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The Effect of a Hypobaric, Hypoxic Environment on Acute Skeletal Muscle Edema after Ischemia-Reperfusion Injury in Rats

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Background. **Clinicians have postulated that decreased atmospheric pressure during air evacuation exacerbates muscle edema and necrosis in injured limbs. The present study investigated whether the mild hypobaric, hypoxic conditions of simulated flight during muscle reperfusion worsened muscle edema and muscle injury in an established animal model.**

Methods. **Twenty male Sprague-Dawley rats underwent tourniquet-induced hind limb ischemia for 2 h. After removal of the tourniquet, rats were divided into two groups ($n = 10$/group), and exposed to either (1) hypobaric, hypoxic conditions (HB) of 522 mm Hg (simulating 10,000 feet, the upper limit of normal aircraft cabin pressure), or (2) normobaric, normoxic conditions (NB) of 760 mm Hg (sea level), for 6 h. Muscle wet weight, muscle dry:wet weight ratios, viability, and routine histology were measured on the gastrocnemius and tibialis anterior muscles. Blood samples were analyzed for percentage hematocrit, leukocyte count, and coagulation status.**

Results. **Ischemia resulted in significant edema in both groups ($P < 0.05$). Normobaric normoxia caused greater edema in the gastrocnemius compared with hypobaric hypoxia; the tibialis anterior was not significantly different between groups. The decrease in body weight for NB and HB was 3.4 ± 1.4 and 10.7 ± 1.2 g, respectively ($P < 0.05$). Hematocrit was 44.7 ± 0.5 and 42.6 ± 0.6 ($P < 0.05$).**

Conclusions. **The hypobaric, hypoxic conditions of simulated medical air evacuation were not associated with increased muscle edema following 2 h of ischemic injury. This suggests that other factors, such as resuscitation, may be the cause of muscle edema in flight-evacuated patients.** Published by Elsevier Inc.

Key Words: **compartment syndrome; muscle injury; altitude; flight; air evacuation; military; extremity trauma.**

INTRODUCTION

Similar to injuries in previous wars, most injuries from the conflicts in Afghanistan and Iraq have been to the extremities, [1–3] resulting primarily from explosions [4]. Explosions can cause fractures, tissue loss, vascular injury, and burns, all of which place extremities at risk for compartment syndrome. A recent retrospective study at Landstuhl Regional Medical Center demonstrated that fasciotomy for extremity compartment syndrome after air evacuation out of the combat theater was associated with increased rates of muscle loss and major amputation [5]. This has led some clinicians to postulate that decreased atmospheric pressure during flight may exacerbate muscle edema in the injured limb, in turn contributing to of the development of compartment syndrome. Although there is only anecdotal evidence to support this contention, it has become of great concern for military medical operations [6].

Our primary objective in this study was to determine the effect of simulated flight on muscle edema in an established model of ischemia-reperfusion injury [7–10]. Additionally, because there have been anecdotal reports that hypoxic conditions of medical air transport may exacerbate muscle injury [11], we examined indices of muscle injury, including viability measurements and routine histology. A major source of damage in ischemia-reperfusion injury is oxidative stress caused by the initial oxidative burst on reestablishment of blood flow [12, 13], with additional oxidative damage

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caused by polymorphonuclear leukocytes (PMNs) as the inflammatory response develops [14]. We therefore also assessed muscle myeloperoxidase (MPO) activity as an index of oxidative stress and PMN infiltration into muscle. Further, we measured alterations in coagulation parameters as air travel has been associated with a hypercoaguable state in healthy volunteers with risk factors, such as use of oral contraceptives, factor V Leiden mutation [15], advanced age, or high body mass index [16], and may pose a similar risk to trauma patients.

METHODS

Animal Care

All animal protocols were approved by the U.S. Army Institute of Surgical Research Animal Care and Use Committee. This study ad hered to National Institutes of Health guidelines for the care and use of laboratory animals (DHHS Publication, NIH, 86 23). Adult male Sprague Dawley rats weighing 403 ± 15 g were obtained from colonies of Harlan Sprague Dawley, Inc. (Indianapolis, IN). Animals were housed in an Association for the Assessment and Accreditation of Laboratory Animal Care approved facility. They were provided with food and water *ad libitum* prior to anesthesia induction and tour niquet application. Animals were habituated to the chamber environ ment and pump noise for 30 min daily for a total of 5 d prior to experimentation.

Experimental Procedures and Monitoring

Rats (n 20) were weighed and anesthetized using 1.5% to 2.5% isoflurane anesthesia, adjusted to maintain a surgical level. Both hind limbs were shaved and animals were instrumented with a lubri cated rectal temperature probe (Physitemp Instruments, Inc., Clif ton, NJ) inserted 5 cm beyond the rectal sphincter. Animals were then placed supine on a warm water flow temperature regulated bed (EX 212; Euthanex Corp., Palmer, PA) and core temperature was maintained at 37 ± 1°C. Blood drainage of the experimental leg was performed by elevation above the level of the heart for 5 min prior to tourniquet inflation. A pneumatic tourniquet was then applied to the proximal aspect of the elevated hind limb and in flated to a pressure of 250 mm Hg. All procedures have been detailed previously [9, 10, 17]. The tourniquets were left in place for 120 min. After 90 min of tourniquet application, an intraperitoneal injection of normal saline (30 cc/Kg body wt) was administered. Saline was administered to all animals to control for extensive fluid loss ob served during the 6 h of simulated flight. The volume was based on the previously observed loss of body weight in unresuscitated rats after simulated flights in pilot studies. Rats were assigned to ei ther the normobaric (NB) or hypobaric (HB) group. Experiments were carried out on two rats per day, alternating between NB and HB.

Flight Simulation

After 10 min of recovery from anesthesia, rats were placed in indi vidual cages (7 in l × 5 in w × 5.5 in h), that were in turn placed in in dividual clear cylindrical acrylic vacuum chambers (12 in dia × 18 in height) (model LVC1218 1121 VC, LACO Technologies, Inc., Salt Lake City, UT) pressurized to simulate an altitude of 10,000 feet (522 mm Hg) or sea level (760 mm Hg), using a custom built vacuum system composed of 2 vacuum pumps (23 series, model 1023 V103, Gast Manufacturing Inc., Benton Harbor, MI) and controlled by a Honeywell controller (model UDC3300; Honeywell, Morristown, NJ) to maintain constant pressure. The chambers allowed a continu ous in flow of room air while the pump maintained the necessary pres sure differential inside the chamber. Both groups started at the ambient barometric pressure of the facility (746 750 mm Hg). The HB rats (n 10, experimental group) reached the target pressure in a period of 30 min, remained at 522 mm Hg for 5 h, then were re pres surized in 30 min, for a total chamber time of 6 h. The NB rats (n 10, control group) were actively pressurized to 760 mm Hg (sea level) in 1 min. The control rats remained at 760 mm Hg for 6 h. These condi tions were designed to simulate air evacuation flights from Iraq to Landstuhl Regional Medical Center in Germany, which lasts from 5 to 7 hours. During these flights, cabin pressure is maintained at an average of 564 mm Hg to 522 mm Hg (the equivalent of 8000 10,000 feet above sea level). Temperature in the room as well as in each chamber was monitored and adjusted to maintain the tempera ture within the thermoneutral range for rats (28 32°C) [18]. While in the cage, rats were allowed to move freely.

Tissue Harvesting

Immediately following simulated flight, rats were removed from the chambers and anesthetized as described previously. Blood sam ples were drawn via cardiac puncture for percent hematocrit (Hct), leukocyte count, and measures of coagulation status [prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen levels, and thrombelastography (TEG)]. Rats were then euthanized by intracardiac injection of euthanasia solution (1.0 mL injection (i.p.) of Fatal Plus). The muscles were excised, trimmed of connective tissue, blotted dry with filter paper, and weighed. Whole muscle weights were obtained on a microbalance (MT 5; Mettler Toledo, Inc., Columbus, OH). The medial gastrocnemius (Gast) and the ti bialis anterior (TA) were divided into 3 cross sectional portions using a custom designed tissue slicer and weighed. The proximal portion was frozen in liquid N_2, the medial portion (4 mm thick) was used for vital staining, and the distal portion of muscle, containing the muscle belly, was pinned at resting length to a tongue depressor, and fixed for 24 h in 10% buffered formalin solution (Fisher Scien tific, Pittsburgh, PA).

Vital Staining

Assessment of muscle viability was determined using 2,3,5 triphe nyltetrazolium chloride (TTC). This method is based on the reduc tion of TTC to water insoluble red formazan by viable mitochondria. Viable myocytes stain red while nonviable myocytes remain unstained. The 4 mm thick medial section was obtained from the Gast and TA muscles and incubated for 1 h according to the method of Belkin *et al.* [19]. The reaction was stopped by placing samples in ice cold PBS, followed by fixation in buffered formalin. TTC staining was assessed using image analysis. Images were ob tained using a Zeiss stereomicroscope (Stemi SV11; Zeiss, Thorn wood, NY) equipped with a Nikon CCD camera (DS 5 M; Nikon, Melville, NY). Images of muscles were digitally isolated and gray scaled. The average gray scale value was determined using Adobe Photoshop, version 7.0 (Adobe Systems Inc., San Jose CA). Tourni quet and contralateral control muscle were captured in a single frame, and the viability index was expressed as the ratio of the av erage gray scale value for the ischemic muscle to untreated contra lateral muscle.

Dry Weight and Wet Weight Determination

Dry weight was determined on the Gast and TA muscles and the right distal lobe of the lung. Tissues were desiccated for 5 d in a drying oven set at 50 °C prior to determination of dry weight. The same por tion of muscle used for TTC was also used for wet to dry weight

determination. Wet to dry weight ratios were determined as previously described [7].

Histology

After fixation and paraffin embedding, muscles underwent cross sectioning and hematoxylin and eosin (H and E) staining. Ischemic and contralateral muscles were graded for edema, degeneration/necrosis, and inflammation by a veterinary pathologist who was blinded to muscle and treatment. Grading was on a 5 point scale (0 to 4), with 0 representing no apparent pathology and 4 signifying extreme pathology.

Complete Blood Count and Coagulation Assays

Prior to euthanization, blood samples were drawn by cardiac puncture for hematocrit, complete blood count (CBC), and coagulation status [(PT, aPTT, fibrinogen, and thrombelastography (TEG)]. The CBC was performed using an ABX Pentra 120 CBC Analyzer (Montpelier, France). PT, aPTT, and fibrinogen were measured using a BCS Coagulation Analyzer (Dade Behring, Deerfield, IL) according to the manufacturer's protocols. TEG was performed on whole blood using the Haemoscope 5000 thrombelastograph (Haemoscope, Niles, IL). The TEG was started and then stopped 30 min after reaching maximal amplitude (MA). TEGs were run in duplicate or triplicate. The following TEG parameters were measured: (1) R time, the time until the onset of clotting, (2) K time, measured from R time to a fixed level of clot firmness, (3) α angle, the rate of clot formation in degrees, and (4) MA, the maximum amplitude or the maximal strength of the clot.

Tissue Antioxidant Status

Tissues were homogenized in 50 mM potassium phosphate buffer pH 7.4. Thiobarbituric acid reactive substances (TBARS), expressed as nanomoles of malondialdehyde per g of tissue, were determined spectrophotometrically in the butanol phase as described by Naito *et al.* [20]. Total antioxidant capacity of tissue (FRAP) was determined spectrophotometrically by evaluating the iron reducing capacity of the tissue as described by Benzie and Strain [21]. Glutathione peroxidase (GPx), and catalase activities were determined kinetically as previously described [22].

Reduced glutathione were determined spectrophotometrically using the enzymatic assay described by Anderson [23]. Total nitrates/nitrites as an estimate of nitric oxide concentration were determined by a commercial kit (Assay Design; Stressgen, Ann Arbor, MI). Protein concentrations were determined with a commercial assay (BioRad Laboratories, Richmond, CA).

Myeloperoxidase activity, as an index of neutrophil infiltration, was determined by a modification of the method of Trush *et al.* [24]. Briefly, tissues were homogenized in 50 mM potassium phosphate buffer pH 6.0 containing 0.5% hexadecyltrimethylammonium bromide. The homogenates then underwent 3 freeze thaw cycles and sonification, followed by incubation at 60°C in a water bath for 2 h to extract myeloperoxidase and reduce interfering substances. Samples were centrifuged at $10{,}000 \times g$ for 30 min at 4°C. Myeloperoxidase activity was determined in the resultant supernatant using o dianisidine as substrate.

Statistical Analysis

Comparisons were made between the injured muscles using Student's t test. Semiquantitative analysis of pathology scores were analyzed using Mann Whitney rank sum test, and are reported as median with range. All statistical comparisons were performed using commercially available software (SigmaStat 3.1; Systat Software Inc., San Jose, CA). Differences were considered to be significant at $P < 0.05$. All data are presented as mean ± standard error of the mean (SEM).

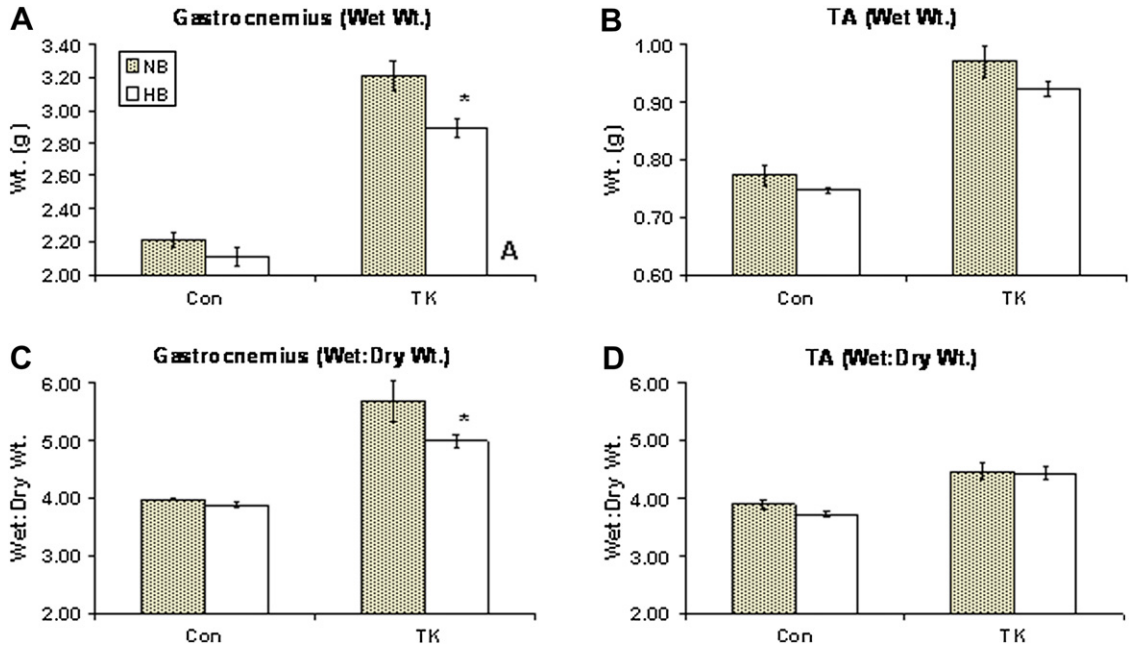

FIG. 1. Muscle wet weights (A), (B) and wet:dry weight ratios (C), (D). Normobaric conditions (NB) resulted in significantly more edema in the tourniquet (TK) injured gastrocnemius muscle compared with the same injured muscle under hypobaric conditions (HB) as indicated by both the significantly greater wet weight (A) and significantly smaller dry:wet weight ratio (C) ($P < 0.05$). In contrast, the wet weight (B) and significantly smaller dry:wet weight ratio (D) were similar between the injured tibialis anterior muscles (TA). There was no significant difference between the uninjured contralateral (Con) muscles for either muscle. (Color version of figure is available online.)

RESULTS

Body Weight

The body weight of NB and HB immediately prior to experimentation was 400 ± 4 g and 407 ± 3 g, respectively. The change in body weight during the treatments was significantly different between treatment groups. Despite fluid replacement, there was an average loss of 3.4 g following NB exposure, and a 10.7 g weight loss following HB exposure ($P < 0.05$).

Muscle Wet Weight and Dry:Wet Weight Ratio

Tourniquet-induced ischemia resulted in significant edema in all muscles, as indicated by both wet weight and dry:wet weight ratios in comparison with the corresponding contralateral control muscle ($P < 0.05$) (Fig. 1). Wet weight of the Gast from NB-exposed rats was significantly greater following tourniquet-induced ischemia compared with HB-exposed rats. In contrast, the TA wet weights were similar between groups.

Muscle Viability

The loss of muscle viability, as indicated by the viability index, was small in both groups, with similar values within the same muscle between groups. The loss of viability in the Gast was 7% and 9% for NB and HB, respectively. The loss of viability in the TA was 18% and 16% after NB and HB exposure, respectively (Table 1).

Muscle Histology

The predominant feature of the histologic examination of the Gast and TA following ischemia was extra- and intracellular edema (data not shown). Polymorphonucleocytes were present at varying degrees in ischemic muscles, and extravascular red blood cells could be visualized in many sections, although there was no systematic difference between HB and NB. Scores based on edema, degeneration, necrosis, and inflammation were significantly higher (indicating greater pathology) in ischemic muscles compared with the untreated contralateral muscles; however, there were no significant differences between HB and NB for either the Gast or TA muscle (Table 2).

TABLE 1

Viability Index Determined as the Ratio of the Average Gray Scale Density of TTC Stained Ischemic: Uninjured Muscle. All Values Represent the Mean ± SEM for 10 Rats Per Group

Trratment	Gast	TA
Normobaric	0.93 ± 0.03	0.82 ± 0.01
Hypobaric	0.91 ± 0.02	0.84 ± 0.01

TABLE 2

Median Pathology Scores for Selected Parameters for Hypobaric (HB) and Normobaric (NB) Groups. All Values are Expressed as the Median and (Range). There Was No Significant Difference Between the Medians of Any Parameter.The Median Values for All Control Legs Were 0 (Not Shown in Table)

Group	Degeneration	Necrosis	Inflammation	Edema
HB	3 (4 0)	0.5 (4 0)	0 (0 3)	2 (0 3)
NB	2 (4 0)	1 (2 0)	0 (0 3)	2 (0 3)

Oxidative Stress

A 2 h ischemia period followed by 6 h reperfusion resulted in no significant changes in TBARS or total antioxidant levels in muscle from rats exposed to either HB or NB (Table 3). In addition, there were no significant differences between treatment groups or between control and tourniquet muscle in GPx or catalase activities, nitric oxide levels, or reduced glutathione concentrations (Table 3). Myeloperoxidase activity was nearly undetectable in all muscles from both treatment groups (data not shown).

Antioxidant Status in the Gast

All values represent the mean ± SEM from 10 rats/group. TBARS, thiobarbituric acid reactive substances; FRAP, total antioxidant potential; GPx, glutathione peroxidase; GSH, reduced glutathione.

Hct and WBC

Rats that underwent tourniquet-induced limb ischemia and reperfusion under NB conditions had a statistically significant 5% higher mean hematocrit compared with the HB group ($P = 0.017$). The Hct values were 44.6 ± 1.8 and 42.5 ± 2.1 for NB and HB, respectively. There was no significant difference between the two groups for WBCs.

Coagulation Parameters

Hypobaric, hypoxic conditions did not impact coagulation. All indices of clotting were similar between NB and HB exposed rats (Table 3).

Lung Dry:Wet Weight Ratio

The treatments did not result in significant differences in the dry:wet weight ratios of the lungs. The

TABLE 3

Antioxidant Status in the Gast. All Values Repersent the mean ± SEM from 10 rats/group

		Contalateral (Control)		Ischemic	
Parameter	Units	Normobaric	Hypobaric	Normobaric	Hypobaric
TBARS	nmol/g	3.9 ± 0.3	3.6 ± 0.3	4.0 ± 0.4	3.9 ± 0.6
FRAP	μmol/g	1.32 ± 0.26	1.09 ± 0.16	1.27 ± 0.29	1.18 ± 0.20
GPx	U/g	3.5 ± 0.7	5.7 ± 0.2	4.8 ± 0.1	5.6 ± 0.3
Catalase	U/g	1655 ± 193	2076 ± 229	1262 ± 127	1575 ± 165
GSH	nmol/g	27.5 ± 1.7	30.3 ± 1.9	27.8 ± 1.9	27.0 ± 1.4

TBARS thiobarbituric acid reactive substanccs; FRAP total antioxidant potcntial; GPx glutathione peroxidase; GSH reduced glutathione

values were 0.218 ± .003 and 0.206 ± 0.009 for NB and HB, respectively.

DISCUSSION

The major finding of this study was that hypobaric hypoxia significantly reduced edema in injured muscle. This is in clear contradiction to the contention that decreased atmospheric pressure during evacuation flights is responsible for exacerbating muscle edema in injured limbs. We purposefully constructed this experiment to test our hypothesis, not in imitation of the usual postoperative or postresuscitation course of combat-wounded personnel, but in a worst case scenario of maximum simulated altitude and exposure during peak edema formation in an animal model. Our study design combined several variables that could contribute to in-flight edema development: large volume isotonic crystalloid resuscitation, flight immediately after injury, highest possible altitude, and no supplemental oxygen. An ischemic time of 2 h was selected to induce predictable moderate edema that would allow for a detectable increase in edema, if present, in the affected muscles [10]. Rats were exposed to the lowest ambient pressure and partial pressure of oxygen that would be expected during air evacuation. Rats began the simulated flight during the 6 h of reperfusion immediately following tourniquet release, which corresponds with the phase of injury in which the majority of edema occurs (unpublished observations). Additionally, all rats were placed in a warm, thermoneutral environment (28–32°C) expected to generate a more severe reperfusion injury than a room temperature environment (22°C) [25, 26].

TABLE 4

Coagulation Parameters Including Thromboelastographic (TEG) Variables in Whole Blood from Rats Exposed to Hypobaric or Normobaric Conditions During Reperfusion All Values Represent the Mean ± SEM from 10 Rats/Group

Parameter	Normobaric	Hypobaric
AVE R	2.04 ± 0.19	1.91 ± 0.30
AVE K	0.83 ± 0.20	0.77 ± 0.08
AVE ANGLE	79.82 ± 0.11	78.49 ± 8.63
AVE MA	71.45 ± 2.20	69.99 ± 7.66

The simulated flight resulted in nearly 3-fold greater loss in body weight following HB compared with NB. Acute hypoxia at high altitude has been shown to result in a decrease in TBW with reductions in both extracellular and intracellular water [27]. It has also resulted in a reduction in plasma and blood volume [27, 28], and an increase in hematocrit [28], in both laboratory animals and humans. In the current study, the differences in body weight between the groups does not appear to be due to a loss of plasma volume as hematocrit values were less in HB compared with NB. The loss in TBW during acute hypoxia at high altitude is due to both increased diuresis [29] and a reduced drive to drink water. The latter has been reported in humans [27], rats [30], and rabbits [28] during acute altitude exposure. Although, in our study, rats were provided with *ad libitum* access to water while in the environmental chambers, rats from the HB group did not consume any water during this time. This was in contrast to NB rats that did drink, although we did not quantify the volume consumed. No attempt was made to measure urine output. HB resulted in significantly less edema in injured Gast muscle (Fig. 1). Taken together, the loss of body weight during simulated flight can likely be attributed to loss of total body water, which in turn affected the magnitude of edema in the injured Gast muscle.

It has also been asserted that air evacuation flights from Afghanistan and Iraq to Landstuhl Regional Medical Center contribute to further necrosis of injured myocytes [11]. However, quantitative analysis of muscle viability using TTC staining and semiquantitative analysis using routine histology revealed no significant differences in the magnitude of injury following HB or NB. Although oxidative stress has been shown to be a major cause of muscle necrosis following various forms of muscle injury, including ischemia-reperfusion [13, 26], cardiotoxin injection [31], blunt trauma [32],

and eccentric injury [33], we found no increase in any index of oxidative stress under the conditions examined.

Taken together, all indices of myocyte injury indicate that the 2 h ischemia did not cause a great deal of injury. The duration of ischemia was chosen to produce limited edema, rationalizing that it would provide the most sensitivity for observing differences between NB and HB if HB worsened edema. Previous work with this model has shown that the magnitude of edema plateaus between 3 and 4 h of ischemia (unpublished observations). Future work involving longer durations of ischemia may be warranted to specifically examine the effect of HB on the progression of muscle injury.

In addition to characterizing the impact of HB on injured muscle, we addressed several other commonly voiced concerns related to possible systemic effects of long distance medical transport of trauma patients, including pulmonary edema and coagulation. Pulmonary edema is a well-studied phenomenon in the context of acute mountain sickness (AMS). The few existing studies of the complications associated with medical transport have been primarily concerned with head injury (cerebral edema) [34] or pulmonary edema in individuals with pulmonary disease [35–37]. We assessed pulmonary edema as it pertains to air transport of trauma patients by measuring the dry:wet weight ratio of lungs following HB and NB and found no significant difference between the two environmental conditions, with statistically similar values between groups. It should be noted that reperfusion in a single limb after even 3 to 4 h of tourniquet-induced ischemia in rats does not produce measurable systemic effects, e.g., pulmonary edema, histological evidence of lung injury, or oxidative damage in uninjured tissue (unpublished observation). A study designed to specifically address the impact of HB on pulmonary edema should involve systemic trauma, such as hemorrhage, or some level of initial lung injury, such as inhalation injury.

Previous studies have indicated that the majority of trauma patients are hypercoagulable after injury [38]. However, to our knowledge, the influence of this hypercoagulable state on the worsening of muscle injury or requirement for delayed fasciotomy in air evacuated patients has not been investigated. Air travel of long duration is reportedly associated with a hypercoaguable state in normal individuals. Several studies suggest that the mild hypobaric hypoxia of commercial air travel is associated with a hypercoaguable state on long flights; this was noted in healthy volunteers with elevated risk factors such as age, high body mass index [16], use of oral contraceptives, and factor V Leiden mutation [15]. To determine if tourniquet-induced muscle ischemic injury represents a similar risk factor, we measured coagulation parameters and found that 2 h of tourniquet-induced ischemia followed by 6 h exposure to HB or NB conditions did not affect standard plasma assays of coagulation (PT, aPTT) or any TEG parameters. This suggests that the coagulation/anti-coagulation balance in these animals was unaltered by this degree of HB. This could be due to the lack of an effect of HB on coagulation, lack of statistical power, or species specific differences. Any of these are possible since the reported higher incidence of DVT in passengers on long distance flights is slight, and risk factors, other than lack of physical activity and seated posture, uncertain. It has recently been reported that evacuation of burn injured patients from Iraq and Afghanistan to Brook Army Medical Center is not associated with an increase in the rate of DVT [39]. In any case, the independent risk associated with hypobaric hypoxia is expected to be small, and it is not surprising that it was not detected in a sample of this size.

CONCLUSION

The results of this study suggest that the hypobaric, hypoxic conditions encountered during air evacuation do not contribute to increased edema in injured muscle. In fact, we found that simulated flight during reperfusion either reduced or caused no change in muscle edema, and did not significantly affect muscle viability in a rat model of tourniquet-induced hind limb injury. This suggests that other factors, such as excessive crystalloid resuscitation, persistent vascular leak unrelated to air pressure, or dependent limb position in flight are the cause of increased muscle edema observed following air transport. Future experiments could explore the effects of hypobaric hypoxia during reperfusion on muscle viability using more severe injury (longer ischemic periods) or combine ischemia with a relevant systemic insult, such as hemorrhage. Nevertheless, the results of the present study do not suggest that air evacuation of injured soldiers, *per se*, contributes to their need for delayed fasciotomies, but suggests causes due to other factors, such as severity of injury and/or a greater volume of resuscitation fluids.

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