MHSRS-26-4227

Force Health Protection at Altitude: Identifying "Invisible" Metabolic Fragility in a Preclinical mTBI Model

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ABSTRACT

Motivation: Warfighters at high altitudes (HA) face higher rates and severity of traumatic brain injury (TBI), but how chronic hypoxia affects mild TBI (mTBI) recovery remains poorly understood.

Goal: Use longitudinal 7T proton MR spectroscopy (MRS) to identify neurochemical biomarkers of HA adaptation and subsequent post-trauma metabolic crisis.

Approach: Mice were acclimated to simulated HA (5,000 m) or sea level (SL) for 12 weeks, subjected to three repetitive closed head injuries to induce mTBI, and monitored via MRS across 14 weeks.

Results: Before injury, chronic HA exposure depleted hippocampal myo-inositol and total choline, creating a baseline of metabolic fragility. Post-mTBI, these HA mice suffered a profound bioenergetic collapse characterized by creatine buffer failure and lower choline levels. Crucially, while sea-level controls began recovering within 14 days, the HA group failed to restore total N-acetylaspartate levels, indicating a stalled neuronal repair process.

Impact: Chronic hypoxia alters brain metabolism and blocks trauma recovery, meaning "invisible" metabolic injuries persist past clinical recovery. Consequently, standard sea-level Return-to-Duty protocols are inadequate; force protection requires environment-aware concussion management to prevent long-term neurodegeneration.

Learning Objectives

Differentiate *between the baseline neurochemical profiles of sea-level and high-altitude populations, identifying the metabolic depletion of myo-inositol and total choline.*

Analyze *the "metabolic crisis" that occurs when mTBI is superimposed on chronic hypoxia, specifically the acute failure of the total creatine energy-buffering pool and the failed recovery of total N-acetylaspartate.*

Discuss *operational implications of substrate competition and microglial activation, focusing on why standard "Return-to-Duty" protocols may be insufficient for altitude-adapted warfighters.*

INTRODUCTION

Transition from sea level (SL) to high-altitude (HA)

- Hypobaric and hypoxic environment → disrupt physiological mechanisms in the brain [1]
- Higher incidence of traumatic brain injury (TBI) at HA [2], and more severe neurological consequences compared to similar injuries sustained at SL [3,4]

Mild traumatic brain injury (mTBI)

- Commonly referred to as concussion, represents the most prevalent form of TBI [5]
- Impaired cognitive function and a constellation of acute symptoms [6]
- impairments in glucose uptake, cerebral blood flow, and white-matter integrity with HA [7]

In this study

- Longitudinally track changing neurochemical levels with ^{1}H-MRS
- Measured how long-term exposure to HA (12 weeks) alters the brain's metabolic baseline, then observed how it reacts to an mTBI over a 14-day recovery period.

METHODS

Experimental timeline (Figure 1A)

- 8-week-old male C57BL/6J mice at SL underwent baseline MR scans (n=10)
- HA group (n=5) were then placed in a hypobaric chamber simulating 5000m elevation
- Exposure lasted 12 weeks, with weekly re-pressurization to SL for cage maintenance
- Ascent and descent were controlled at 200m/min to ensure adequate acclimatization
- After 12 weeks, mice were anesthetized & underwent unilateral mTBI via closed head injury (CHI) [8]
- CHI was induced using an electromagnetically controlled cortical impact device, targeting a site near Bregma (3.0mm lateral, –2.0mm posterior, 1.5mm depth, left hemisphere)
- Impact parameters: a 5mm tip, 4m/s velocity, 15° -angle, and 100ms dwell time.

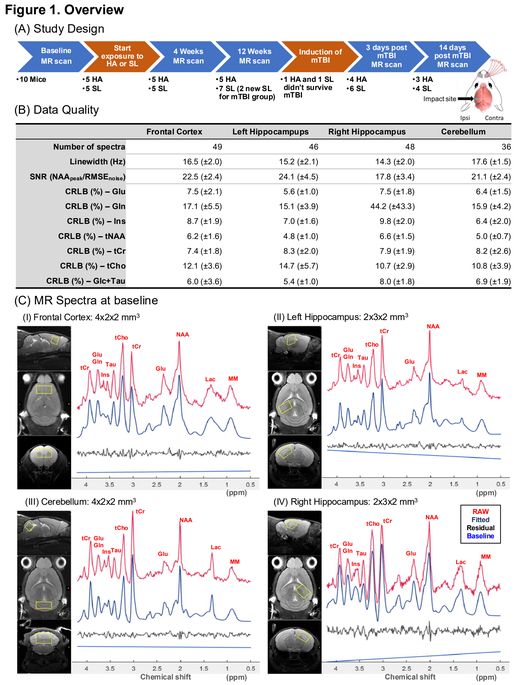
MR imaging

- 7T Bruker Biospec 70/20 with a birdcage transmit coil and a 4-ch receive array
- MR spectra with PRESS (TR/TE = 2500/16.5ms, 256 transients, Rx bandwidth = 3301Hz)
- 4 volumes-of-interest (VOIs): frontal cortex (4 × 2 × 2mm³), left hippocampus (2 × 3 × 2mm³), right hippocampus (2 × 3 × 2mm³), and cerebellum (4 × 2 × 2mm³)
- Multi-echo T2-w RARE (TR/TE = 4000/10–110ms; RARE factor = x2; 4avg.; time = 15min)

Spectral data

- Processed using MRspa for frequency, phase, and eddy-current correction
- Metabolite quantification with LCModel (v6.3.1N) using a simulated basis set
- Statistical comparisons between HA and SL groups, as well as across post-injury time points, were conducted using two-tailed unpaired t-tests with unequal variance

Figure 1. Overview



	Frontal Cortex	Left Hippocampus	Right Hippocampus	Cerebellum
Number of spectra	49	46	48	36
Linewidth (Hz)	16.5 (±2.0)	15.2 (±2.1)	14.3 (±2.0)	17.6 (±1.5)
SNR ($NAA_{peak}/RMSE_{noise}$)	22.5 (±2.4)	24.1 (±4.5)	17.8 (±3.4)	21.1 (±2.4)
CRLB (%) – Glu	7.5 (±2.1)	5.6 (±1.0)	7.5 (±1.8)	6.4 (±1.5)
CRLB (%) – Gln	17.1 (±5.5)	15.1 (±3.9)	44.2 (±43.3)	15.9 (±4.2)
CRLB (%) – Ins	8.7 (±1.9)	7.0 (±1.6)	9.8 (±2.0)	6.4 (±2.0)
CRLB (%) – tNAA	6.2 (±1.6)	4.8 (±1.0)	6.6 (±1.5)	5.0 (±0.7)
CRLB (%) – tCr	7.4 (±1.8)	8.3 (±2.0)	7.9 (±1.9)	8.2 (±2.6)
CRLB (%) – tCho	12.1 (±3.6)	14.7 (±5.7)	10.7 (±2.9)	10.8 (±3.9)
CRLB (%) – Glc+Tau	6.0 (±3.6)	5.4 (±1.0)	8.0 (±1.8)	6.9 (±1.9)

Figure 1. (A) Experimental timeline illustrating key interventions (orange) and measurement times (blue). The number of animals assessed at each time point and the equipment used for interventions are also shown. (B) Overview of MRS quality metrics shown as mean (± standard deviation). (C) Sample MR spectra at baseline and LCModel outputs from (I) frontal cortex, (II) left hippocampus, (III) cerebellum, and (IV) right hippocampus, shown alongside corresponding volume-of-interest placements on T2-weighted images.

Figure 2. High Altitude (HA) vs Sea Level (SL)

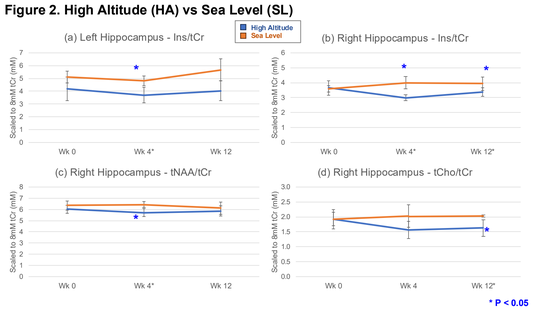


Figure 2. Mean metabolite levels and standard deviations (ratio to tCr, normalized to 8 mM) across time points for high altitude (HA, N = 5) and sea level (SL, N = 5) groups (same animals for all time points). Significant group differences ($P < 0.05$; 2-tailed, unpaired t-test with unequal variance) were observed for Ins in both hippocampi (a and b), and for tNAA and tCho in the right hippocampus (c and d). Some metabolite alterations appeared transient, while others persisted beyond 12 weeks.

RESULTS AND DISCUSSION

- All mice successfully underwent HA / 2 died within 3 days of CHI, and 2 more by day 14
- Summary metrics for linewidth, signal-to-noise ratio, and Cramér-Rao lower bounds of key metabolites are presented in Figure 1b
- Figure 1c displays representative VOIs and corresponding spectra

HA exposure

- Ins/tCr levels different between HA and SL in both hippocampal formation (Figure 2)
- Right hippocampus also showed significant changes in tNAA/tCr and tCho/tCr levels
- Reflects disrupted astroglial osmoregulation, membrane synthesis, and metabolic stress, reflecting an energy-conservation phenotype under chronic hypobaric-hypoxia

Post-mTBI neurochemical profiles

- In the aggregate of HA and SL (Figure 3) tNAA/tCr, and tCr appears to be most affected following CHI, in line with changes in creatine levels as previously reported [9,10]
- Cross-sectional comparisons between HA and SL (Figure 4) show the most prominent post-mTBI differences in Ins and tCho
- Cross-sectional analysis was limited by sample size, and further investigation is needed

Figure 3. mTBI – Aggregate analysis

Figure 3. Mean metabolite levels and standard deviations (left column - ratio to tCr, normalized to 8mM; right column - concentrations) for Glu, Gln/Glx, Ins, tNAA, tCr, tCho and Glc+Tau at various time points following mTBI, aggregating data from both high altitude (HA) and sea level (SL) groups. N values for pre-injury, 3-day and 14-day time points were: frontal cortex, 10/9/7; left hippocampus, 7/10/6; right hippocampus, 10/10/8; and cerebellum, 5/6/7. Significant differences ($P < 0.05$; 2-tailed, unpaired t-test with unequal variance) were observed between pre- and post-injury groups across brain regions, with tNAA/tCr showing the most pronounced change.

Figure 4. mTBI – HA vs SL

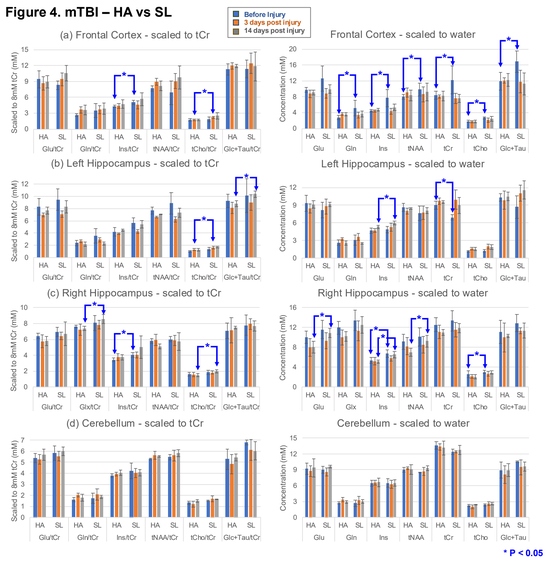


Figure 4. Mean metabolite levels and standard deviations (left column - ratio to tCr, normalized to 8mM; right column –concentration) for Glu, Gln/Glx, Ins, tNAA, tCr, tCho and Glc+Tau at various time points following mTBI for HA and SL groups. For the HA and SL groups, N values at the pre-injury, 3-day, and 14-day time points were 4/3/3 and 6/6/4 for the frontal cortex, 4/4/3 and 3/6/3 for the left hippocampus, 4/4/3 and 6/6/5 for the right hippocampus, and 3/3/3 and 2/3/4 for the cerebellum, respectively. Significant differences ($P < 0.05$; 2-tailed, unpaired t-test with unequal variance) were observed between pre- and post-injury groups across brain regions, with tCho showing the most pronounced group differences. Note that the pool of animals is different compared to the previous Figure 2, accounting for the differences between the two figures.

Figure Abbreviations: SNR - signal-to-noise ratio; **Linewidth** – full-width-half-maximum of water; **CRLB** – Cramér–Rao lower bounds; **mTBI** – mild traumatic brain injury; **HA** – high altitude; **SL** – sea level; **Glu** – glutamate; **Gln** – glutamine; **Ins** – myo-Inositol; **tNAA** – total NAA (sum of NAA and NAAG); **tCr** – total creatine (sum of Cr and PCr); **tCho** – total choline (sum of PCho and GPC); **Glc+Tau** – sum of glucose and taurine.

CONCLUSIONS

- The study identified region-specific neurochemical changes in mice following high-altitude exposure and mild traumatic brain injury [11]
- Significant alterations in various metabolites, notably tNAA/tCr, tCr, Ins, and tCho were observed, with some effects persisting over time
- However, the limited sample size prevented comprehensive cross-group comparisons
- A more in-depth analysis with a larger cohort is needed to establish clear profiles of the neurochemical biomarkers in HA adapted brains following mTBI

DISCLAIMERS

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COI: The authors do not have any conflicts of interest related to the content of this presentation.

ACKNOWLEDGMENTS

The author(s) declared that financial support was received for this work and/or its publication. Initial infrastructure for high-altitude research was supported by a concept award from the Air Force Medical Support Agency. Ongoing support for this work was funded by the U.S. Department of Defense (DoD) and the Uniformed Services University of the Health Sciences (USUHS) in the Military Traumatic Brain Injury Initiative (MTBI2), U.S. DoD Program Project 308430 USUHS, Intramural HP-70-12224 USUHS DoD, Defense Health Agency (DHA) award HT9425-25-1-0704, and the internal funds from the Department of Radiology and Bioengineering at USUHS DoD.

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