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TITLE: Measuring Glial Metabolism in Repetitive Brain Trauma and Alzheimer's Disease

PRINCIPAL INVESTIGATOR: Alexander Lin, PhD

CONTRACTING ORGANIZATION: Brigham and Women’s Hospital

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14. ABSTRACT

The overall aim of the study is to better understand glial metabolism within the context of repetitive brain impacts and Alzheimer's disease and its potential findings in veterans. In preparation for the start of the study, we have conducted a number of studies to quantify the improvement moving from 3T to 7T. We also present data comparing military and sports-related repetitive brain trauma and show that changes in glutamate show similar patterns. These results support our initial hypothesis and illustrate the importance of measuring glial metabolism using indirect detection of 13C-labeled metabolism.

15. SUBJECT TERMS
Repetitive brain trauma, glial metabolism, glutamate, multinuclear spectroscopy, chronic traumatic encephalopathy, Alzheimer's disease, 13C acetate

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CONTENTS

1. INTRODUCTION:

Glutamate is a key compound in cellular metabolism with its most important role as a neurotransmitter with which the brain utilizes 80% of energy consumption to maintain this important cycle. Elevated levels of glutamate have been shown to be predictive of outcome in severe traumatic brain injury and our preliminary data from existing studies, have shown that glutamate remains elevated in the chronic stages of repetitive brain trauma (RBT) as well. Current methods of measure brain glutamate using spectroscopy is not specific to different cell types or the dynamic changes that undergo metabolism. We have developed a novel, non-invasive, quantitative method of measuring the dynamic rates of glutamate using ^{13}C-labeled acetate, the primary fuel for glial cells, which can be tracked through the cerebral glutamate synthesis cycle using magnetic resonance spectroscopy. Our goal is to utilize infusion of ^{13}C-labeled acetate in our existing cohort of retired NFL athletes with and without increased glutamate, subjects with Alzheimer's disease (AD), military veterans with a history of traumatic brain injury, and age-matched controls to measure the effect of repetitive brain trauma upon glutamate metabolism. Our hypothesis is that increased glutamate found in these players, will be reflected in up-regulation of glial pathways. The result of the study would be to identify the dysfunctional pathways that underlie glutamate excitotoxicity in sports-related brain trauma. These dysfunctions will provide precise targets for existing glutamate medications that are known to modulate specific pathways. Therefore we anticipate not only providing a better understanding of the metabolic mechanisms of sports-related head injury but also to provide data that will be useful for the development of much needed treatments for this devastating disease.

2. KEYWORDS:

Repetitive brain trauma, glial metabolism, glutamate, multinuclear spectroscopy, chronic traumatic encephalopathy, sports-related brain injury, military-related brain injury, Alzheimer's disease, ^{13}C acetate

3. ACCOMPLISHMENTS:

3.1. Major Goals

Our overall aim will be to better understand glial metabolism within the context of RBT and AD and its potential findings in veterans. Specifically, we will:

Aim 1: Determine the mechanism (excitoxicity?) that results in increased cerebral glutamate and glutamine (Glx) levels by comparing glial metabolic rates in NFL athletes with the highest levels of Glx and those with the lowest levels.

Aim 2: Determine the mechanism (neurodegeneration?) that results in decreased cerebral Glx levels by comparing glial metabolic rates in NFL athletes with the lowest levels of Glx and AD patients.

Aim 3: Identify the specific metabolic pathway that results in alternations of cerebral Glx levels in military and healthy controls as well as in comparison with NFL and AD subjects.

Aim 4: Correlate the glial and glutamate metabolic rates with additional measures obtained in the parent studies including of a) serum, CSF, and genetic biomarkers in the NFL subjects and b) neurocognitive

measures in all cohorts. The result of the study would be to identify the underlying physiological changes in glial metabolism in RBT such as neuroinflammation and glutamate excitotoxicity thus providing targets for much needed treatments as well as provide a safe, non-radioactive test to monitor these treatments.

3.2. Goal Accomplishments

Due to the lengthy delay in obtaining IRB approval, a stop order has been in effect until IRB approval could be obtained. On October 11th, the Partners Healthcare Institutional Review Board approved the subject protocol. The locally approved protocol was sent to the U.S. Army Medical Research and Development Command Office of Research Protections and Human Research Protection Office for review. Approval from HRPO was obtained on December 13th, 2019 and the stop work was halted shortly after so that the study could proceed forward.

We immediately initiated efforts for recruitment of subjects with our collaborators at Boston University to obtain NFL subjects and AD subjects. In order to recruit military and healthy controls, we also re-established collaborations with collaborators at the US Army Research Institute of Environmental Medicine (USARIEM) who we had previously collaborated on DOD award W81XWH-10-1-0835 which had formed the basis for the preliminary data for this study.

3.2.1. 7T Voxel Locations

We also re-initiated contact with Cambridge Isotopes for the preparation of the 13C-labeled acetate to be used in the study. As it would take several months to prepare the pharmacy grade substance, we placed the order and proceeded with the preliminary data collection necessary for the start of the study. One of the tasks was to determine which brain region would be the focus of the 1H MRS data acquisition. There are several brain regions that have been implicated in traumatic brain injury and neurodegeneration studies, these would include: 1) locus coeruleus (LOC), 2) anterior cingulate cortex (ACC), 3) dorsa-lateral pre-frontal cortex (DLPFC), 4) ventral medial pre-frontal cortex (VMPFC), and posterior cingulate gyrus (PCG) as shown in Figure 1

Five subjects were scanned three times each to determine the reproducibility and robustness of each of the brain regions.

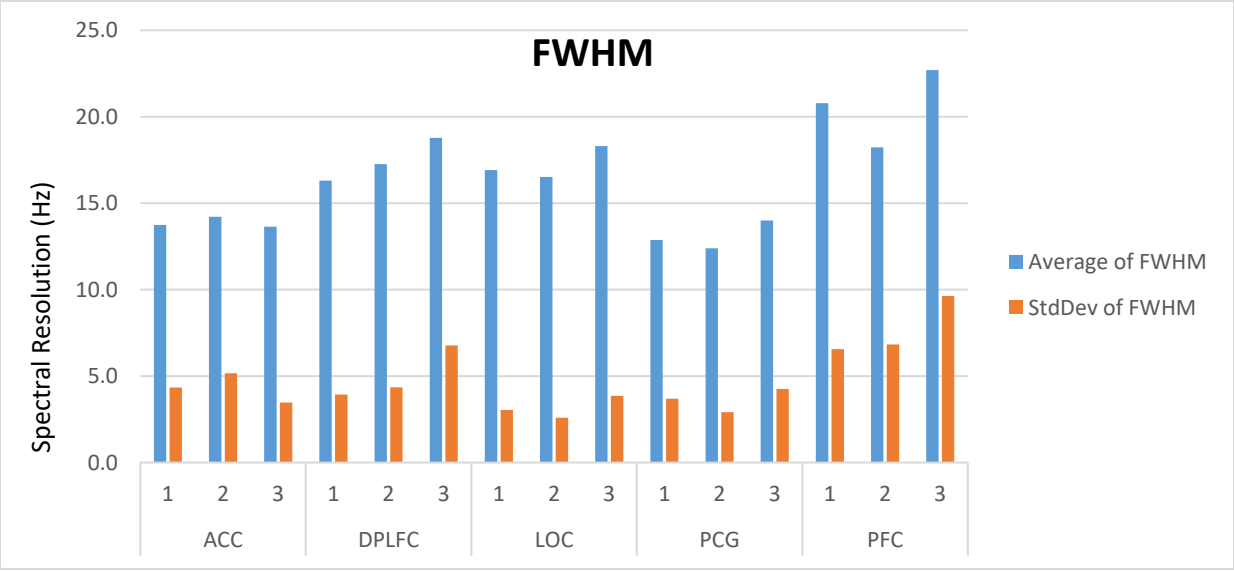

Figure 2. Full width half max measurements of multiple brain regions in healthy controls.

The timing of this groundwork study was opportune as all of the work was completed prior to the onset of the COVID pandemic. On March 13th, 2020, all elective medical studies were shutdown due to the coronavirus. As it was unclear how long this shutdown would last, we placed the 13C acetate preparation on hold as it has a limited shelf life.

3.2.3. Glutamate Cohort Comparisons

Starting June 8th, 2020, all COVID restrictions at Brigham and Women's Hospital were lifted such that clinical research could recommence. We immediately began scheduling subjects to be scanned for the study. Within this quarter, we recruited and scanned 10 of 20 NFL subjects, 5 of 10 AD subjects, and 14 of 15 military subjects, and 12 of 15 controls as determined by our study design. As the baseline scans for all of these subjects were part of other funded studies, there was no cost to this study to obtain baseline glutamate and glutamine levels in these scans. The results are summarized in the table below:

Cohort	Glutamate	Glutamate %SD	Glutamine	Glutamine %SD
NFL	7.57 ± 0.25 mM	2.2 ± 0.4%	2.75 ± 0.25 mM	5.0 ± 0.8%
AD	5.78 ± 0.11 mM	3.0 ± 0.2%	2.29 ± 0.09 mM	5.0 ± 0.3%
Military	7.15 ± 1.36 mM	2.3 ± 0.6%	3.00 ± 0.83 mM	4.9 ± 1.1%
Control	7.04 ± 0.35 mM	2.1 ± 0.4%	2.23 ± 0.13 mM	5.0 ± 0.5%

These results show that glutamate levels in the NFL are highest and lowest in AD subjects. The military cohort has a greater variability of glutamate. The glutamate %SD is the Cramer-Rao lower bound of the fit of the glutamate signal to the raw data. Generally, a %SD value above 20% is considered poor which demonstrates that the low values observed above across all cohorts is considered excellent data quality.

3.2.4. Preliminary Results of Glutamate Metabolism Measures

We are excited to finally obtain some results! We conducted glutamate metabolism studies at the 7T scanner where we utilized the functional MRS approach described in previous reports where in short, we acquire repeated measures of short echo proton spectroscopy over a period of 15 minutes. We then reconstruct the MRS data and measure the glutamate and glutamine concentration in each of the repeated measures. The baseline metabolite concentration is subtracted from the concentration measurement at each time point which then provides a time course of the change in glutamate and glutamine. The results of the test in healthy control are shown in Figures 3 and 4.

These results show change in the glutamate and glutamine signals as the 13C label enters the glutamate-glutamine cycle through the tricarboxylic acid cycle. The rate of the decrease can be calculated using a bi-exponential fit to determine the rate of synthesis of glutamate and glutamine. As expected the change in glutamate occurs somewhat faster than the change in glutamine as has been shown in 13C direct detection studies.

We have collected data in five healthy controls thus far. We are eager to apply this methodology to NFL athletes and patients suffering from Alzheimer's disease but due to the pandemic, travel restrictions have not allowed for the parent DIAGNOSE-CTE study to restart recruitment. It is our hope that with the development of COVID vaccinations that this should help ease restrictions so that the study can start again. We have also obtained a list of subjects that were previously scanned that live locally. We will contact these subjects to schedule them for this study.

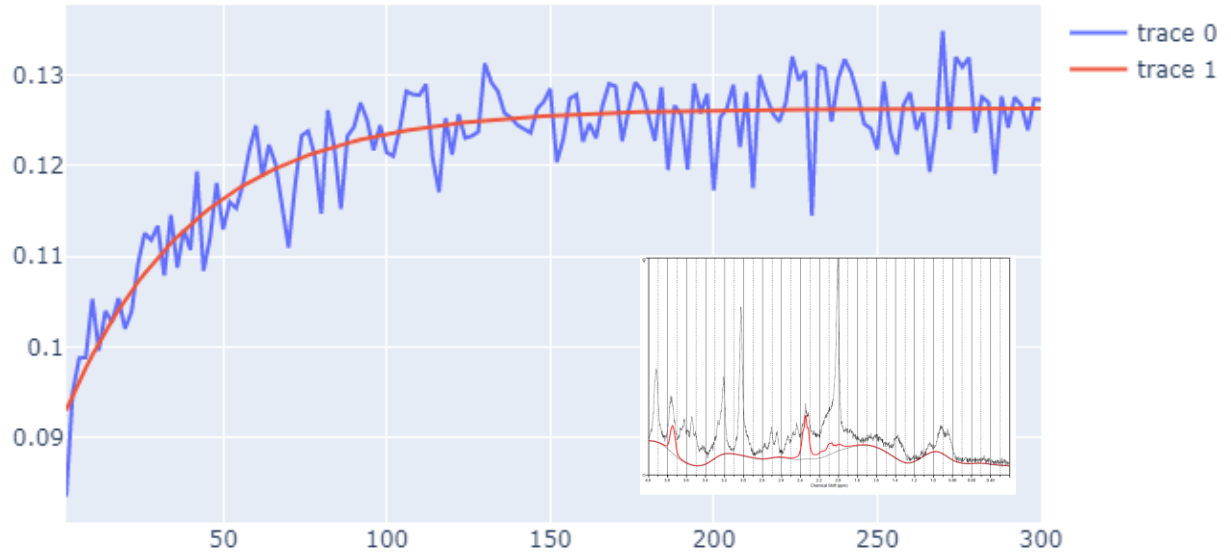

Figure 3. Plot of glutamate signal in sequential spectra acquired over a 15 minute period. The red line indicates the exponential fit of the data. The inset shows the fitting of the glutamate signal at a single timepoint within the 1H MRS spectrum acquired at 7T.

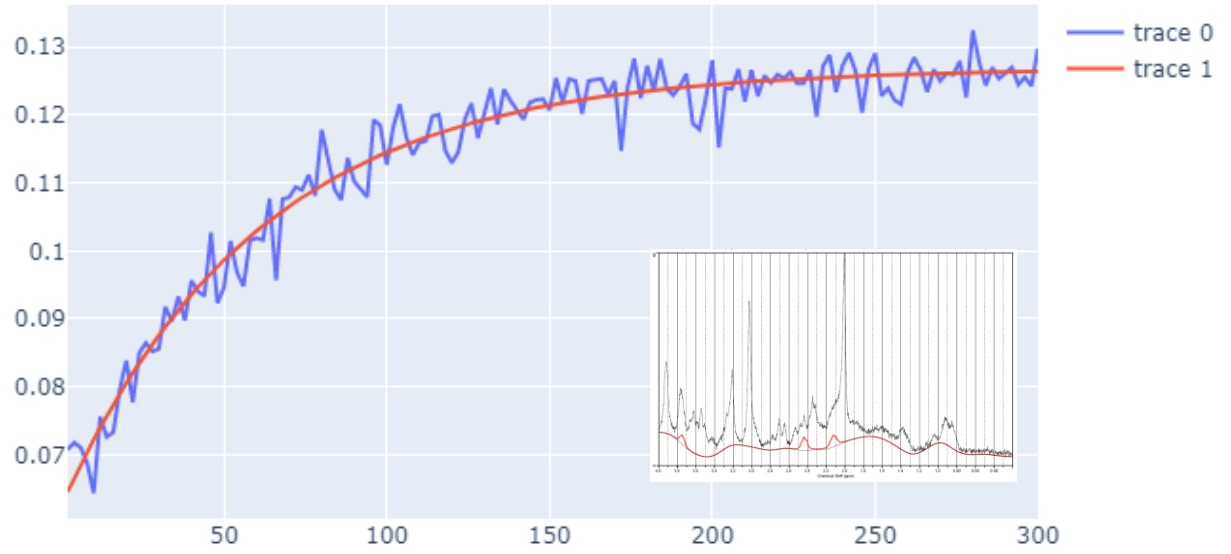

Figure 4. Plot of glutamine signal in sequential spectra acquired over a 15 minute period. The red line indicates the exponential fit of the data. The inset shows the fitting of the glutamine signal at a single timepoint within the 1H MRS spectrum acquired at 7T.

3.3. Training and Professional Development

While this study does not have a component for training and professional development, we have had Dr. Eduardo Coello from the Technical University of Munich as a post-doctoral student for several years. Eduardo is an MR physicist with extensive experienced with 7T spectroscopy and pulse sequence programming. He has been a major asset to the study while also benefiting from exposure to clinical research. Although not involved directly in the study, we have also recruited a senior post-doctoral student, Dr. Katherine Breedlove who has had extensive experience with concussion research having been part of NCAA-DOD CARE Consortium at University of Michigan and University of Delaware in her previous post-doc positions and her PhD at Purdue University. She is a certified athletic trainer who has focused her career on the use of neuroimaging in sports-related concussion.

3.4. Results Dissemination

We have published several papers on NFL athletes that are the subject of this study.

1. Alosco ML, Tripodis Y, Rowland B, Chua AS, Liao H, Martin B, Jarnagin J, Chaisson CE, Pasternak O, Karmacharya S, Koerte IK, Cantu RC, Kowall NW, McKee AC, Shenton ME, Greenwald R, McClean M, Stern RA, **Lin AP**. A magnetic resonance spectroscopy investigation in symptomatic former NFL players.Brain Imaging Behav. 2020 Oct;14(5):1419-1429.
2. Lepage C, Muehlmann M, Tripodis Y, Hufschmidt J, Stamm J, Green K, Wrobel P, Schultz V, Weir I, Alosco ML, Baugh CM, Fritts NG, Martin BM, Chaisson C, Coleman MJ, **Lin AP**, Pasternak O, Makris N, Stern RA, Shenton ME, Koerte IK. Limbic system structure volumes and associated neurocognitive functioning in former NFL players. Brain Imaging Behav. 2019 Jun;13(3):725-734.
3. Alosco ML, Tripodis Y, Koerte IK, Jackson JD, Chua AS, Mariani M, Haller O, Foley ÉM, Martin BM, Palmisano J, Singh B, Green K, Lepage C, Muehlmann M, Makris N, Cantu RC, **Lin AP**, Coleman M, Pasternak O, Mez J, Bouix S, Shenton ME, Stern RA. Interactive Effects of Racial Identity and Repetitive Head Impacts on Cognitive Function, Structural MRI-Derived Volumetric Measures, and Cerebrospinal Fluid Tau and Aβ. Front Hum Neurosci. 2019;13:440.

We have also presented results related to NFL athletes at various conferences:

1. Starr T, Alosco M, Stern R, Scherrer B, Lin AP. Evidence of Increased N-acetyl-aspartate After Repetitive Head Injury. *MRS Workshop (2019)*
2. Charney M, Starr T, Alosco M, Martin B, Liao HJ, Koerte I, Shenton M, Stern R, Lin AP. Neuropsychological Changes Associated with Brain Inflammation in Subjects with Suspected CTE. *3rd Annual Chronic Traumatic Encephalopathy Conference (2019)*
3. Lin AP, Koerte I. Magnetic Resonance Spectroscopy of Sports-related Head Injury: A Cross-Sectional Comparison. *American Academy of Neurology 71st Annual Meeting. Philadelphia, PA; May 4- May 10, 2019.*
4. Charney M, Kochseik J, Starr T, Alosco M, Martin B, Liao HJ, Kaufman D, O'Donnell L, Bioux S, Zhang F, Makris N, Shenton M, Stern R, Koerte I, Lin AP. Cohort Stratification by Clinical Symptoms through Unsupervised Learning Reveals Metabolic and Microstructural Brain Alterations in Former American Football Players. *International Society for Magnetic Resonance in Medicine 27th Annual Meeting and Exhibition. Montreal, Canada; May 11-16, 2019.*
5. Starr T, Charney M, Alosco M, Qu J, Coello E, Liao HJ, Koerte I, Kaufmann D, Shenton M, Stern R, Lin AP. Decreased Brain Temperature in Former NFL Athletes. *International Society for Magnetic Resonance in Medicine 27th Annual Meeting and Exhibition. Montreal, Canada; May 11-16, 2019.*

3.5. Next Reporting Period Plan

The goal for the next year is to collect data in NFL and AD subjects with the hope that the pandemic will soon end so that subjects can travel again to participate in the study.

4. IMPACT:

4.1. Principle Discipline

Indirect detection of 13C-acetate using 7T proton spectroscopy has not been utilized before in other studies and therefore will provide highly novel and impactful results. As more and more sites move to higher field strengths, there has not been major developments in our field aside from the improved resolution of images. This method takes advantage of several innovative improvements and could potentially provide a new area of data acquisition that has not been fully explored. We are convinced that publications from this study will be eminently publishable in high impact journals given its technical novelty and important clinical implications.

4.2. Other Disciplines

While the technical improvements impact the radiological fields, the availability of a method to assess glial metabolism, specifically that of glutamate, will provide a non-invasive insight that will not only advance our understanding of glial changes in repetitive brain trauma and neurodegenerative diseases but will also provide potential targets for treatments for in this field that can impact disciplines of pharmaceutical research and ultimately for clinical treatment of long-term impacts of repetitive head trauma.

4.3. Technology Transfer

The availability of the dynamic MRS post-processing algorithms described in this and previous progress reports will be open to the public through open-source software will allow for the ready transfer of the technology developed in this study to the scientific community. All too often the scientific community has difficulty in reproducing methods that are developed “in-house” as the details are often not available. With an open-source approach, much like the aim for FITBIR, these methods will be available for the whole of the scientific community and could provide potential benefits both in our field and other disciplines.

Society

The publication of these novel MRS methods will provide advances in the field of research that can potentially impact society through improved diagnosis and detection of disease.

5. CHANGES/PROBLEMS:

5.1. Changes in approach

There have been no changes to the approach.

5.2. Actual or anticipated problems

The COVID pandemic began at the beginning of this study period. During that time all MRI research scans were suspended however these restrictions were lifted in June. This allowed us to collect data in several healthy subjects.

5.3. Impact on expenditures

Due to the delay of the start of the study, we have significantly reduced spending on the grant. A no-cost extension was approved to carry out the study this year.

5.4. Significant changes in use or care of human subjects

None.

6. PRODUCTS:

6.1. Publications, conference papers, and presentations

1. Alosco ML, Tripodis Y, Rowland B, Chua AS, Liao H, Martin B, Jarnagin J, Chaisson CE, Pasternak O, Karmacharya S, Koerte IK, Cantu RC, Kowall NW, McKee AC, Shenton ME, Greenwald R, McClean M, Stern RA, **Lin AP**. A magnetic resonance spectroscopy investigation in symptomatic former NFL players.Brain Imaging Behav. 2020 Oct;14(5):1419-1429.
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7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	Alexander Lin
Project Role:	Principal Investigator
Research Identifier:	orcid.org/0000-0001-8236-880X
Nearest Person Month Worked	4
Contribution to Project	Dr. Lin has been addressing the concerns of the IRB and MR safety committees by conducting tests of the 13C coil for safety purposes. The results of the tests have been sent to the committee for evaluation.

Name:	Huijin 'Vicky' Liao
Project Role:	Study Coordinator
Research Identifier:	https://www.linkedin.com/in/huijun-vicky-liao-3b682451
Nearest Person Month Worked	4
Contribution to Project	Ms. Liao has assisted Dr. Lin with the acquisition of the 7T spectroscopy data and assisted with the submission of the IRB documentation.

Name:	Eduardo Coello
Project Role:	Postdoc
Research Identifier:	n/a
Nearest Person Month Worked	3
Contribution to Project	Dr. Coello has been preparing for the start of the project by developing a 7T basis set specific to our system.

Name:	Robert Stern
Project Role:	Subaward PI
Research Identifier:	orcid.org/0000-0002-5008-077X
Nearest Person Month Worked	1
Contribution to Project	Dr. Stern has assisted with the recruitment process as described above.

Name:	Michael Alosco
Project Role:	Subaward Study Coordinator
Research Identifier:	n/a
Nearest Person Month Worked	2
Contribution to Project	Dr. Alosco has been in contact with both NFL and AD participants through his own work at BU and will assist with recruitment of subjects for this study.

7.1. Change in Personnel

Nothing to Report.

7.2. Other Organizations

Organization Name: Boston University School of Medicine

Location of Organization: Boston, Massachusetts

Partner's contribution to the project:

- Collaboration: Drs. Robert Stern and Michael Alosco are our collaborators and are responsible for recruitment of subjects, acquisition of neuropsych results, and upload of that data into FITBIR.

8. SPECIAL REPORTING REQUIREMENTS

Nothing to Report.

9. APPENDICES:

N/A