AWARD NUMBER: CDMRPL-16-0-DM167040

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

PRINCIPAL INVESTIGATOR: LCDR Carolyn Gosztyla

CONTRACTING ORGANIZATION: Naval Medical Research Center, Silver Spring, MD

REPORT DATE: October 2021

TYPE OF REPORT: ANNUAL

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2021	ANNUAL REPORT	01Oct2020-30Sep2021

4. TITLE AND SUBTITLE
Preclinical Evaluation of the Effects of Aeromedical Evacuation on Military-Relevant Casualties

5a. CONTRACT NUMBER
CDMRPL-16-0-DM167040

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
LCDR Carolyn Gosztyla
Dr. Francoise Arnaud

E-Mail: carolyn.e.gosztyla.mil@mail.mil/ francoise.arnaud.ctr@mail.mil

5d. PROJECT NUMBER
DM167040

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Naval Medical Research Center
503 Robert Grant Avenue
Silver Spring, MD

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Aeromedical evacuation is associated with several stressors that may cause harm during casualties' transport. In addition to these stressors, timing, oxygen supplementation and altitude may have additional effects that are currently unknown. The purposes of this proposal are to better evaluate the effect of these additional variables and define an adequate timing, oxygen supplementation level and best appropriate altitude to maintain normal organ physiology. This proposal includes the use of two different animal models, rats and swine, using different and complement strategies to better understand the effects of aeromedical evacuation. So far, our results showed the feasibility of our models to monitor the effects of aeromedical evacuation on neurobehavioral damage, inflammatory response and hemodynamic changes. Specifically, we showed no changes in behavioral or pathological changes in the short term of transport after injury in the rat model but noticed modification in the inflammatory response. In addition, we established a swine model that can be used to monitor hemodynamic changes during aeromedical evacuation with different levels of oxygen and altitude.

15. SUBJECT TERMS
Traumatic brain injury; hemorrhagic shock; aeromedical evacuation; oxygenation; altitude; timing of evacuation

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	Unclassified	13	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION:

Current practice in Operation Enduring Freedom commonly includes transport of the critically injured patient to the Continental United States (CONUS) soon after stabilization and initial surgery. In general, service members can be returned to the US medical treatment facility in five-to-seven days. Aeromedical transport is associated with obvious concerns that include hypobaria, hypoxemia, air trapped within a body cavity, vibration, and hypothermia. Current guidelines for critical care air transport teams (CCATT) note that basic physiology parameters during transport are to be supported; to include adequate oxygen saturation, ventilation, blood pressure etc. However, these parameters may be difficult to achieve. The impact of hypobaria on the transport of critically ill patients is unknown. Applying resuscitation guidelines for trauma developed over decades for ground-based scenarios to aeromedical transport is simply based on expert opinion. This grant incorporates three projects that address specific operational issues regarding optimization of aeromedical evacuation standards. In animal models of combat trauma, we will address the effects of timing, altitude, and oxygen supplementation during aeromedical evacuation.

2. KEYWORDS:

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

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Current practice in Operation Enduring Freedom commonly includes transport of the critically injured patient to the Continental United States (CONUS) soon after stabilization and initial surgery. In general, service members can be returned to the US medical treatment facility in five to seven days. Aeromedical transport is associated with obvious concerns that include hypobaria, hypoxemia, air trapped within a body cavity, vibration, and hypothermia. Current guidelines for critical care air transport teams (CCATT) note that basic physiology parameters during transport are to be supported; these include adequate oxygen saturation, ventilation, and blood pressure etc. However, these parameters may be difficult to achieve. The impact of hypobaria on the transport of critically ill patients is unknown. Applying resuscitation guidelines for trauma developed over decades for ground-based transport scenarios to aeromedical transport is simply based on expert opinion.

This grant incorporates three projects that address specific operational issues regarding optimization of aeromedical evacuation standards. In animal models of combat trauma, we will address the effects of timing, altitude, and oxygen supplementation during aeromedical evacuation.

	Timeline	Method	NMRC
Specific Aim 1: Evaluation of the timing of aeromedical evacuation in rat and swine models of TBI and polytrauma	Months		
Major Task 1: IACUC/ACURO approval	1-3	Writing	Complete
Major Task 2: Rat blast/AE timing experiments	4-20	Animal experiment	Complete
Major Task 3: Swine TBI/polytrauma AE timing experiments	50-62	Animal experiment	Cancelled

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

Aim 1/ Major Task 3: Swine TBI/polytrauma AE timing experiments. To date, no experiments have been performed towards this aim in swine. A new protocol has been drafted based on preliminary results from the rat study, which includes the following groups of animals listed in the table below. Data from these animals will include: hemodynamic data (blood pressure, heart rate), biochemical analysis (electrolytes, blood gases and blood cell count), organ function, inflammation markers and organ histopathology.

Group	Treatment	Normobaria (sea level), number of animals	Hypobaria (8,000 ft.), number of animals
1	Sham (instrumentation without injury)	8	8
2	Traumatic Brain Injury (TBI) – Flight Day 1	8	8
3	Acute respiratory distress syndrome (ARDS) – Flight Day 1	8	8
4	Traumatic Brain Injury (TBI) – Delayed Flight	8	8
5	Acute respiratory distress syndrome (ARDS) – Delayed Flight	8	8

A revised statement of work was approved for removal of this task. .

Aim 2/ Major Task 2: Swine supplemental O_2/AE experiments. Twenty animals were used previously on this study (protocol 17-OUMD-24LS), with the last experiment in July 2019. The data obtained were highly variable protocol revision was needed to produce more consistent results with the TBI. A new protocol was submitted and approved by IACUC (20-OUMD-28LS). Large animal experiments resumed in February 2021 and five animals have been used. Two of them were pilot animals and three of them underwent full experimental protocol. An additional 15 animal TBI experiments were performed to the end of September proving valuable data. Also, a new implantable tissue oxygenation device has used with preliminary results showing good correlation to external oxygenation measurements. Experiment with Sham (instrumentation - no injury) is ongoing.

Group	Treatment	21% (number of animals)	40% (number of animals)	54% (number of animals)	100% (number of animals)
1	Instrumentation –no injury (Normobaria)	4	4	4	4
2	TBI and Hemorrhage Shock (HS; Normobaria)	4	4	4	4
3	Instrumentation –no injury (Hypobaria; 8,000 ft.)	4	4	4	4
4	TBI and Hemorrhage Shock (HS; Hypobaria; 8,000 ft.)	4	4	4	4

Aim 3/ Major Task 2: Swine altitude experiments. This part of the project has not been initiated. A new protocol was submitted to IACUC and was approved. The animals are grouped as shown in the table below. Data will include: neurophysiological parameters (i.e., ICP, CPP and brain oxygenation), hemodynamics (i.e. blood pressure, cardiac index, systemic and pulmonary pressures) blood gas and biochemical (acid/base, inflammatory mediators, serum enzymes) and histology (H&E, Fluorojade). Animals from Aim2 at normobaria or hypobaria (8,000 ft.) will be served as controls for Aim 3.

A revised SOW was approved to change the number of animals per group and animal experiments are continuing.

Group	Treatment	40% from Aim 2 (number of animals)
1	Instrumentation –no injury (Hypobaria; 4,000 ft.)	4
2	TBI-HS – Hypobaria (4,000 ft.)	4

A revised SOW was approved to change the number of animals per group and animal experiments are continuing.

What was accomplished under these goals?

During this reporting period

- Aim #1: Major task 3 has been removed
- Aim #2

- A total of 21 swine experiments with swine for various oxygen supplementation levels were performed; 18 of them generated valuable data, three animals had preexisting medical conditions that precluded successful data collection.

- Aim #3
  - Experimentation at 4000ft is in progress.

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

This project so far provided several one-on-one training activities for employees who work as scientists or research assistants on this project. Through literature search and regular discussion groups within our team we were able to significantly increase their knowledge platform in regards to battlefield care and general and flight physiology. Additionally, 2 medical students from USUHS were trained in research conduct, laboratory techniques and the potential effects of flight on injuries.

How were the results disseminated to communities of interest?

Nothing to report.

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

- Complete protocol closeout and documentation for Aim #1, complete manuscript for rat data.
- Continue animal experiments for Aim# 2 and 3 for all groups in the protocol as outlined in the recently approved revised statement of work.

4. IMPACT:

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

This project will likely have an impact on revisiting current practices in patient transport and aeromedical evacuation, as well as standard operating procedures during aeromedical transport. USAF leadership is currently evaluating results from this and other studies of this laboratory to re-assess aeromedical evacuation practices.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

This project will likely have an impact on revisiting current practices in patient transport and aeromedical evacuation, as well as standard operating procedures during aeromedical transport.

What was the impact on society beyond science and technology?

Nothing to Report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

There have been some issues with swine supply with a number of animals coming into NMRC with structural cardiac and pulmonary issues making them unfit for use on this protocol. A new supplier is being sought. The impacted animals are listed in the following tables with the time of early death and the reason for early death identified at time of necropsy.

Date	Included	Animal #	Group	Death	Reason
07/20/21	NO	55709	54% Inj-H	End	Dilated cardiomyopathy
08/10/21	NO	55795	54% Inj-N	T150	Aortic stenosis
08/18/21	NO	55871	54% Inj-N	T-30	Sudden cardiac arrest pre-injury, PRRSV (+)
09/08/21	NO	55963	54% Inj-H	T150	Cuff leak
09/14/21	NO	56041	100% Inj-N	T30	Possible pneumonia, enlarged mediastinal lymph node
09/16/21	NO	56043	54% Inj-H	T60	Pericarditis, pericardial effusion, pleural adhesions

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

Rapid resolution for the swine supply issue is underway.

Changes that had a significant impact on expenditures

There were no changes that impacted expenditure during this reporting period.

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

Significant changes in use or care of human subjects

N/A

Significant changes in use or care of vertebrate animals

N/A

Significant changes in use of biohazards and/or select agents

N/A

6. PRODUCTS:

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

Nothing to report.

Journal publications.

Nothing to report.

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

Nothing to report.

Other publications, conference papers and presentations.

Nothing to report.

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

Nothing to report.

- **Technologies or techniques**

Nothing to report.

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

Nothing to report.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Personnel	Role	Person month worked
LCDR Carolyn Gosztyla	PI	4
Col Debra Malone	AI	1
Dr. Francoise Arnaud	Scientist: project management	4
Dr. Yaron Dayani	Scientist: data analysis	2
Michael Hammett	Research Assistant: hematology	2
William Porter	Chamber Operator	2
Fang Zhou Yang	Research Assistant: animal data	4
Natalie Coschigano	Research Assistant: animal data	4
Dr. Ye Chen	Scientist: molecular biology	2
Dr. Syndey Dishman	Postdoctoral research resident	3
Dr. William Hennrikus	Postdoctoral research resident	1

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

Nothing to report.

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

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

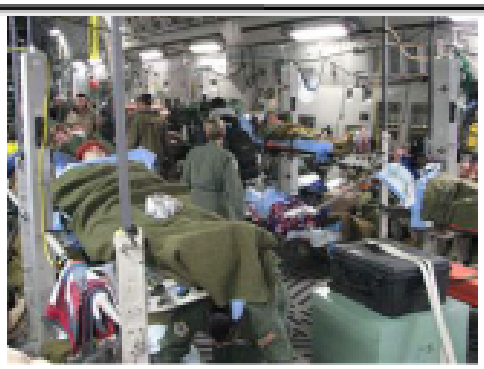
Joint En Route Care Award –Intramural
Log Number DM167040 -Project 1
PI: LCDR Carolyn Gosztyla, Dr. Stephen T. Ahlers **Org:** NMRC/USUHS **Award Amount:** $1,176,000

Study/Product Aim(s)

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

Approach

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



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

Timeline and Cost

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

Updated: 22OCT2021

Goals/Milestones
FY17 Goals
☑ IACUC/ACURO protocol written, submitted and approved
FY18 Goals
☑ Begin rat blast experiments
☑ Complete rat blast experiments
FY19 Goals
☑ Initiate swine experiments
☑ Data analysis rat study
FY20 Goals
☐ IACUC/ACURO protocol written, submitted and approved
FY21 Goals
☐ Finalize rat study
FY22 Goals
☐ Manuscript preparation and Final study report
Comments/Challenges/Issues/Concerns: swine timing aim was removed
Budget Expenditure to Date: $1,176,000

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

Joint En Route Care Award –Intramural

Log Number DM167040 -Project 2

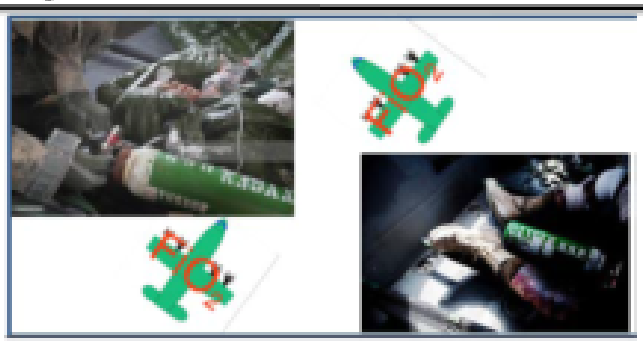
PI: LCDR Carolyn Goszyla **Site-PI:** Dr. Françoise Arnaud, Dr. Richard Mahon **Org:** NMRC/USUHS **Award Amount:** $577,610

Study/Product Aim(s)

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

Approach

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



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

Timeline and Cost

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

Updated: 22OCT2021

Goals/Milestones

FY17 Goals

- ☑ IACUC/ACURO protocol written, submitted and approved
- ☑ Initiate supplemental O2/Aero-Evacuation experiments

FY18 Goals

- ☑ Finalize Normo and Hypo settings
- ☑ Collect physiology and laboratory data

FY19/20 Goal

FY21/22 Goals

- ☐ Complete experiments
- ☐ Data analysis
- ☐ Write report
- ☐ Submit manuscript

Comments/Challenges/Issues/Concerns: Revised animal number

Budget Expenditure to Date: $577,610

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

Joint En Route Care Award –Intramural

Log Number DM187040 -Project 3

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

Study/Product Aim(s)

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

Approach

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

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

Timeline and Cost

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

Updated: 22OCT2021

Goals/Milestones

FY19 Goals

FY20 Goals

☐ IACUC/ACURO protocol written, submitted and approved

FY21 Goals

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

☑ Begin in vivo experiments

☑ **FY21/22Goals**

☐ Complete in vivo experiments (N = 16)

☐ Batched biosample analysis, histopathology

☐ Final database

☐ Statistical Analysis

☐ **FY22 Goal**

☐ Manuscript preparation and submission

Comments/Challenges/Issues/Concerns: Revised animal number

Budget Expenditure to Date: $919,589

9. APPENDICES: