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

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CONTRACTING ORGANIZATION: Naval Medical Research Center, 503 Robert Grant Avenue, Silver Spring, MD 20910

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14. ABSTRACT

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

15. SUBJECT TERMS

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

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

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:** *Provide a brief list of keywords (limit to 20 words).*

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

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

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

*Revised as of 10/01/2020 = Y5Q1 = Month 49

	Timeline	Method	Status
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	
Major Task 4: Data analysis/manuscript/final report	68-74	Statistics/ writing	

	Timeline	Method	Status
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	
Specific Aim 3: Physiological consequences of 4,000 and 8,000 ft. altitude aeromedical evacuation on swine with traumatic brain injury and hemorrhagic shock			
Major Task 1: IACUC/ACURO approval	45-55	Writing	Ongoing
Major Task 2: Swine AE/altitude experiments	55-68	Animal experiment	
Major Task 3: Data analysis/manuscript/final report	68-74	Statistics/ writing	

* Revised timeline is pending approval of no-cost-extension at the end of period of performance.

Aim1/ Major Task 3: Swine TBI/polytrauma AE timing experiments. No animals were used on this task. A new protocol has been drafted based on preliminary results from the rat study, which includes the following groups of animals. Data from these animals will include: all hemodynamics data (blood pressure, heart rate), blood analysis (electrolytes, blood gases and blood cell count), organ function (Enzymes), inflammation markers and organ histopathology (H&E, marker of ischemic lesions and cell infiltrates).

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

Aim2/ Major Task 2: Swine supplemental O_2/AE experiments. Twenty animals were used on this study (protocol 18-OUMD-24LS), last one on July/2019. Nonetheless the data obtained were variable and not deemed considered quality data. Thus, a new protocol was submitted and approved by IACUC (20-OUMD-28LS). Animals and groups that will be used in this study are as follows. Data from these animals include: neurophysiological parameters (i.e. ICP, CPP, and brain oxygenation), hemodynamics (i.e. blood pressure, cardiac index, systemic and pulmonary pressures) blood gas (oxygen transport variables), biochemical (acid/base, inflammatory mediators, serum enzymes) and histology (H&E, Fluoro Jade).

Group	Treatment	40% (number of animals)	54% (number of animals)	74% (number of animals)	100% (number of animals)
1	Instrumentation –no injury (Normobaria)	8	8	8	8
2	TBI and Hemorrhage Shock (HS; Normobaria)	8	8	8	8
3	Instrumentation –no injury (Hypobaria; 8,000 ft.)	8	8	8	8
4	TBI and Hemorrhage Shock (HS; Hypobaria; 8,000 ft.)	8	8	8	8

Aim3/ Major Task 2: Swine altitude experiments. This part of the project has not been initiated yet and no animals were used. A new protocol was submitted and approved. The animals are grouped as follows. 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, Fluoro Jade).

Group	Treatment	40% (30%) or best rate, based on results from Aim 2 (number of animals)
1	Instrumentation –no injury (Hypobaria; 4,000 ft.)	8
2	TBI-HS – Hypobaria (4,000 ft.)	8

*Animals from Aim2 at normobaria or hypobaria (8,000 ft.) will be served as controls for Aim3.

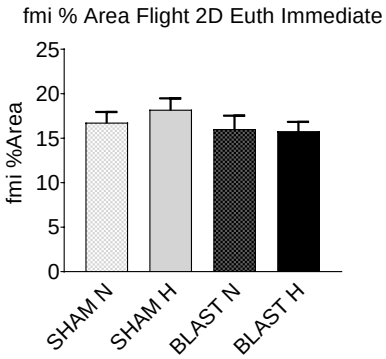
What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Specific Aim 1: Evaluation of the timing of aeromedical evacuation in rat (Rattus norvegicus) and swine (Sus scrofa domestica) models of TBI and polytrauma:

We are currently working on the first experimental phase where we examine the timing of aeromedical evacuation in rat animal models. We have completed all rat animal work and are planning the swine study based on further analysis from the first part.

In the rat study, we exposed rats to blast overpressure (3 consecutive blasts at 110kPa on Day 1) and then delayed the simulated 6-hour flight (altitude at 8000ft cabin pressure) for 2, 7 or 14 days post blast in order to assess any changes on motor function and physiology after both blast and flight. The euthanasia time point for all groups has been either eight days or immediately after flight, and we have been collecting brain, heart, lung, liver, intestine and kidney tissues for molecular analyses as well as plasma from blood for cytokine expression and CBC. Tissues were processed and stained for histopathological analysis and brain injury. At this time, we have completed all animal work and analyzed histopathological and behavioral data and brain injury for all groups. Contrary to our hypotheses, we have not found any significant injury or deficits in motor behavior among the animals exposed to blast and hypobaric flight conditions. Injury scores and behavioral performance on the Rotarod test were consistent across all groups. In addition, we have analyzed cytokine expression from plasma from several cohorts. These samples were processed with a commercial Luminex (Multiplex) kit that probed for a panel of various cytokines/chemokines. Although there was high variability among the cytokine results, we have seen that the effect of blast and hypobaria last for days after the exposure. In addition, immunohistochemistry analysis of the brain using GFAP marker showed no remarkable injury between the different groups (Fig. 1-4). We are currently waiting for additional immunohistochemistry, cell death analysis and additional histopathology data to further determine the effect of blast and delayed flight on organ damage.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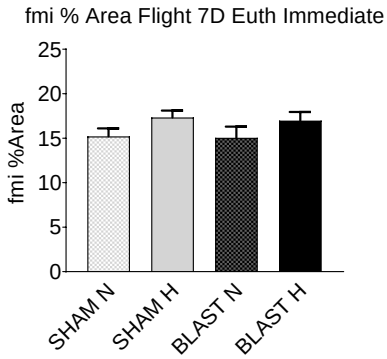
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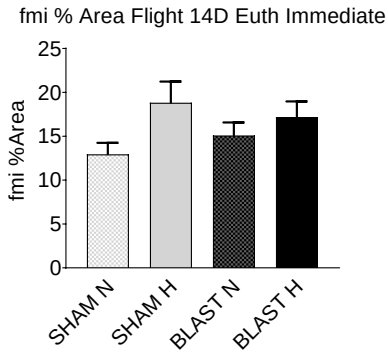
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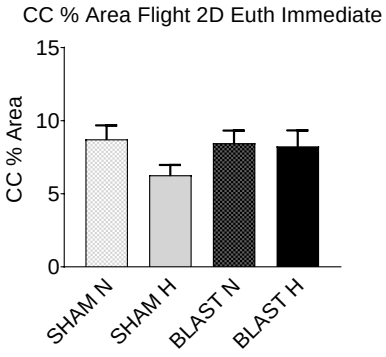
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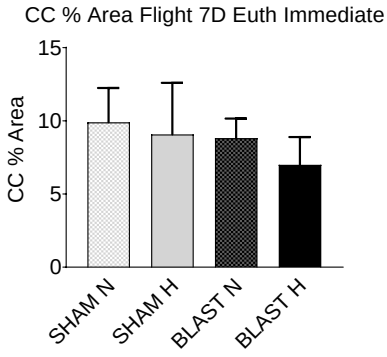
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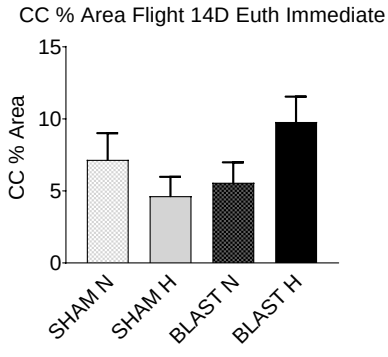
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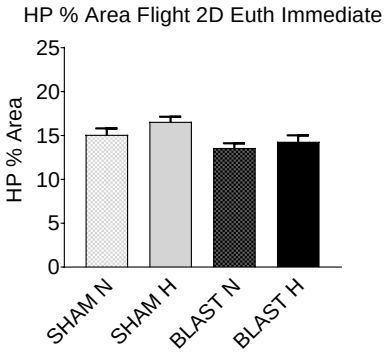
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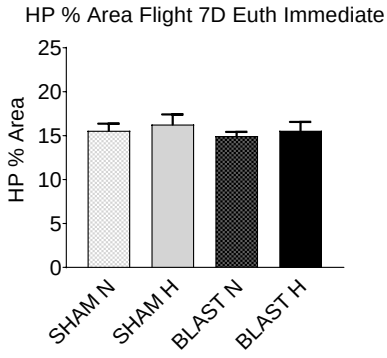
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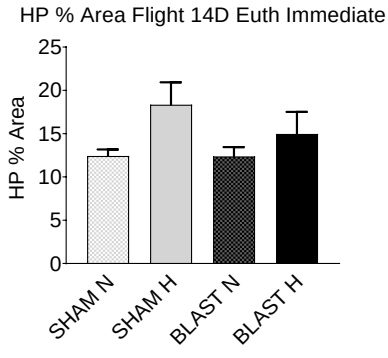
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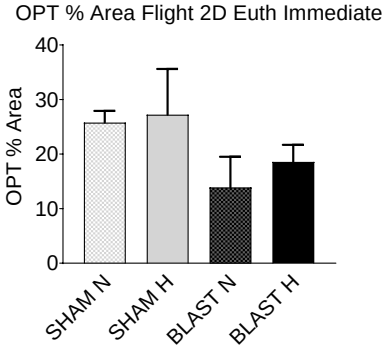
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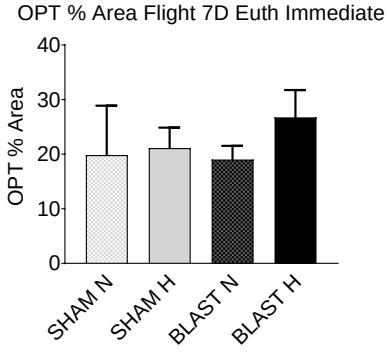
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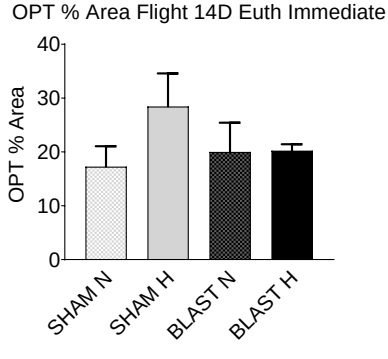
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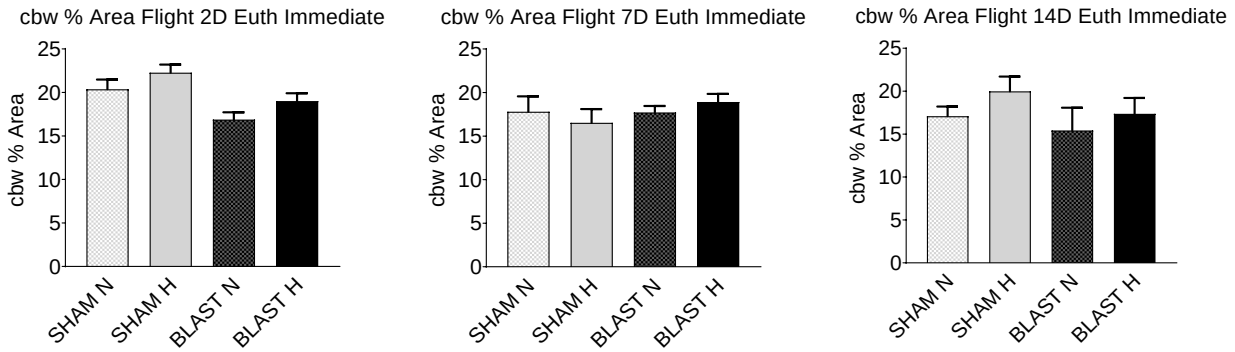
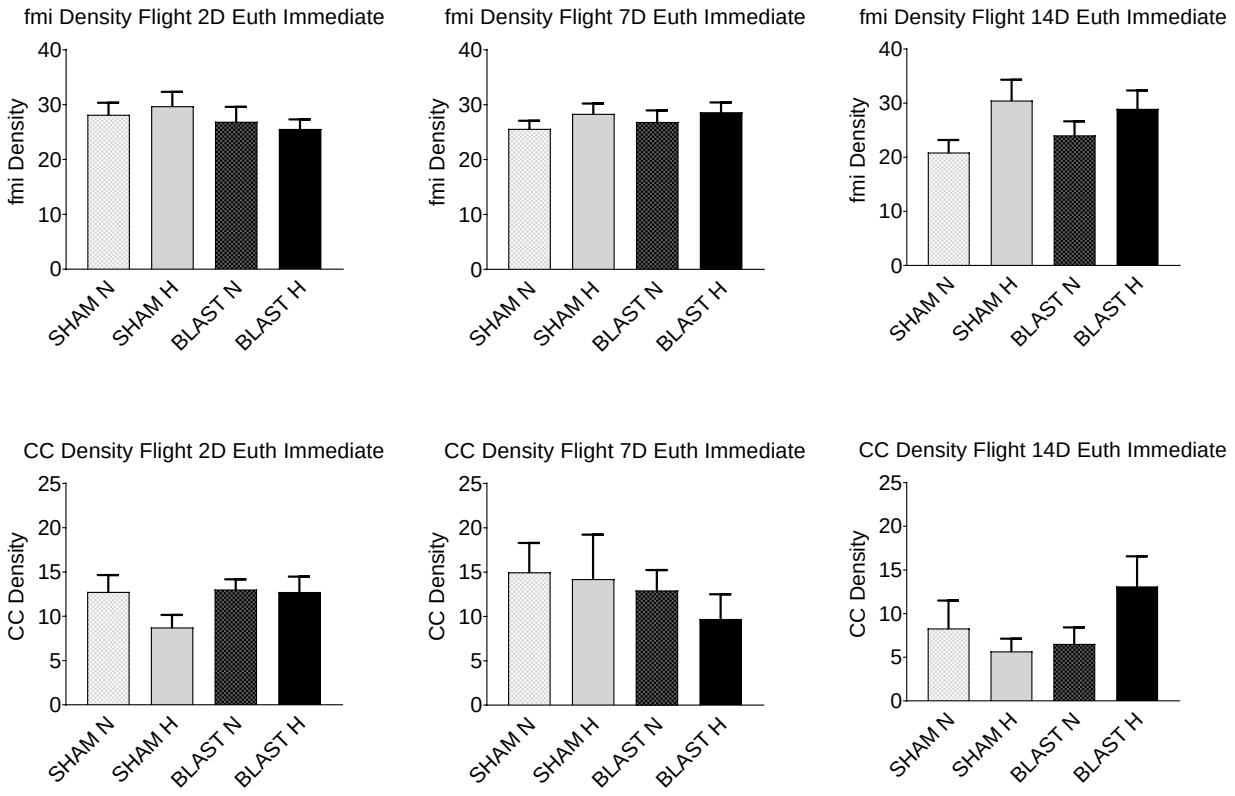


Figure 1: GFAP staining in the cohorts euthanized immediately after flight (percentage). Anesthetized animals were exposed to polytrauma injury on day 1 (BLAST) or not (SHAM) followed by flight simulation (H) or sea level control (N) two, seven or fourteen days after (2D, 7D and 14D, respectively). All animals were euthanized immediately after the flight (Euth Immediate). Data are shown as percentage of the area that exhibited labeling in the forceps minor (fmi), corpus callosum (CC), hippocampus (HP), optic tract (OPT) and at the cerebellum white matter (cbw). Error bars, standard error of the mean.



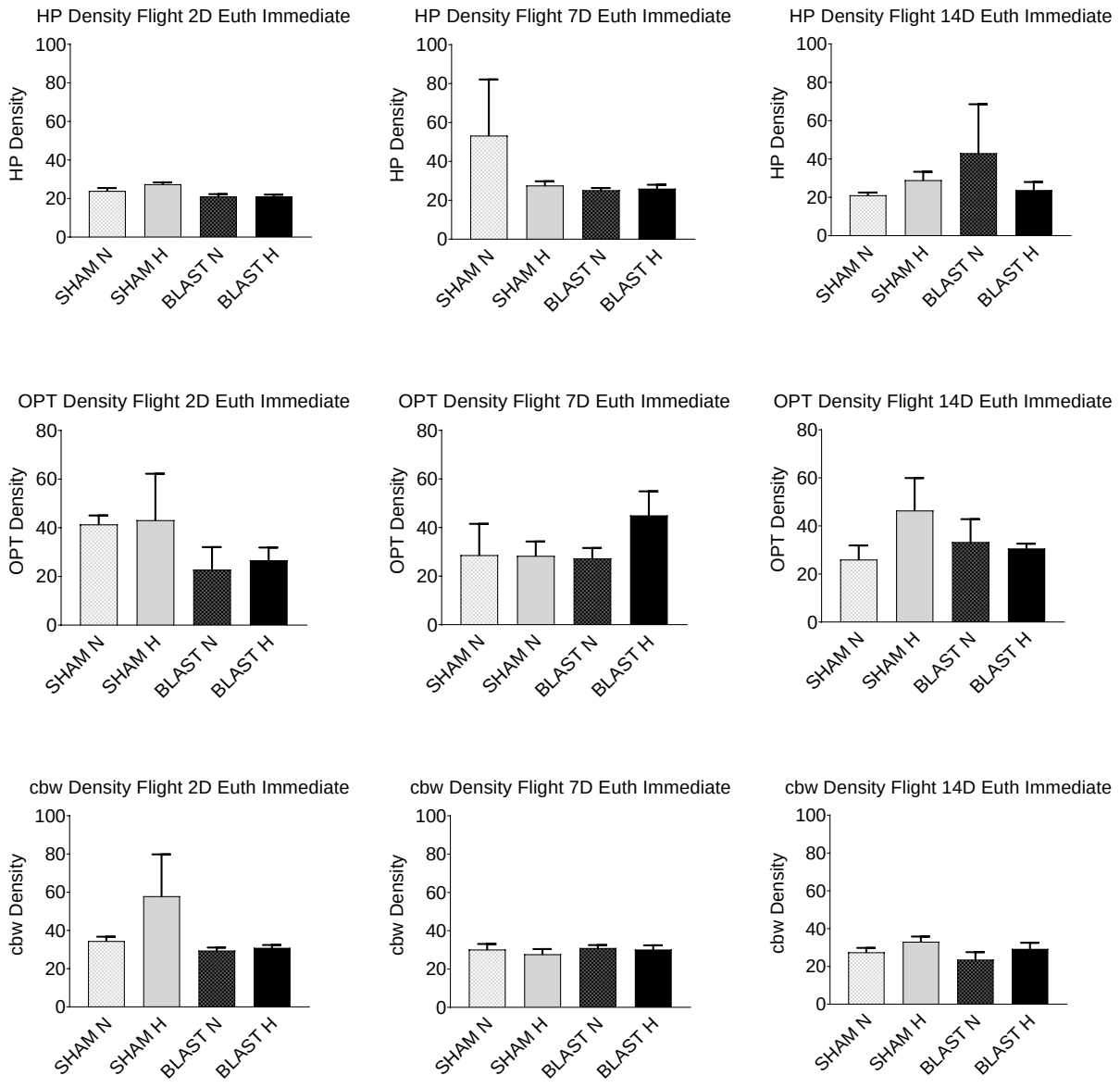
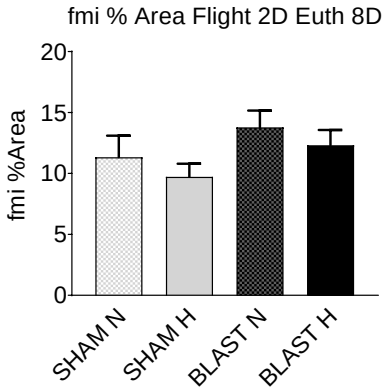


Figure 2: GFAP staining in the cohorts euthanized immediately after flight (density). Data from Figure 1 are shown as staining density. Error bars, standard error of the mean.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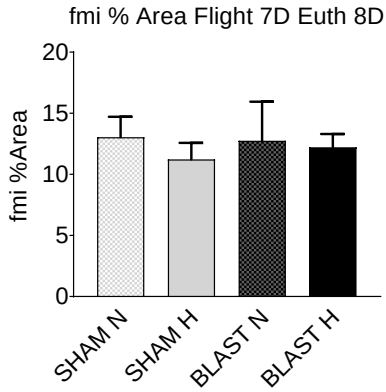
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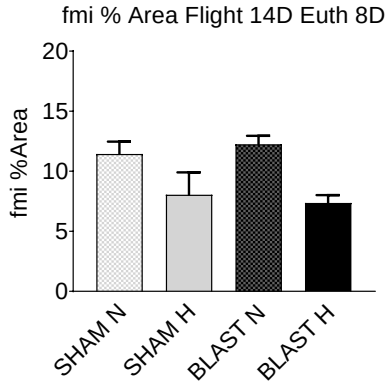
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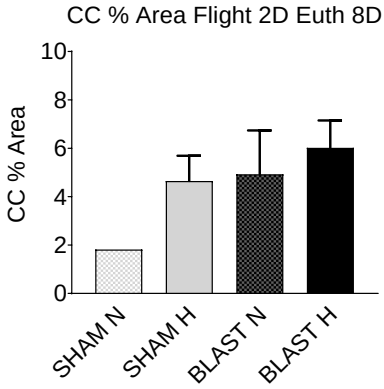
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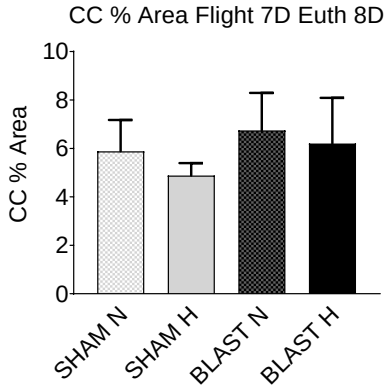
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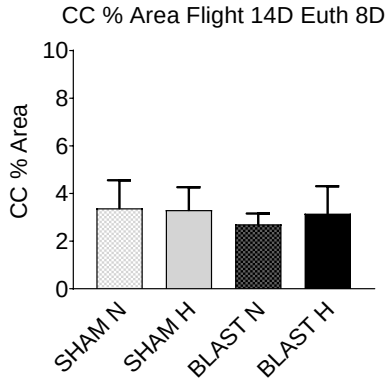
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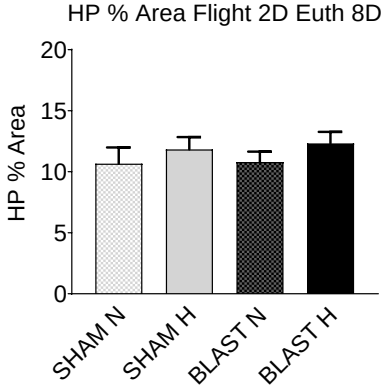
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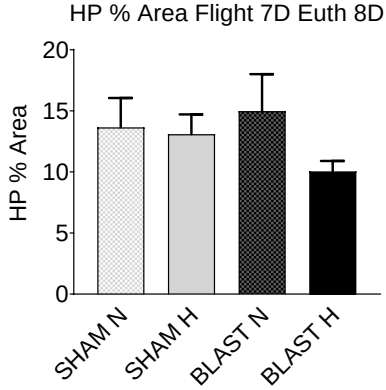
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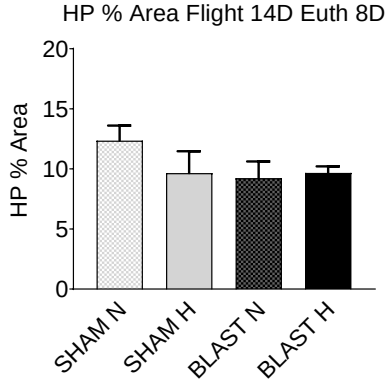
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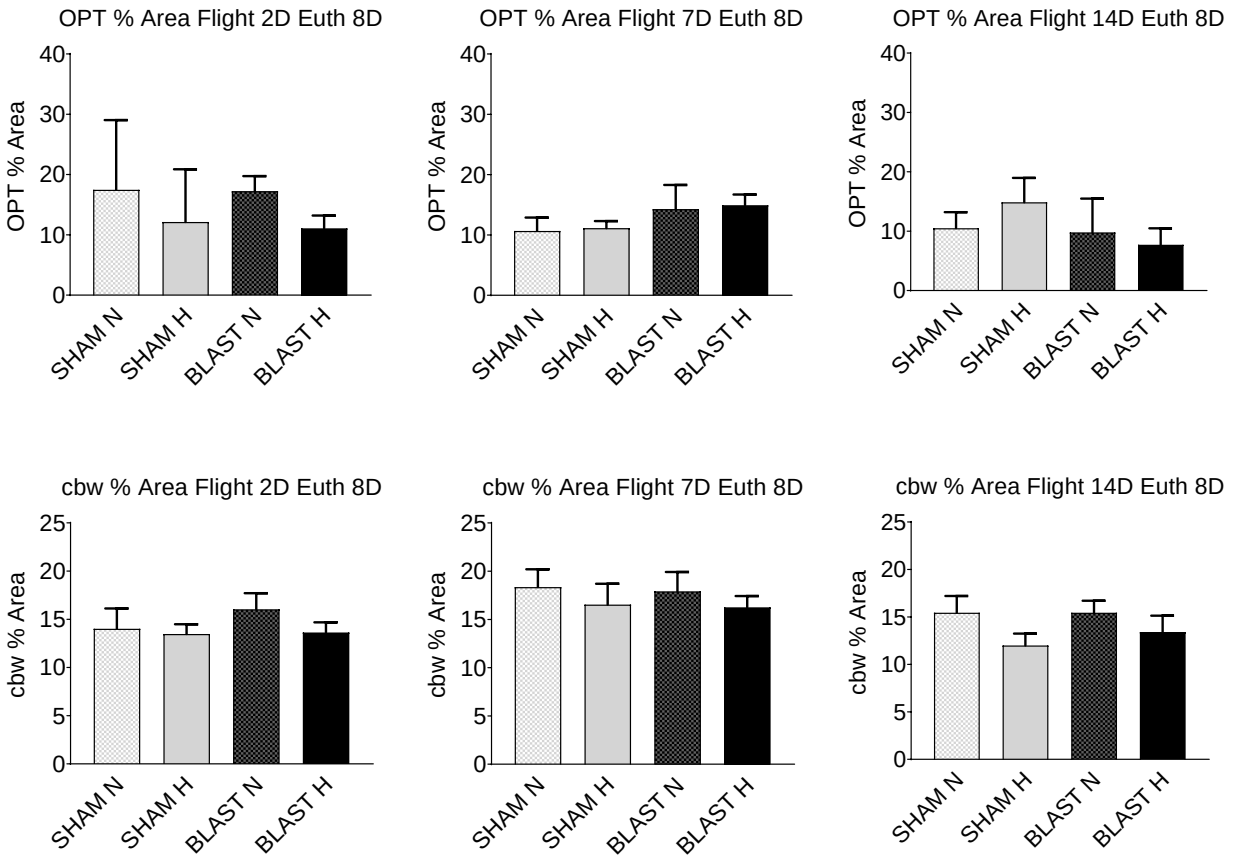
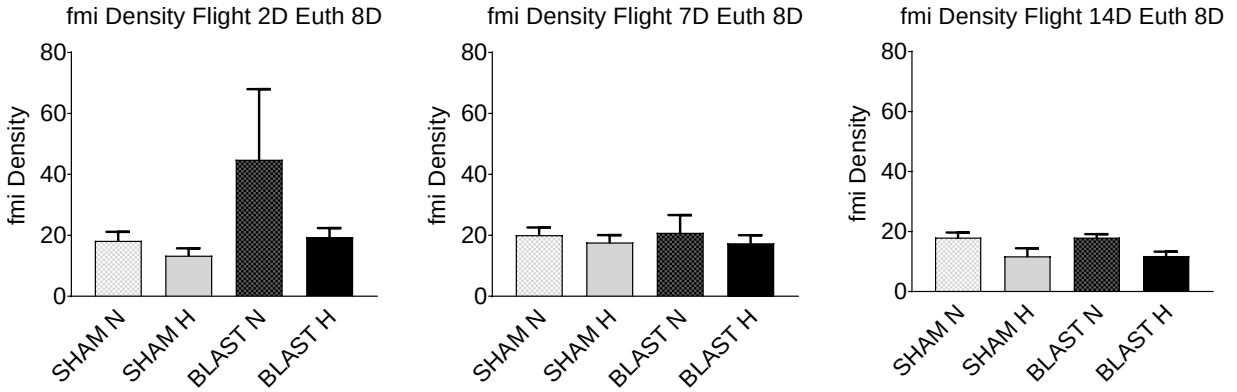
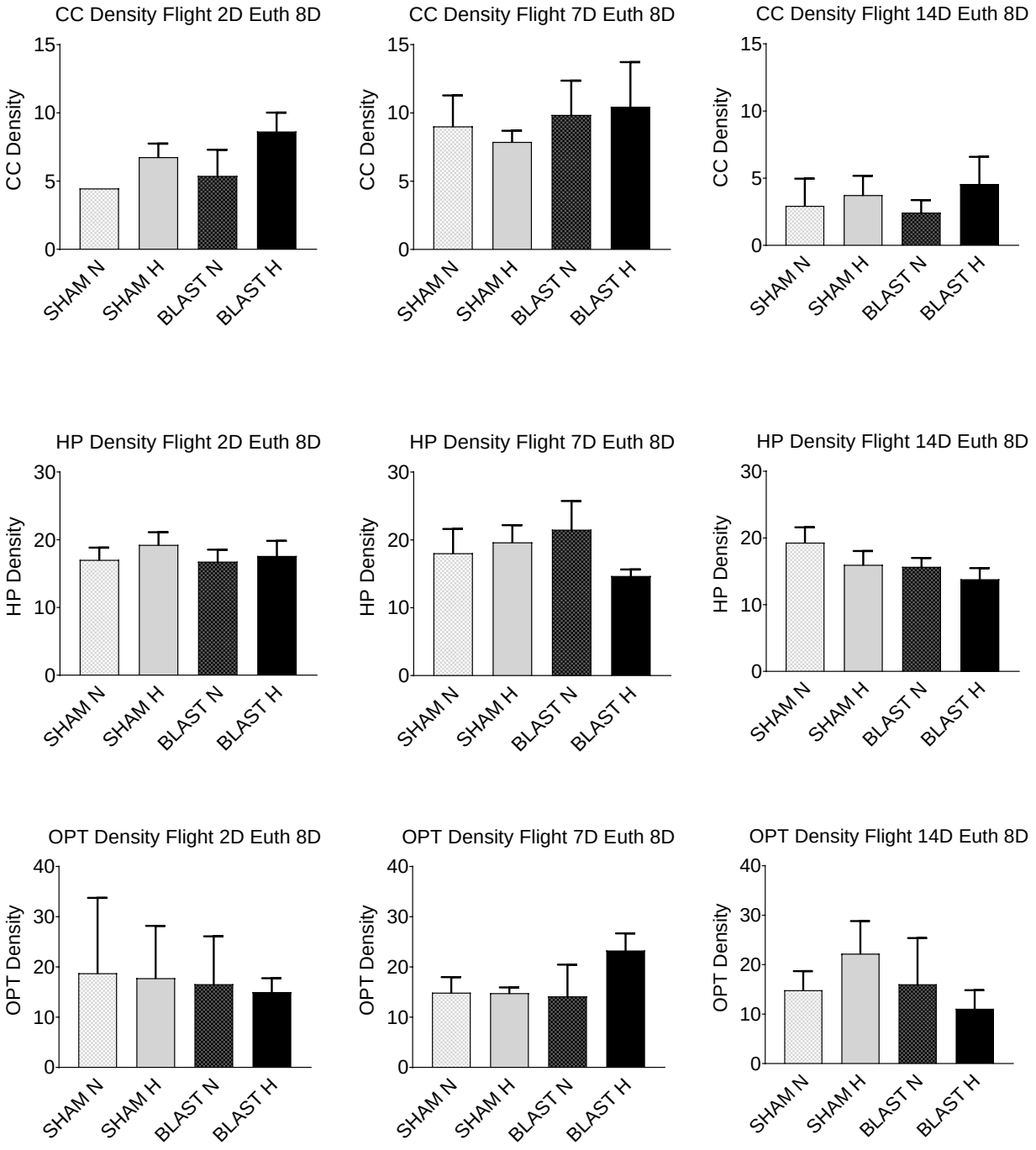


Figure 3: GFAP staining in the cohorts euthanized eight days after flight (percentage). Anesthetized animals were exposed to polytrauma injury on day 1 (BLAST) or not (SHAM) followed by flight simulation (H) or sea level control (N) two, seven or fourteen days after (2D, 7D and 14D, respectively). All animals were euthanized eight days ((Euth 8D) after the flight. Data are shown as percentage of the area that exhibited labeling in the forceps minor (fmi), corpus callosum (CC), hippocampus (HP), optic tract (OPT) and at the cerebellum white matter (cbw). Error bars, standard error of the mean.

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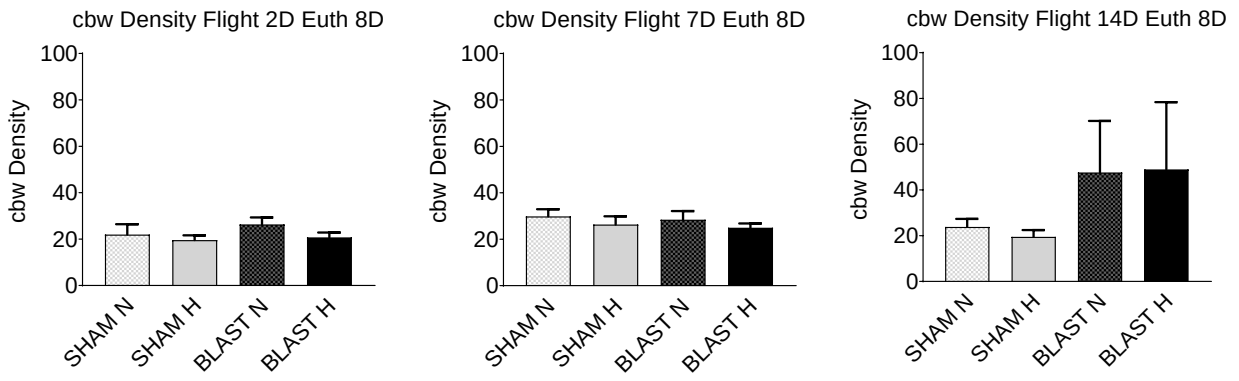

Figure 4: GFAP staining in the cohorts euthanized eight days after flight (density). Data from Figure 3 are shown as staining density. Error bars, standard error of the mean.

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):

Twenty animals were used to set up the system and tune the correct setting and parameters. Since the animals are under full anesthesia and ventilation, it was important to investigate which ventilation works best for the study (e.g. spontaneous breathing, volume assisted or pressure assisted). In addition, adjusted ventilation parameters such as PEEP were examined. Furthermore, arterial and venous blood samples were collected from these animals at different time points and conditions and the oxygenation parameters (oxygen delivery consumption, extraction) were analyzed and compared. The last animal experiment for this work was performed on July 2019. Data continued to be analyzed until June 2020. Briefly, these experiments consisted of swine under anesthesia that were subjected to traumatic brain injury (TBI) and a 30% estimated blood volume hemorrhage (HS). Following resuscitation and stabilization, animals underwent a simulated aeromedical evacuation in a hypobaric chamber at 0 versus 8000 ft. for 4 hours. Measurements of the FiO_2 in the chamber were performed from the pre-mixed flow of air and O_2 that entered the ventilator (VersaMed) and the outgoing air mixture that was delivered under a set flow and pressure towards the animal tube. FiO_2 levels ranged from 21% to 100%.

Figure 5 shows delivery of FiO_2 as measured by the ventilator and in the chamber. Level of FiO_2 was similar in both cases, validating our system.

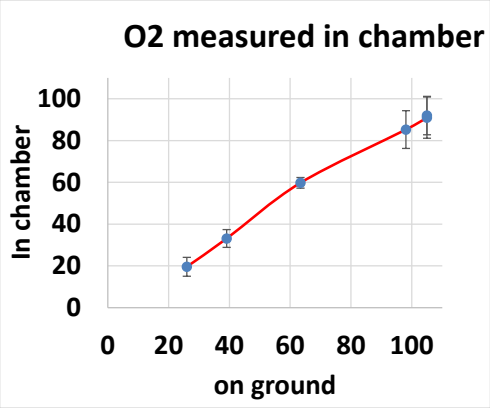

Figure 5: correlation between air mixtures from the ventilator to that measured towards the animals.

Blood samples were collected from different time points and arterial and venous partial pressures of oxygen (PaO_2 and PvO_2, respectively), oxygen delivery (DO_2) or extraction ($ExtO_2$), were calculated (Fig 6). Also, these parameters were also associated with some hemodynamic parameters such as heart rate (HR, Fig. 7).

Figure 6: O2 content and O2 delivery at various FiO2.

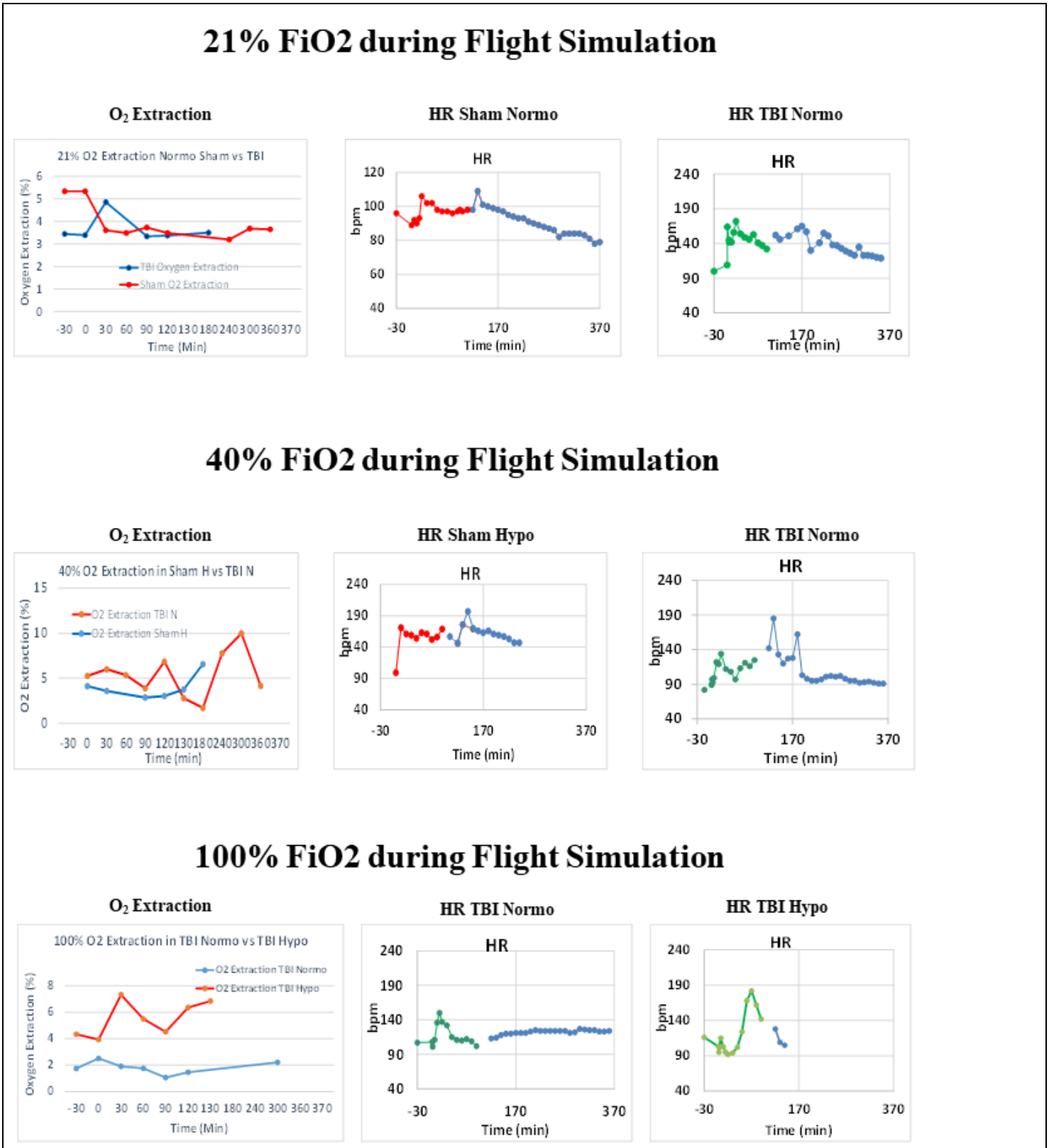

Figure 7: O_2 extraction and hemodynamics at normobaria (Normo) and hypobaria (Hypo)

Not all conditions were fulfilled due to low number of animals to meet the requirements of all combinations of injury and altitude, (i.e. hypo vs normo vs Sham vs TBI). Twenty animals were used in protocol 18-OUMD-24LS (last animal was used on July 2019 and data were analyzed until June 2020).
The oxygen delivered (Oxygen delivery) to the Sham and TBI-injured animals was utilized similarly (oxygen extraction) by the animals whether the experiment was performed on ground and at 8000 ft. Oxygen content, delivery and extraction were equivalent in these TBI animals. The amount of oxygen provided seems largely sufficient. The hemodynamics are not affected with comparable patterns during the 4 hour of evacuation.
In conclusion, this large animal model to test the supplementation of oxygen during aero-evacuation was found to be a valid model. Observations for aeromedical evacuation (AE) are not sufficient at this time to substantiate the effects of oxygen supplementation during aeromedical transport. Experiments are still in progress to determine the optimal level of oxygen under hypobaria.
Specific Aim 3: Physiological consequences of 4,000 and 8,000 ft. altitude aeromedical evacuation on swine with traumatic brain injury and hemorrhagic shock

The IACUC protocol was submitted on June 30 and was approved on November 24 2020. Protocol was submitted to ACURO for approval.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

This project so far provided several one-on-one training activities for new employees who will work as junior 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 third year medical students from USUHS and 4 High School summer students were trained in research conduct, laboratory techniques and the potential effects of flight on injuries.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

Specific Aim 1: Evaluation of the timing of aeromedical evacuation in rat and swine models of TBI and polytrauma:
We plan to continue data analysis until the next reporting period. We will conduct several molecular assays on tissues and blood in addition to reviewing histological data in order to determine the physiological effects of the various flight times post blast TBI.

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):
Upon authorization to perform large animal experiments, we are anticipated to resume normobaric and hypobaric experiments for sham and TBI injured animals.

IMPACT: D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."
Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

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?
If there is nothing significant to report during this reporting period, state "Nothing to Report."
Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report.

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Animal studies were delayed due to the reconstruction and relocation of the hypobaric chamber and site closure due to COVID-19 pandemic restrictions. A no cost extension will be requested to complete the study. Change of PI to LCDR Carolyn Gosztyla.

Specific Aim 1: Evaluation of the timing of aeromedical evacuation in rat and swine models of TBI and polytrauma	
Specific Aim 1: Evaluation of the timing of aeromedical evacuation in rat and swine models of TBI and polytrauma	
Major Task 3: Swine TBI/polytrauma AE timing experiments	Not started
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 2: Swine supplemental O_2 /AE experiments	Initiated with pilot animals
Specific Aim 3: Physiological consequences of 4,000 and 8,000 ft. altitude aeromedical evacuation on swine with traumatic brain injury and hemorrhagic shock	
Major Task 1: IACUC/ACURO approval	Approved by IACUC
Major Task 2: Swine AE/altitude experiments	Not Started

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

The relocation of the hypobaric chamber and lately the impact of COVID-19 on the management of animal experimentation delayed the scheduling of our work for about one year. We are currently working closely with the veterinary staff to resume animal work. Completion of the oxygen supplementation arm of the study will be attempted first, assuming that surgical activities will resume as early as next year in accordance with the veterinary support program (VSP). Therefore, it may take a year to complete the experiments with 64 swine at a rate of one swine per week. For the timing experiment, the earliest possible start of these experiments may not be before June 2021 and may also require a year to be completed. The entire project may be completed by the end of 2022.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

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
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

N/A

Significant changes in use or care of vertebrate animals

A new protocol was drafted for the timing arm of the study.

Significant changes in use of biohazards and/or select agents

N/A

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

Nothing to report.

Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report.

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report.

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

Nothing to report.

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

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report.

- **Technologies or techniques**

  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report.

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

  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report.

- **Other Products**

  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change"

Personnel	Role	Person month worked
LCDR Carolyn Gosztyla	New- PI	2
Dr. Anke Scultetus	Old -PI	1
Col Debra Malone	AI	1
Noemy Carballo	Senior Research Assistant: animal	4
Dr. Francoise Arnaud	Scientist: project management	4
Dr. Yaron Dayani	Scientist: data analysis	2
Jordan Hubbell	Research Assistant: animal, data	4
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

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

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. However, our lab showed that hypobaria reduces brain tissue oxygenation in TBI swine. This study will evaluate the effects 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							
Data analysis, final report, manuscript preparation							
Estimated Budget ($K)							

Updated: 23NOV2020

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
☐ Continue swine experiments
FY22 Goals
☐ Complete swine experiments
☐ Data analysis swine study
☐ Manuscript preparation and Final study report
Comments/Challenges/Issues/Concerns: None
Budget Expenditure to Date: $800k

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 Gosztyla **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 aero-medical 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 (FiO2of 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: 23NOV2020

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:

Budget Expenditure to Date: $510k

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 DM167040 -Project 3

PI: LCDR Carolyn Gosztyla **Site-PI:** Col Debra Malone, MC, USAF, Dr. Françoise Arnaud **Org:** NMRC/USUHS Award Amount: $577,610

Study/Product Aim(s)

- 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).
- Determine if the type and severity of the injury (TBI or TBI + HS) is affected by altitude.

Approach

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

US Navy combat nurse Lt. Cdr. Eric Gryntends to a critically injured civilian en route to hospital. The hypobaric chamber at the Center for Hypobaric Experimentation, Simulation and Testing (CHEST) will simulate such transport in swine. It has been successfully used for two swine studies in 2016.

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: 23NOV2020

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-life experiments (N = 72)

FY21/22Goals

☐ Complete In-life experiments (N = 72)

☐ Batched biosamples analyzed and Histopathology

☐ Final database (Cleaned and locked)

☐ Statistical Analysis

☐ **FY22 Goal**

☐ Manuscript preparation and submission

Comments/Challenges/Issues/Concerns:

Budget Expenditure to Date: $20k

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*