrhage, edema or congestion.

DISCUSSION

This study evaluated one transport condition, altitude (hypobaria), on acute physiological parameters and the histopathology of selected organs from anesthetized, mechanically ventilated swine. We performed these experiments in order to obtain a baseline understanding of the clinical impact of hypobaria so that we can assess the efficacy and safety of hypobaric transport for wounded warriors. Given the data demonstrating that healthy individuals flying at cabin pressures equivalent to altitudes of 1,526 to 2,441 m (5,000 to 8,000 ft.) can compensate for the lower partial pressure of inspired oxygen (~108 mm Hg) (14) and given the lack of critical symptoms in flight crew personnel, it was not surprising that there were minimal to no immediately life threatening clinical changes (hemodynamic, neurologic, oxygen transport, blood gas, chemistry, or complete blood count data) in these healthy swine. However, the pigs exposed to hypobaric conditions had significantly more histopathological changes in their lungs compared to normobaric control pigs. The overall Lung Injury Score was significantly worse in the HYPO group and this increased score was largely due to an increased number of histopathological categories. Specifically, in the HYPO group, there was more

edema/fibrin/hemorrhage, more congestion, evidence of microatelectasis, and increased neutrophilic and histiocytic inflammation.

While there is no direct evidence to link the results of our data to the occupational hazards of aircrew, an association can be drawn and should not be disregarded. As an example, the prevalence of medical symptoms from 323 Israeli Air Force personnel was recently reviewed and respiratory symptoms were the second most frequent symptom exhibited (behind spinal pain); with a frequency of one in five aviators (22%) (5). More precisely, these respiratory symptoms included rhinorrhea (17%), cough (10%) and dyspnea (1%). The symptoms associated with the respiratory pathology found in our study are unknown since these pigs were anesthetized but coughing and dyspnea are conceivable. Furthermore, the pathological changes in the hypobaric pigs developed acutely, were mild and, most likely, were reversible as were the symptoms in this clinical report. In addition, short exposure to high altitude has been shown to increase pulmonary edema in healthy, awake, spontaneously breathing humans after only one day at 4,559 m (14,957 ft.), supporting the possibility of alveolar-capillary damage (15) with additional evidence that pulmonary edema (16) and pulmonary oxidative stress (17) are triggered by altitude alone.

On the other hand, an earlier assessment of these Israeli Air Force aviators found no correlation between any of their symptoms and the hours of flying or type of plane, leading the authors to conclude that flight itself might not be a trigger for their symptoms (18). Altitude was not directly investigated in this early Israeli study. In contrast, the only difference between the hypobaric transport group and the normobaric control group in our study was, indeed, flight or, more precisely, being at 2,441 m (8,000 ft.) altitude for four hours. Thus, it may not be the hours

in a plane or the type of plane but the exposure to certain altitudes for unspecified amounts of time.

The potentially confounding effects of anesthesia, mechanical ventilation, and breathing dry inspired gases (vs. humidified gases when awake) occurred in this swine study and could be additive or synergistic with any effects of altitude alone. A short duration of anesthesia and controlled ventilation is known to cause alveolar damage, atelectasis, and impaired gas exchange (19-21). Awake animal models are needed to more appropriately mimic air crew scenarios and determine the underlying mechanisms of the occupational medical health hazards they face. Nonetheless, even if the effects of anesthesia and controlled ventilation make the results in this study incomparable to flight crews, the results demonstrate that transport at altitude may have detrimental effects on wounded warriors being aero-medically evacuated. These wounded warriors may receive in-flight sedation and respiratory support (e.g., a sedated soldier requiring mechanical ventilation due to a severe head injury). When one then adds on the impact of transporting a wounded warrior with lung injury, the combined effects of hypobaria, anesthesia and controlled ventilation may be significant enough to affect morbidity and mortality.

One puzzling result of this study was the increased renal inflammation in the normobaric group compared to the hypobaric group. The scoring between the two groups was similar (scores ranging from 0 to 1) and all pigs had low scores (minimal inflammation), with no outlier in either group. There is no clear explanation for this outcome and renal pathology should continue to be evaluated in future studies.

It is clear that our study raises more questions than it answers. It is unknown when the adverse pulmonary pathological changes demonstrated in this study began to develop, although there was a visual trend of some parameters changing after 2 h of hypobaric transport. It is

unknown if the pathological changes become more severe with longer flights or higher altitude, if it is transient and spontaneously resolves, or if it progresses to a more severe condition.

-Military and civilian aircraft are pressurized in a similar fashion to a cabin pressure equivalent to 8,000ft. At the discretion of the medical officer in charge of the aeromedical evacuation, the aircraft can be further pressurized to 4,000 ft or even lower if the condition of a specific patient necessitates; but this is not routinely done. Research in this area is just emerging and future studies are planned to assess the effects of different transport altitudes on injuries. While helicopters are not pressurized, their altitude at flight for aeromedical transport in theatre is usually 3,000-4,000ft. However, this can vary depending on the starting altitude at ground level. For instance, while the ground level in Iraq is at sea level, the ground level in Afghanistan varies widely with base altitudes of up to 10,000 ft. It is therefore not unusual for helicopters to fly as high as 14,000 ft with unpressurized cabins. While these altitudes are reached for much shorter duration, their effect on aeromedical transport is certainly worthy of future investigations.

In summary, data from this study suggest that hypobaria, itself, may have an adverse effect on even healthy individuals and that lungs may be the most sensitive organ to the effects of hypobaria. However, these swine were anesthetized and mechanically ventilated which is a critical difference between this study and the effect hypobaria may have on aircrew personnel. Nonetheless, whether the pathology observed in the lungs of the "flight" animals are due to hypobaria alone or hypobaria plus anesthesia/ventilation, the results may have important implications for our wounded warriors, especially those with lung injury and/or disease. It is paramount to continue focused research studies, including awake models, and systematically modify the transport factors (e.g., duration, altitude) to better characterize and understand the clinical implications of these findings. Aeromedical evacuation, as currently practiced, needs to

be fully investigated to determine if any changes are necessary to improve the outcomes of our transported wounded warriors and maintain the health of the flight crew.

Disclaimers

Authors are employees of the U.S. Government. This work was prepared as part of their official duties. Title 17 U.S.C. § 105 provides that 'Copyright protection under this title is not available for any work of the United States Government.' Title 17 U.S.C. § 101 defines a U.S. Government work as a work prepared by a military service member or employee of the U.S. Government as part of that person's official duties. The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, the Uniformed University of the Health Sciences , nor the U.S. Government.

Conflict of interest statement and Source of Funding

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Meetings

This manuscript will be presented (D. Malone) as an oral quick shot at the 31st Annual Scientific Assembly for the Eastern Association for the Surgery of Trauma (EAST) held in Lake Buena Vista, FL from January 9-13, 2018.

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Author Contributions

The following authors contributed to this manuscript in the following manner: Anke Scultetus (study design, data collection, data analysis, data interpretation, critical revision), Michelle Jefferson (histopathological analysis), Ashraful Haque (literature search, data collection, data analysis, data interpretation, writing), Lam Thuy Vi Tran Ho (data collection, data analysis), Biswajit Saha (data collection, data analysis), Steve Chun (data collection), Brittany Hazzard (data collection, data analysis), Charles Auker (data interpretation), Paula Moon-Massat (data interpretation, writing), Richard McCarron (study design, critical revision), Debra Malone (data interpretation, study design, critical revision),

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BACKGROUND

Recent military conflicts have provided evidence that rapid evacuation of combat casualties to definitive care is of paramount importance to achieve diminished morbidity and mortality (1). However, despite growing interest in determining the potential damage of long-range flight on the wounded warrior(2), it has been assumed that the flight crew can adequately compensate for the physiological changes that occur during such flights. Nonetheless, commonly reported medical symptoms for military aircrew include headache, decreased cognitive function(3, 4), fatigue(5), barodontalgia (6), sensorineural hearing loss(7) and, most commonly, spinal pain and respiratory symptoms (5, 8-13). The etiologies of these occupational health concerns are multifactorial and may include altitude-induced hypoxia, pressure changes, vibrations, noise, and air-quality as well as characteristics related to the mission (i.e., time and length of travel).

The overall goal of our laboratory is to identify which factors associated with aeromedical transport conditions can be attenuated to improve the safety of injured patients. We previously provided evidence that hypobaric conditions exacerbate cerebral hypoxia and worsen traumatic brain injury (TBI)(2). Evaluation of how such transport conditions affect the pathophysiology of healthy individuals is an equally important step in assessing hypobaric transport conditions. The study reported herein uses a healthy swine model and a realistic military timeline to simulate hypobaric transport conditions (i.e., from Afghanistan to Germany). The study aim was to investigate if there were acute cardiopulmonary, neurological or biochemical changes and/or histological evidence of a detrimental effect of hypobaria on healthy swine. Results will not only improve our understanding of studies using injury models or

clinical trauma data but also expand our knowledge of how such transport may affect the aircrew exposed to these conditions.

METHODS

The study protocol was reviewed and approved by the Walter Reed Army Institute of Research/Naval Medical Research Center Institutional Animal Care and Use Committee in compliance with all applicable Federal regulations governing the protection of animals in research. All animals used in the protocol were maintained under the surveillance of a veterinarian.

Yorkshire swine (31.8 ± 1.6 kg [mean ± SEM]; both genders; Animal Biotech Industries, Danboro, PA) were sedated (33 mg/kg IM ketamine and 0.05 mg/kg IM atropine) prior to mask induction with isoflurane to facilitate endotracheal intubation. Maintenance anesthesia was initially with isoflurane but was changed to total intravenous anesthesia using propofol (250-350 µg/kg/min IV) and fentanyl (5—10 µg/kg/h IV) once venous access was established. Swine were ventilated using synchronized intermittent mechanical ventilation mode (Apollo®, Draeger Medical Inc. Telford, PA) with an FiO_2 of 0.4 and a PEEP of 5 cm H_2O. Ventilator settings were adjusted to maintain end-tidal carbon dioxide ($ETCO_2$) between 35-40 mm Hg. Animals received 3 mL/kg/h IV 0.9% saline as a maintenance fluid to make up insensible fluid loss.

Before surgical procedures, buprenorphine (0.01mg/kg IM) was administered for preemptive analgesia. Catheters were placed to permit invasive hemodynamic monitoring. A 7.5 Fr Swan-Ganz CCOmbo V pulmonary artery catheter (Edwards Lifesciences LLC, Irvine, CA) was advanced down a jugular vein until proper waveforms were observed for measurements of mean pulmonary artery pressures (MPAP), central venous pressure (CVP), and cardiac output (CO), as well as provided access for collection of mixed venous blood and a thermistor for

monitoring body temperature. Femoral arterial and venous catheters were placed for blood pressure monitoring, blood sample collection, and infusion of maintenance fluids. A Foley catheter was placed into the bladder via the urethra (female) or directly inserted into the bladder via cut down (male) to monitor urine production. Non-invasive monitoring included ECG and pulse oximeter measurements. Body temperature was supported by supplemental heat *via* a warming blanket.

To obtain intracranial pressure (ICP) measurements, pigs were turned to a prone position and a 5-mm diameter craniotomy was performed in the left anterior parietal region and a Codman Microsensor™ Intraparenchymal catheter was inserted into the superficial brain tissue. The other end of the catheter was connected to the Codman Express™ (Codman Neuro, Raynham, MA) for ICP monitoring.

Study design. Experiments were conducted under normobaric (ground) conditions and hypobaric (aeromedical evacuation) conditions using an altitude chamber that was specifically designed and built for large animal research. Normobaric control conditions were defined as ambient laboratory atmospheric pressure in Silver Spring, MD 121 m (397 ft, 14.5 psi). Hypobaric conditions were defined as an atmospheric pressure equivalent to an altitude of 2,441 m (8,000 ft., 10.9 psi). Prior to simulated transport, animals were randomly assigned to Normobaric (NORMO, N = 6) or Hypobaric conditions (HYPO, N = 6). After instrumentation animals were observed in the OR for 1 hour and then moved to hypobaric chamber. Following baseline measurements at normobaric conditions in both groups (T0), the HYPO group "ascended" to 2,441 m (8,000 ft.) over a 15-min time span, after which pigs remained at "transport" altitude for the duration of the experiment. Once altitude was reached, all pressure transducers were recalibrated. All equipment, including the ventilator, was located within the

hypobaric chamber. During this time, to simulate transport conditions, fluids were continued, and ventilator parameters were adjusted, but no additional treatment was allowed. In the chamber, animals were ventilated using an iVent 201 transport ventilator (GE HealthCare,Chicago, IL). After the last measurement at T240, HYPO pigs were brought to normobaric conditions prior to euthanasia. Actual normobaric and hypobaric pressures were measured and converted to altitude using an avionic digital pressure gauge (CPA 2501, Mensor Corp., San Marcos, TX).

Measurements. Physiological (heart rate, MAP, MPAP, CVP, CO, oxygen saturation, respiration rate, $ETCO_2$) and neurological (ICP) data were collected every 5 minutes for the entire study. Arterial and mixed venous blood samples were collected every 30 minutes for determination of hemoglobin (Hb), pH and blood gases, oxygen saturation, electrolytes, and indirect assessment of tissue oxygenation (lactate, pH, base excess and HCO_3^-). Calculations from these data included Cerebral Perfusion Pressure (CPP), Cardiac Index (CI), SVRI, and oxygen transport variables (oxygen contents, oxygen delivery [DO_2], oxygen consumption [VO_2] and oxygen extraction [O_2ER]) using standard formulae. A blood sample for a Complete Blood Count (CBC) was collected at T0, T120 and T240 and analyzed using a Pentra $60C^+$ cell counter (Horiba ABX Diagnostics, Irvine, CA).

Following euthanasia at the end of T240, a complete gross necropsy was performed and tissue samples from various organs were paraffin-embedded for later staining with standard H&E stain and batch analysis by a board-certified pathologist blinded to the identification of the study groups. The specific organs and type of pathology assessment for each organ are provided in Table I but, in general, tissues were evaluated for hemorrhage, inflammation, edema, and necrosis. Tissue sections were graded on a scale of 0 –5 for the severity or degree of histologic

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Surprisingly, the overall injury scores for the kidney were reversed from the lung; with a higher score in the NORMO group than the HYPO group (Table IV). This difference between the groups was due to a significantly increased renal inflammation score in the NORMO group compared to the HYPO group (1.00 ±0.18 vs. 0.33 ± 0.17, respectively) as there were no differences between them for renal hemorrhage, edema or congestion.

DISCUSSION

This study evaluated one transport condition, altitude (hypobaria), on acute physiological parameters and the histopathology of selected organs from anesthetized, mechanically ventilated swine. We performed these experiments in order to obtain a baseline understanding of the clinical impact of hypobaria so that we can assess the efficacy and safety of hypobaric transport for wounded warriors. Given the data demonstrating that healthy individuals flying at cabin pressures equivalent to altitudes of 1,526 to 2,441 m (5,000 to 8,000 ft.) can compensate for the lower partial pressure of inspired oxygen (~108 mm Hg) (14) and given the lack of critical symptoms in flight crew personnel, it was not surprising that there were minimal to no immediately life threatening clinical changes (hemodynamic, neurologic, oxygen transport, blood gas, chemistry, or complete blood count data) in these healthy swine. However, the pigs exposed to hypobaric conditions had significantly more histopathological changes in their lungs compared to normobaric control pigs. The overall Lung Injury Score was significantly worse in the HYPO group and this increased score was largely due to an increased number of histopathological categories. Specifically, in the HYPO group, there was more

edema/fibrin/hemorrhage, more congestion, evidence of microatelectasis, and increased neutrophilic and histiocytic inflammation.

While there is no direct evidence to link the results of our data to the occupational hazards of aircrew, an association can be drawn and should not be disregarded. As an example, the prevalence of medical symptoms from 323 Israeli Air Force personnel was recently reviewed and respiratory symptoms were the second most frequent symptom exhibited (behind spinal pain); with a frequency of one in five aviators (22%) (5). More precisely, these respiratory symptoms included rhinorrhea (17%), cough (10%) and dyspnea (1%). The symptoms associated with the respiratory pathology found in our study are unknown since these pigs were anesthetized but coughing and dyspnea are conceivable. Furthermore, the pathological changes in the hypobaric pigs developed acutely, were mild and, most likely, were reversible as were the symptoms in this clinical report. In addition, short exposure to high altitude has been shown to increase pulmonary edema in healthy, awake, spontaneously breathing humans after only one day at 4,559 m (14,957 ft.), supporting the possibility of alveolar-capillary damage (15) with additional evidence that pulmonary edema (16) and pulmonary oxidative stress (17) are triggered by altitude alone.

On the other hand, an earlier assessment of these Israeli Air Force aviators found no correlation between any of their symptoms and the hours of flying or type of plane, leading the authors to conclude that flight itself might not be a trigger for their symptoms (18). Altitude was not directly investigated in this early Israeli study. In contrast, the only difference between the hypobaric transport group and the normobaric control group in our study was, indeed, flight or, more precisely, being at 2,441 m (8,000 ft.) altitude for four hours. Thus, it may not be the hours

in a plane or the type of plane but the exposure to certain altitudes for unspecified amounts of time.

The potentially confounding effects of anesthesia, mechanical ventilation, and breathing dry inspired gases (vs. humidified gases when awake) occurred in this swine study and could be additive or synergistic with any effects of altitude alone. A short duration of anesthesia and controlled ventilation is known to cause alveolar damage, atelectasis, and impaired gas exchange (19-21). Awake animal models are needed to more appropriately mimic air crew scenarios and determine the underlying mechanisms of the occupational medical health hazards they face. Nonetheless, even if the effects of anesthesia and controlled ventilation make the results in this study incomparable to flight crews, the results demonstrate that transport at altitude may have detrimental effects on wounded warriors being aero-medically evacuated. These wounded warriors may receive in-flight sedation and respiratory support (e.g., a sedated soldier requiring mechanical ventilation due to a severe head injury). When one then adds on the impact of transporting a wounded warrior with lung injury, the combined effects of hypobaria, anesthesia and controlled ventilation may be significant enough to affect morbidity and mortality.

One puzzling result of this study was the increased renal inflammation in the normobaric group compared to the hypobaric group. The scoring between the two groups was similar (scores ranging from 0 to 1) and all pigs had low scores (minimal inflammation), with no outlier in either group. There is no clear explanation for this outcome and renal pathology should continue to be evaluated in future studies.

It is clear that our study raises more questions than it answers. It is unknown when the adverse pulmonary pathological changes demonstrated in this study began to develop, although there was a visual trend of some parameters changing after 2 h of hypobaric transport. It is

unknown if the pathological changes become more severe with longer flights or higher altitude, if it is transient and spontaneously resolves, or if it progresses to a more severe condition.

Military and civilian aircraft are pressurized in a similar fashion to a cabin pressure equivalent to 8,000ft. At the discretion of the medical officer in charge of the aeromedical evacuation, the aircraft can be further pressurized to 4,000 ft or even lower if the condition of a specific patient necessitates; but this is not routinely done. Research in this area is just emerging and future studies are planned to assess the effects of different transport altitudes on injuries. While helicopters are not pressurized, their altitude at flight for aeromedical transport in theatre is usually 3,000-4,000ft. However, this can vary depending on the starting altitude at ground level. For instance, while the ground level in Iraq is at sea level, the ground level in Afghanistan varies widely with base altitudes of up to 10,000 ft. It is therefore not unusual for helicopters to fly as high as 14,000 ft with unpressurized cabins. While these altitudes are reached for much shorter duration, their effect on aeromedical transport is certainly worthy of future investigations.

In summary, data from this study suggest that hypobaria, itself, may have an adverse effect on even healthy individuals and that lungs may be the most sensitive organ to the effects of hypobaria. However, these swine were anesthetized and mechanically ventilated which is a critical difference between this study and the effect hypobaria may have on aircrew personnel. Nonetheless, whether the pathology observed in the lungs of the "flight" animals are due to hypobaria alone or hypobaria plus anesthesia/ventilation, the results may have important implications for our wounded warriors, especially those with lung injury and/or disease. It is paramount to continue focused research studies, including awake models, and systematically modify the transport factors (e.g., duration, altitude) to better characterize and understand the clinical implications of these findings. Aeromedical evacuation, as currently practiced, needs to

be fully investigated to determine if any changes are necessary to improve the outcomes of our transported wounded warriors and maintain the health of the flight crew.

Disclaimers

Authors are employees of the U.S. Government. This work was prepared as part of their official duties. Title 17 U.S.C. § 105 provides that 'Copyright protection under this title is not available for any work of the United States Government.' Title 17 U.S.C. § 101 defines a U.S. Government work as a work prepared by a military service member or employee of the U.S. Government as part of that person's official duties. The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of Defense, the Uniformed University of the Health Sciences , nor the U.S. Government.

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Meetings

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Author Contributions

The following authors contributed to this manuscript in the following manner: Anke Scultetus (study design, data collection, data analysis, data interpretation, critical revision), Michelle Jefferson (histopathological analysis), Ashraful Haque (literature search, data collection, data analysis, data interpretation, writing), Lam Thuy Vi Tran Ho (data collection, data analysis), Biswajit Saha (data collection, data analysis), Steve Chun (data collection), Brittany Hazzard (data collection, data analysis), Charles Auker (data interpretation), Paula Moon-Massat (data interpretation, writing), Richard McCarron (study design, critical revision), Debra Malone (data interpretation, study design, critical revision),

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Table I. Organs evaluated histologically from anesthetized healthy swine (N = 6 each condition) after 4 h simulated "transport" at normobaric (sea level) and hypobaric (2,441 m; 8,000 ft.) conditions.

Organ	Histological Evaluation
Lungs (right cranial, middle and caudal lobe; accessory lobe and left cranial and caudal lobe)	Edema/fibrin/± hemorrhage, subpleural
	Edema/fibrin/± hemorrhage, interlobular
	Edema/fibrin/± hemorrhage, alveolar
	Congestion, alveolar septa
	Inflammation (neutrophilic), alveolar septa/lumen
	Inflammation (histiocytic), alveolar septa
	Necrosis/cellular debris, alveolar septa
	Microatelectasis
	Hyaline membranes
Liver, Kidney (left and right), adrenal gland and pancreas	Hemorrhage
	Edema
	Congestion
	Inflammation/infiltrate, mononuclear and/or neutrophilic
	Glomerular tufts (kidney only)
Brain (cerebrum and coronal section at level of basal ganglia, hypothalamus, mammillary body, lateral geniculate nucleus of hippocampus and rostral	Meningeal fibrin and/or edema +/-hemorrhage
	Meningeal congestion
	Intraventricular hemorrhage
	Submeningeal & neuropil (intraparenchyma)

colliculus)*	hemorrhage
	Fibrin thrombi
	Neuropil rarefaction/ Neural tissue edema (spongiosis)
	Axonal degeneration
	Gliosis
	Neuropil loss or necrosis
	Inflammation/infiltrate, mononuclear and/or neutrophilic

In addition to an overall brain injury score from these regions, data was also evaluated individually in these areas: cerebral cortex, basal nuclei, thalamus-hypothalamus, hippocampus, and mesencephalon.

Table II: Selected time points of arterial blood gas analysis (mean±sem).

Parameter	Group	T0	T60	T120	T180	T240
Hemoglobin (g/dL)	Hypo	7.9±0.3	7.7±0.3	7.7±0.5	6.7±0.6	7.4±0.4
	Normo	7.8±0.2	7.7±0.2	7.8±0.3	7.6±0.3	7.6±0.4
pH (U)	Hypo	7.393±0.024	7.458±0.020	7.448±0.021	7.468±0.017	7.467±0.023
	Normo	7.431±0.023	7.423±0.021	7.415±0.015	7.432±0.014	7.422±0.015
PaO_2 (mm Hg)	Hypo	170±4	181±8	177±6	167±8	177±4
	Normo	188±8	193±7	195±8	202±11	193±15
$PaCO_2$ (mm Hg)	Hypo	49±4	40±2^	40±2*	32±3*^	41±4
	Normo	43±2	45±2	47±2*	44±2*	47±1
Lactate (mmol/L)	Hypo	0.8±0.1	0.7±0.0	0.7±0.0	0.5±0.0	0.6±0.0
	Normo	1.2±0.2	1.0±0.1	0.9±0.1	0.8±0.1	0.6±0.1
Base Excess (mmol/L)	Hypo	4.6±0.7	3.8±0.5	3.3±0.8	-0.2±2.9	3.5±1.6
	Normo	4.0±1.0	4.4±0.8	4.8±0.9	4.6±1.1	6.3±1.3

* indicates a significant difference ($p \leq 0.05$) between the groups.

^ indicates a significant difference ($p \leq 0.05$) from T0 within a group.

Table III: Selected time points of hemodynamic data (mean±sem). There were no significant differences between groups or over time for any parameter.

Parameter	Group	T0	T60	T120	T180	T240
Heart Rate (beats/min)	Hypo	102±8	94±8	94±7	95±5	100±6
	Normo	113±17	111±14	122±16	116±17	121±12
Cardiac Index ($L/min/m^2$)	Hypo	6.9±0.9	5.7±0.6	5.8±0.6	6.0±0.6	6.3±1.2
	Normo	5.4±0.7	5.4±0.4	5.8±0.5	5.4±0.5	5.7±0.5
CPP (mm Hg)	Hypo	78±6	85±4	86±3	83±4	92±6
	Normo	72±6	80±5	78±6	75±6	75±7
CVP (mm Hg)	Hypo	5±1	6±1	6±1	4±1	6±2
	Normo	3±1	4±1	4±0	4±0	3±0
MPAP (mm Hg)	Hypo	21±2	20±3	20±2	20±3	21±1
	Normo	18±1	18±1	19±1	19±2	20±2
SVRI ($mmHg/L/min/m^2$)	Hypo	14±3	19±2	18±2	17±3	18±5
	Normo	16±1	17±1	16±1	17±2	16±1

CPP = cerebral perfusion pressure, CVP = central venous pressure, MPAP = mean pulmonary artery pressure, SVRI = systemic vascular resistance index

Table IV. Injury scores (mean ±sem) for selected organs and brain regions of swine undergoing 4 hour transport under normobaric (NORMO) or hypobaric (HYPO) conditions.

Organ	Healthy Transport Group	Injury scores
Lung	HYPO	10.78 ±1.22*
	NORMO	2.31 ±0.71*
Kidney	HYPO	0.92 ±0.33*
	NORMO	1.92 ±0.30*
Brain	HYPO	1.98 ±0.26
	NORMO	1.70 ±0.24
Liver	HYPO	0.50 ±0.34
	NORMO	1.50 ±0.43
Adrenal	HYPO	0.00 ±0.00
	NORMO	0.33 ±0.21
Pancreas	HYPO	0.00 ±0.00
	NORMO	0.00 ±0.00
Brain Region Injury Scores		
Cerebral Cortex	HYPO	5.17 ±1.30
	NORMO	2.83 ±1.17
Basal Nuclei	HYPO	0.80 ±0.58
	NORMO	0.00 ±0.00
Thalamus-Hypothalamus	HYPO	0.80 ±0.20
	NORMO	1.83 ±0.48

Hippocampus	HYPO	1.20 ±0.37
	NORMO	2.33 ±0.42
Mesencephalon	HYPO	1.67 ±0.33
	NORMO	1.50 ±0.43

* Asterisk indicates a significant difference ($p \leq 0.05$) between the groups.

Figure 1. MAP (top) and ICP (bottom) data (mean±sem) from healthy anesthetized pigs under hypobaric (HYPO) or normobaric (NORMO) conditions. Time 0 and 240 were at normobaric conditions for both groups while intervening time points simulated transport at 8,000 ft. altitude for the HYPO group.

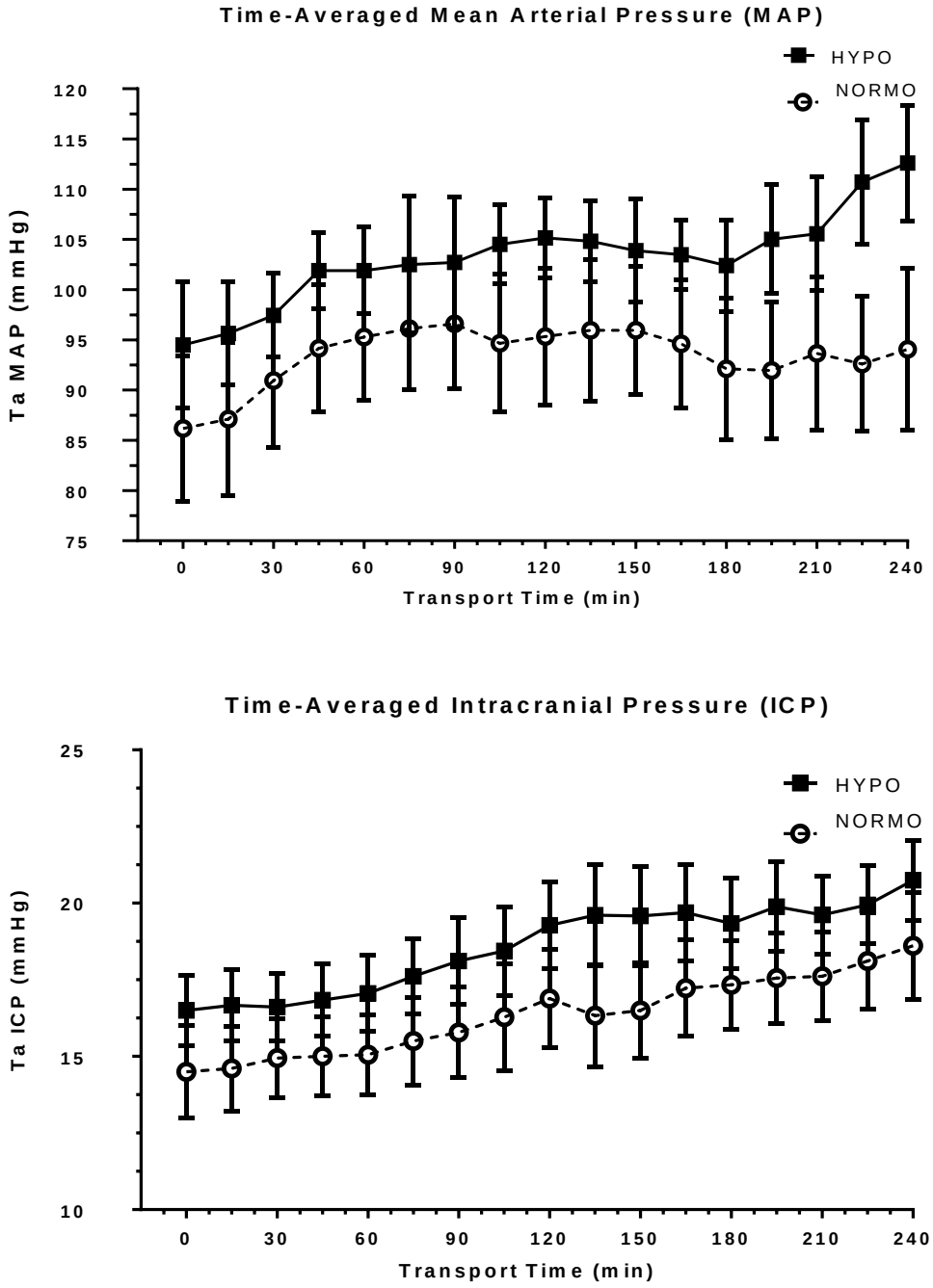

Figure 2. A.) Normobaric conditions. Lung, H&E, 4x. B) Hypobaric conditions. Lung, H&E, 4x. In B, there is increased cellularity with moderate evidence of congestion, edema and hemorrhage (arrow) and microatelectasis when compared to A.

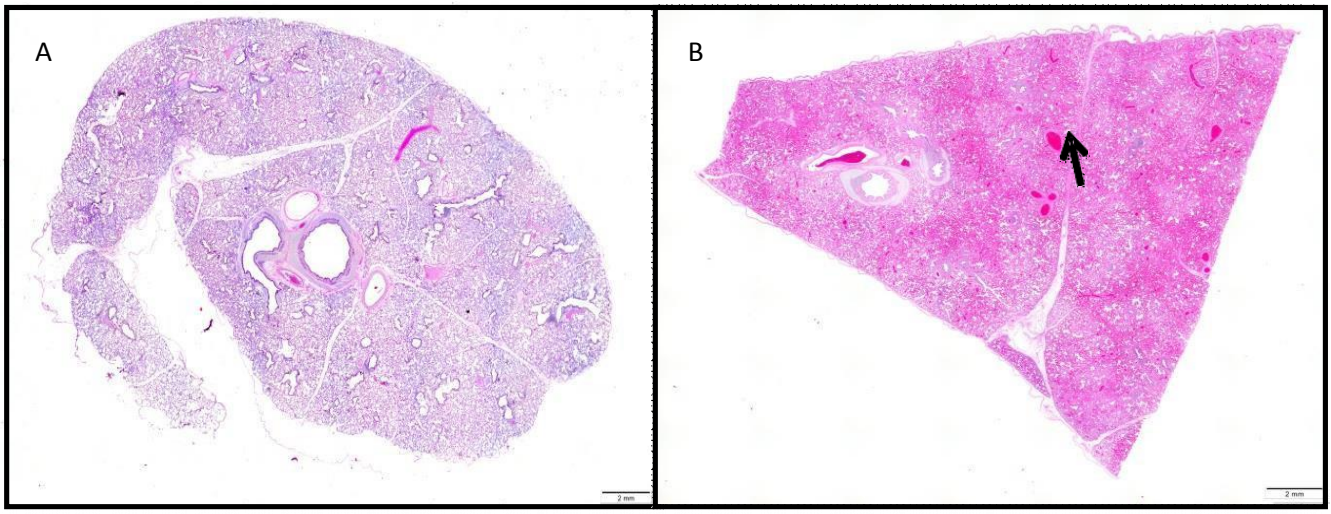
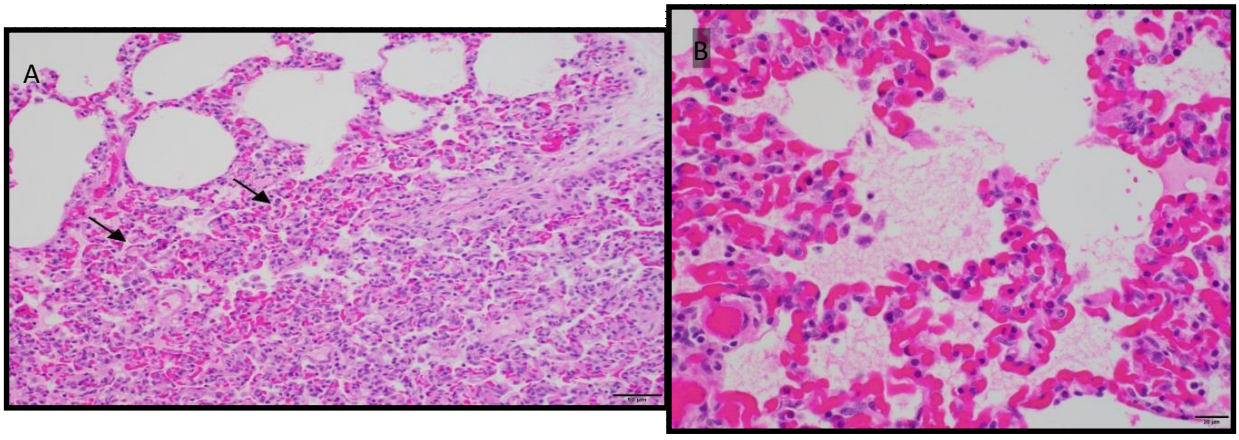
Figure 3. A.) Hypobaric conditions. Lung, H&E, 20x. Microatelectasis with scattered inflammation in the alveolar lumen and septa (arrows). B) Higher magnification of A, 40x showing marked congestion, alveolar lumen fibrin and edema.