Quantitative analysis of metabolites in mouse brain following heat exposure using magnetic resonance spectroscopy

Brian Andrews-Shigaki1,2, Yifan Chen1, Asamoah Bosomtwi1, Reed Selwyn1, and Haiying Tang2

1Military & Emergency Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD, United States; 2Radiology & Radiological Sciences, Uniformed Services University of the Health Sciences, Bethesda, MD, United States

INTRODUCTION
Heat injury continues to be a significant problem confronting the military, particularly during recruit training, and remains a common cause of preventable non-traumatic related death (1,2). Current research on heat stress (injury before the onset of heat stroke) has focused on physiological parameters such as core temperature (3). Although exposure to heat stress causes physiological dysfunction, the effects of heat stress on the brain remain undetermined. In this research we analyzed changes in metabolites from selected brain regions in mice following heat exposure using proton Magnetic Resonance Spectroscopy (1H-MRS).

METHODS
Five mice (C57BL/6J, 10-12 weeks, 20-25g) were used in this experiment. All mice were implanted with temperature telemetry probes and were exposed to 39.5°C in an environmental chamber for up to 3hrs. Each mouse was scanned twice, before implantation and 24hrs following heat exposure. In-vivo single voxel 1H-MRS was performed on a Bruker BioSpec system (Bruker NMR, Inc., Billerica, MA) consisting of a 7-Tesla (T), 20-cm horizontal bore, superconducting magnet (MagneX Scientific, Abingdon UK) with a Biospec 70/20 console and Paravision software. An Autopac mouse positioning and physiological monitoring system, 86mm quadrature transmit coil and a 4-channel phase array mouse head coil were used. Mice were anesthetized with isoflurane during the preparation period and during the scan using a ‘flow through’ nose cone (1.5-2.0%). Single voxel 1H-MRS data was acquired using Point RESolved Spectroscopy (PRESS, TR/TE = 2500/20ms, averages = 256, voxel size = 3-5mm³), with and without water suppression, localized at the hippocampus, hypothalamus, and cerebellum. Spectra were processed in LCModel (S.W. Provencher), with eddy current correction and water-scaling, to quantify brain metabolites N-acetylaspartate (NAA), glutamate+glutamine (Glx), choline (Cho), creatine (Cr) and myo-inositol (mI) in the selected regions. Concentrations between groups were statistically analyzed in MATLAB (The Math Works, Natick, MA) and GraphPad (GraphPad, La Jolla, CA) for significance.

RESULTS
The major results of this experiment are summarized in the table. During heat exposure, peak core temperatures reached between 40.5–42.4°C. In the hypothalamus (Figure), heat-exposed mice showed a significant decrease in NAA (17%, p=0.0275) and Cr (7%, p=0.0751) with no significant changes in mI, Cho or Glx. In contrast, results show significant increases in Glx (19.40%, p=0.0431) in the Cerebellum. Hippocampus showed no significant changes for any metabolite.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Hypothalamus</th>
<th>Cerebellum</th>
<th>Hippocampus</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Cr]</td>
<td>-6.86% (0.0751)</td>
<td>5.61% (0.4199)</td>
<td>-10.46% (0.2791)</td>
</tr>
<tr>
<td>[mI]</td>
<td>5.61% (0.4199)</td>
<td>18.05% (0.1816)</td>
<td>10.69% (0.3783)</td>
</tr>
<tr>
<td>[Cho]</td>
<td>-8.29% (0.3108)</td>
<td>2.91% (0.9248)</td>
<td>4.92% (0.7518)</td>
</tr>
<tr>
<td>[NAA]</td>
<td>-16.92% (0.0275)</td>
<td>3.45% (0.7609)</td>
<td>3.39% (0.7716)</td>
</tr>
<tr>
<td>[Glx]</td>
<td>-10.46% (0.2791)</td>
<td>19.40% (0.0431)</td>
<td>2.75% (0.9020)</td>
</tr>
</tbody>
</table>

Table: Metabolite quantification results. Average % change between pre and post heat exposure. P-value from paired t-test.

DISCUSSION
Neuronal damage occurs prior to the onset of heat stroke. Injury from heat stress is not homogeneously distributed in the brain and is localized to the hypothalamus and cerebellum. Increase of Glx in the cerebellum is unexpected and should be investigated further. These neuronal markers for heat stress will be vital for future heat injury and recovery studies.

ACKNOWLEDGEMENTS
Grant support: USUHS R091FX.

REFERENCES
2. Davis JM, Murphy EA, Carmichael MD and Davis B. Quercetin increases brain and muscle mitochondrial biogenesis and exercise tolerance. Am J Physiol Regul Integr Comp Physiol 296: R1071-R1077, 2009