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A Longitudinal Assessment of Structural and Chemical Alterations in MMA Fighters

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We thank the reviewer for their comments. All critiques have been addressed in the revised manuscript as indicated in our point-by-point response below. We have also bolded all changes in the revised manuscript to facilitate review.

Comments to the Author
The authors have addressed many of the minor issues discussed in the initial review however there remains several major issues:

1. It is unfortunate that a more focused analysis of the MRSI data is not possible as this leaves the rather large looming issue of why there were no significant differences found between individuals with high exposure to repetitive head injuries and healthy controls. In the reviewer response, the authors state that their method is sensitive to small overall GM and WM changes as shown in previous work yet there is no difference observed here. This discrepancy requires a greater focus in the discussion apart from the fact that longitudinal changes are found. Is this due to some history of head injury in the healthy controls? Is the method utilized insensitive to these group differences?

We politely disagree with the reviewer. Our previous response indicated that within subjects designs are more sensitive than between subjects design. As a general rule, between subjects variance is large in any biological system relative to potential treatment and/or group effects. Thus, our previous response is consistent with current observations and what is currently stated in the manuscript (i.e., that within subject comparisons are likely to be more powerful). We have also provided effect sizes so that others in the community can determine the sample size necessary for achieving between-group significance based on our current data. There is no reason to believe that the methods utilized in the current paper would be insensitive to group differences if 1) our sample size were larger and 2) between group effect sizes were of sufficient magnitude.

The previous version of the results section indicates that a subset of HC (6/14) did have a history of head injury. This has also been included as a limitation in the current discussion section per the reviewer's suggestion.

2. Also in relation to their previous studies using the same method, changes in Cr and Glx are observed but not observed in this cohort of subjects. It would be important to discuss why these changes are not observed in this particular cohort of subjects.

We have revised the discussion to include potential reasons why findings may not have replicated across current and previous studies (single mTBI versus repetitive mTBI, diet and other issues inherent to more unique samples such as MMA fighters).

3. While the authors are highly experienced in MR spectroscopy, it would be helpful to include measures that allows the reader to assess the quality of the data.

a. The authors have described that they have measured NAA line width and SNR and they are not significantly different between the MMA and HC, the mean and standard deviations of both of those measures are still not reported in the text or tables.

We agree with the reviewer that this information is important to the readers as well as reviewers. Therefore, the mean and standard deviation of these measures are now included in the paper as requested by the reviewer.

b. The number of spectra rejected per scan based on the quality control criteria are not described. This would also help assess the overall data quality.

The average number of spectra rejected is now included in supplemental materials parsed by metabolite and group. As expected, few spectra were rejected in our high quality data set.

4. The authors argue that myoinositol was not analyzed due to lack of significance in previous studies by the authors. However given that repetitive brain injury is a very different injury than the single acute mTBI previously studied, it would be important to include this additional analysis. In addition, several other papers, including the one they cite (Kierans 2014) have shown changes in mI.

We agree with the reviewer that repetitive mTBI may results in differences not apparent in single mTBI and have included this information in the discussion. We have also added both choline and myoinositol as supplementary analyses in our paper per the reviewer's request.

5. Figure S1: The location of the MRSI slab remains somewhat unclear. Are the axial images the top and bottom of the MRSI slab? If so, this should be described in the figure legend. It would also be helpful to show the slab thickness in the sagittal image.

We have updated the figure legend to indicate that the position of the axial images. We have also updated the sagittal image to reflect the slab thickness.

A Longitudinal Assessment of Structural and Chemical Alterations in MMA Fighters

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Keywords: Volumetrics; spectroscopy; concussion; repetitive injury; longitudinal

Abstract

Growing evidence suggests that temporally proximal acute concussions and repetitive sub-concussive head injuries may lead to long-term neurological deficits. However, the underlying mechanisms of injury and their relative time-scales are not well documented in human injury models. The current study therefore investigated whether biomarkers of brain chemistry (MR spectroscopy: N-acetylaspartate, combined glutamate and glutamine, total creatine**, choline compounds and myo-inositol**) and structure (cortical thickness, white matter/sub-cortical volume) differed between mixed martial artists (MMA; N=13) and matched healthy controls without a history of contact sport participation (HC; N=14). A subset of participants (MMA=9; HC=10) returned for follow-up visits, with MMA (N=3) with clinician-documented acute concussions also scanned serially. As expected, MMA self-reported a higher incidence of previous concussions and significantly more cognitive symptoms during prior concussion recovery. Fighters also exhibited reduced memory and processing speed relative to controls on neuropsychological testing coupled with cortical thinning in the left posterior cingulate gyrus and right occipital cortex at baseline assessment. Over a 1 year follow-up period, MMA experienced a significant decrease in both white matter volume and N-acetylaspartate concentration, as well as relative thinning in the left middle and superior frontal gyri. These longitudinal changes did not correlate with self-reported metrics of injury (i.e., fight diary). In contrast, HC did not exhibit significant longitudinal changes over a 4 month follow-up period ($p > 0.05$). Collectively, current results provide preliminary evidence of progressive changes in brain chemistry and structure over a relatively short time period in individuals with high exposure to repetitive head hits. These findings require replication in independent samples.

Introduction

The neurobehavioral effects of concussion (mild traumatic brain injury; mTBI) can be relatively brief (several weeks) or devastating (lifetime impairment), and likely vary both as a function of the magnitude of injury[1] as well as the number of temporally proximal insults.[2] Previous research suggests that history of concussion is associated with increased baseline symptomatology, that a single concussive event dramatically increases the risk of repeat concussions and that repeat concussions (e.g., within the same season) increase the risk of long-term cognitive and psychiatric dysregulation.[3-5] Moreover, individuals with a history of repetitive mTBI (professional athletes and military personnel) exhibit a unique pattern of neuropathology upon autopsy, as well as an increased incidence of dementia and emotional dysregulation during their lifetime.[6-8] Similar findings of increased neurobehavioral symptoms and neuropathology have also been reported in animal models of repetitive head trauma relative to single injuries.[2;9] Finally, there is recent evidence of *in vivo* neurophysiological changes in brain function due to sub-concussive injuries,[10;11] a topic yet to be fully investigated using measures of brain chemistry and structure.

^{1}H-MRS provides a potential measurement of brain injury within both gray (GM) and white (WM) matter.[12] The N-acetylaspartate (NAA) signal, often measured as the sum of NAA and the weaker and poorly resolved N-acetyl-aspartylglutamate (NAAG) signal, is frequently observed to be decreased in acute and semi-acutely injured mTBI patients.[13-15] A reduced ratio of NAA to total creatine (creatine and phosphocreatine; Cre) has been observed in the prefrontal GM and WM in athletes following concussion,[16-20] with the decrease in NAA reportedly greater for first time injuries.[17] Importantly, both cross-sectional[21] and longitudinal[16;19;20] studies have suggested that NAA increases (i.e., improves) post-injury, presumably as a function of recovery.

Similar findings of decreased NAA and subsequent recovery have been observed in animal models in brain regions without axonal injury or histological evidence of cell death,[22] suggesting that ^{1}H-MRS may be more sensitive than anatomical imaging for detecting trauma. However, not all studies have reported alterations in NAA following mTBI,[23;24] and to our knowledge no studies have examined longitudinal changes in metabolites as a function of participation in sports activities with high-risk of repetitive injury.

Other work suggests that Cre, the signal from mobile choline-containing molecules (Cho)**, myo-inositol (mI)** and the combined signal (Glx) from glutamate (Glu) and glutamine (Gln) may also be promising markers of long-term repetitive injury following mTBI. Findings from previous studies on Cre are mixed, with reports of both increased[21;25] and decreased[26] Cre in WM during the semi-acute stage of mTBI. Others have reported both decreased Glu or Glx in the primary motor cortex[16] and WM[21] following injury, as well as increased Glx in GM[21;25] and deep GM nuclei.[23] **In addition, increased mI has been reported in the putamen following single-episode mTBI,[23] but not in global WM or GM.[18;27]** Finally, increased Cho/Cre has also been observed during the semi-acute injury stage in WM,[28] GM[13] and sub-cortical structures.[28]

Emerging evidence from studies on professional athletes[7;8] suggests a higher incidence of frontal and medial temporal lobe atrophy in individuals with CTE, and atrophy is commonly noted in more severe forms of TBI.[29] Previous work with a chronically symptomatic (81.3% with post-traumatic stress disorder), mixed injury (mild and moderate TBI) cohort have reported longitudinal differences in whole brain parenchyma and cerebral white matter 1-2 years post injury.[30] Others report atrophic whole-brain changes for complicated mTBI patients 6 months post-injury.[31] Typical mTBI patients show no evidence of atrophy during similar or even shorter post-injury times,[31-33] although atrophy has been observed at one year post-injury.[33]

Atrophic changes may also occur as a function of repetitive injury. For example, smaller subcortical volumes have been documented in professional fighters,[34] and fight exposure has been found to inversely correlate with subcortical volume in a mixed sample of boxers and MMA fighters.[35] Changes in subcortical structure volume in this sample were also inversely correlated with measures of impulsivity.[36] Furthermore, a study of noncombative martial artists reported increased grey matter volume in pre- and supplementary motor areas.[37] Finally, a recent study indicated decreased hippocampal volumes in football players relative to athlete controls, with history of mTBI also a contributing factor for volume loss.[38] To our knowledge, there have been few studies examining atrophic changes on a longitudinal basis in high-risk athletes.

Based on our previous work in single-episode mTBI,[21;32] we conducted a preliminary investigation on whether biomarkers of brain chemistry (spectroscopy: NAA, Glx and Cre) and structure (atrophy: cortical thickness, total white matter volume, and thalami/hippocampi volume) were different between high-risk athletes (mixed-martial artists; MMA) and healthy controls without a history of contact sport participation. **Supplementary analyses were conducted on both Cho and mI.** Our second aim examined whether these biomarkers changed over a one-year period, presumably as a function of participation in a sport with a high risk for repetitive mTBI. Where feasible, case study data is also presented to determine how these metrics were affected by acute mTBI.

Methods

Participants:

A total of 14 mixed martial arts (MMA) and 16 healthy controls (HC) were recruited for the current study. All fighters were recruited from a local training gym in Albuquerque, New Mexico. To be included in the study, MMA fighters were required to be between the ages of 18 and 45, have no contraindications for MRI scanning and to be participating in a regular training routine, including professionally organized fighting events as well as weekly sparring. The majority of controls were directly recruited to match patients in terms of gender, age (±4 years) and education (±4 years). HC were also required to be physically active (strenuous exercise at least 2 times per week), and have no history of regular, organized participation in a contact sport.

Participants were excluded if there was a history of mental illness, history of neurological disorders, a previous traumatic brain injury with more than 30 minutes of loss of consciousness and a recent history of substance abuse. One MMA was diagnosed with a pre-existing neurological disorder by an independent physician during the course of the study and was subsequently eliminated, leaving a total of 13 MMA (11 males, 2 females; 28.2±4.9 years old; 13.9±1.7 years of education). Two HC were excluded prior to analyses due to failures with data acquisition (e.g., MRI-induced claustrophobia), leaving a total of 14 matched (12 males, 2 females) HC (28.1±5.1 years old; 15.8±2.3 years of education) in the final sample. All participants provided informed consent according to institutional guidelines at the University of New Mexico.

All participants were evaluated both clinically and with an extensive neuroimaging battery. MMA fighters were asked to return for a repeat scan approximately 1 year from their initial visit (mean = 385.9±32.2 days), and all fighters were asked to complete a fight history

diary (professional fights, sparring events, concussions) using a method similar to the Timeline Followback Calendar.[39] Fighters completed the diary every 3-5 months by phone or at the time of a clinical visit. HC were evaluated identically to MMA in terms of clinical and neuroimaging assessments, with the exception that the follow-up visit occurred between three to six months post baseline (mean = 169.3±37.3 days). HC were also not required to complete a fight diary. Clinical and imaging protocols occurred the same day for the majority (>90%) of visits. A total of 5 MMA participants self-reported experiencing acute mTBI during the course of the 1 year follow-up period. Three of the self-reported mTBIs were confirmed within 24 hours by a clinical neuropsychologist (A.M.). One of the 3 participants experienced multiple concussions, which was also confirmed by the study team. These subjects were rescanned serially (acute (within 72 hours); N = 3, semi-acute (1 month); N = 2, and early chronic (approximately 4 months); N = 3) post-injury. Structural images from each scan were reviewed by a certified radiologist for any abnormal findings and are summarized in Supplemental Table S1.

Clinical Assessment:

Composite indices were calculated for the following cognitive domains: attention (Paced Auditory Serial Addition Test [PASAT], Stroop color-word scores, and Wechsler Adult Intelligence Scale-Third Edition [WAIS-III] digit span), working memory (WAIS-III letter number sequence, and Wechsler Adult Intelligence Scale-Third Edition [WAIS-III] digits backward), processing speed (WAIS-III digit symbol coding, Trails A), executive function (Trails B and Controlled Oral Word Association FAS-test), and memory (Hopkins Verbal Learning Test- Revised Edition: immediate recall, long-delay free recall, discrimination index). The Wechsler Test of Adult Reading (WTAR) was also used to provide an estimate of overall

pre-morbid intellectual functioning. The Test of Memory and Malingering (TOMM) allowed assessment of participant effort and cooperation.

Self-report for measures of somatic, cognitive and emotional complaints common in concussion (Neurobehavioral Symptom Inventory; NBSI), history of previous concussions and the degree of symptoms associated with previous concussions (a modified version of the Rivermead Post-Concussion Symptoms Questionnaire; RPSQ) and general emotional status (State-Trait Anxiety Index and Beck Depression Inventory-Second Edition; STAI and BDI-II, respectively) were also assessed. Balance was assessed with the balance error scoring system (BESS). For all measures, raw data were converted to T-scores using published age-specific norms whenever possible.

Image Acquisition:

All imaging sequences were collected on a Siemens 3 Tesla TrioTim scanner with a 12 channel head coil. High resolution T_1-weighted [TE (echo time) = 1.64, 3.5, 5.36, 7.22, 9.08 ms, TR (repetition time) = 2.53 s, TI (inversion time) =1.2s, 7° flip angle, number of excitations (NEX) = 1, slice thickness = 1 mm, FOV (field of view) = 256 mm, resolution = 256 x 256], T_2-weighted [TE = 77.0 ms, TR = 1.55 s, flip angle 152˚, NEX = 1, slice thickness = 1.5 mm, FOV = 220 mm, matrix = 192x192, voxel size = 1.15x1.15x1.5 mm^3], FLAIR [TE = 88.0 ms, TR = 10.4 s, flip angle 160˚, NEX = 1, slice thickness = 3 mm, FOV = 256 mm, matrix = 320x320, voxel size = 0.8x0.8x3 mm^3], SWI [TE = 20.0 ms, TR = 28 ms, flip angle 15˚, NEX = 1, slice thickness = 1.5 mm, FOV = 240 mm, matrix = 256x256, voxel size = 1.0x0.9x1.5 mm^3] and MRA [TE = 3.59 ms, TR = 20 ms, flip angle 18˚, NEX = 1, slice thickness = 0.5 mm, FOV = 200 mm, matrix = 384x384, voxel size = 0.5x0.5x0.5 mm^3] images were collected for all

participants at the baseline visit. All anatomical scans were repeated at the follow-up visits with the exception of the MRA.

The ^{1}H-MRSI sequences used in the current study were identical to previous publications.[21;25] Specifically, phase-encoded versions of a point-resolved spectroscopy sequence (PRESS), both with and without water presaturation (TE = 40ms, TR = 1500ms, slice thickness = 15mm, FOV = 220 × 220 mm, circular k-space sampling (radius = 24), total scan time = 572s), were collected for all participants for all visits. The nominal voxel size was 6.875 × 6.875 × 15 mm after zero-filling in k-space to 32 × 32 samples. The ^{1}H-MRSI volume of interest (VOI) was prescribed off the T2 image and was located approximately 1.8 cm above the lateral ventricles roughly parallel to the anterior-posterior commissure axis (Supplementary Figure S1). The volume of interest (VOI) was dependent on head size but typically ranged between 75 and 95 cm^3 and was demarcated with strong saturation bands to reduce chemical shift artifacts (as prescribed automatically by the standard Siemens spin echo ^{1}H-MRSI sequence). Additional saturation bands were manually placed around the VOI to further reduce signal contamination. This was followed by automatic shimming. To eliminate the chemical shift artifact between the unsuppressed water signal and the NAA methyl signal, the transmitter was set to the frequency of the NAA methyl peak during the acquisition of the metabolite spectra and to the frequency of the water peak during the acquisition of the unsuppressed water spectra. The outermost rows and columns of the volume of interest were excluded from analysis to reduce the inclusion of voxels with chemical shift artifact for other metabolites of interest.

T_1 Structural Data

SPM (v5) was used to segment the T_1 data into cerebral spinal fluid (CSF), WM and GM probability maps. The FreeSurfer reconstruction pipeline (version 5.3) was used to generate

cortical thickness values and regions of interest (ROI) based on standard labels. All FreeSurfer results were visually inspected for quality assurance and corrected when necessary. The FreeSurfer longitudinal pipeline was used to maximize between-visit image registration, as well as account for the variable time between visits for fighters and controls.[40] Specifically, a "symmetrized" percent change (SPC) value was derived from the rate of change of thickness using the formula $\frac{(V2-V1)/(Time2-Time1)}{0.5(V1+V2)}$. Cortical thickness maps were projected to surface-based vertices, smoothed with 10 mm FWHM Gaussian kernel and compared across groups. Results were corrected for multiple comparisons at $p < 0.05$ (vertex-wise and cluster-wise) using Monte Carlo simulations in the FreeSurfer package. Volumetric analyses focused on overall white matter, hippocampi and thalami volumes, with total intracranial volume serving as a covariate.

^{1}H-MRSI Processing:

A Hamming filter with a 50% window width was first applied to the ^{1}H-MRSI data followed by a 2D spatial Fourier transformation. The time domain ^{1}H-MRSI data were analyzed using LCModel (v6.2-0T) on a voxel-wise basis with tissue water as a concentration reference.[41] LCModel spectra model were corrected for partial volume effects (CSF, WM, and GM content) and for T_1 and T_2 relaxation effects based on SPM segmentation results using previously described methods (Supplementary Figure S2 for example spectra).[42] Linear regression was then used to estimate each metabolites' concentration in "pure" tissue by fitting all voxels against the normalized GM fraction derived from segmentation values (e.g., extrapolation to GM = 0 for pure WM and to GM =1 for pure GM). **Voxel spectra with Cramer-Rao lower bounds greater than 20% were eliminated prior to fitting the regression (see Supplemental Table 2). Additional quality assurance metrics included the mean line-width (full-width at half**

maximum) and mean signal-to-noise ratio for the NAA fit (as calculated by LCModel), as well as the percentage of usable spectra following Cramer-Rao lower bounds filtering.

Quality Assurance Protocol

Outlier analyses were performed on all data prior to analyses. One HC was an outlier on their Visit 1 TOMM score and exhibited poor performance on other neuropsychological domains across both visits. However, the participant responded consistently on other clinical measures (i.e., reverse scoring items), and their quality assurance metrics for their imaging data were within normal ranges. Therefore, this participant's neuropsychological data were eliminated from all further analyses while demographic, clinical (i.e., self-report) and imaging data were retained.

One MMA was an outlier on GM NAA concentration and on mean NAA line-width measure. Another MMA was an outlier on GM Glx concentration, and both participants were removed from the specific metabolite analyses. **There were no outliers on any of the other metabolites (Glx, Cre, Cho or mI).** The amount of overlap between the aligned baseline and follow-up visits PRESS voxels was calculated to verify that tissue sampling was comparable across visits. Two MMA participants exhibited minimal voxel overlap (< 60%) at the one year follow-up visit, and supplementary longitudinal ^{1}H-MRSI analyses were conducted without data from these participants to verify primary results. Effect sizes are reported using either Cohen's d or a repeated measures effect size that correctly accounts for correlation between time-points given the limited sample size.[43]

Results

Neuropsychological and Clinical measures:

Please see Table 1 for all clinical data. There were no differences between the two groups on age, sex, levels of testing effort, balance performance or hand preference ($p > 0.10$). Both educational attainment ($t_{25} = 2.47$, $p = .021$) and estimated premorbid intelligence ($t_{1,24} = 2.29$ $p = .031$) were greater for HC relative to fighters. The composite indices of attention, working memory, memory, processing speed, and executive functioning were correlated to varying degrees (r's ranged from 0.01 to 0.71). A MANCOVA examining difference in cognitive functioning (WTAR as a covariate) was not significant for the multivariate effect of group ($p > 0.10$), although univariate tests indicated significantly reduced performance for MMA fighters for both memory ($F_{1,23} = 5.75$, p = 0.025) and processing speed ($F_{1,23} = 4.45$, p = 0.046).

Baseline self-reports of somatic, cognitive and emotional issues common in concussion (NBSI) were strongly correlated (r's ranged from 0.80 to 0.90). A MANOVA indicated that multivariate effect of group was non-significant ($p > 0.10$), although univariate tests indicated significantly elevated somatic complaints ($F_{1,25} = 4.39$ p =0 .046) in MMA fighters.

As expected, a higher proportion of MMA fighters reported experiencing at least one previous mTBI ($X^2 = 10.56$, $p = 0.001$; 6/14 HC; 13/13 MMA), and also reported a significantly (Mann-Whitney U = 170.00, z = 4.00; $p < 0.001$) higher average life-time incidence of mTBI (HC = 0.43±0.5; MMA = 2.23±1.1). A MANOVA examining the average acute somatic and cognitive symptoms resulting from previous head injuries (modified version of the Rivermead Post-Concussion Symptoms Questionnaire) returned a significant multivariate effect of group ($F_{2,16} = 4.57$, p = 0 .027), with univariate tests indicating that MMA fighters reported significantly more cognitive (RPQ1-3; $F_{1,17} = 9.51$, p = 0 .007) symptoms during the recovery from their previous mTBIs.

Please see Table 1 for a semi-quantitative analysis of self-reported fight history for the MMA participants during the course of the study.

Baseline ^{1}H-MRSI:

There were no significant differences ($p > 0.10$) between the groups on NAA line-width (MMA = 0.035 +/-0.003; HC = 0.036 +/-0.004), signal-to-noise ratio (MMA = 16.171 +/-0.778; HC = 15.916 +/- 1.008) or percentage of usable spectra (Supplemental Table 2). One-way ANOVAs were conducted to examine group differences in WM and GM metabolites (NAA, Cre and Glx), using the Bonferroni method to control for false positives within each tissue type. The univariate effects of diagnosis were not significant ($p > 0.10$) for either GM or WM metabolites (GM Cohen's d: -0.38 to 0.17; WM Cohen's d: -0.25 to 0.60). **Supplemental analyses examining Cho and mI were also negative ($p > 0.10$).**

Baseline Structural Results:

There were no significant differences in total intracranial volume between groups at the baseline visit ($p > 0.10$). Three ANCOVAs examining for significant volumetric differences for total WM ($p > 0.10$; Cohen's d: -0.62) or in key subcortical structures ($p > 0.10$; Cohen's d: thalami = -0.03; hippocampi = 0.22) were not significant following Bonferroni correction. Results from the FreeSurfer analyses indicated that MMA fighters had significantly reduced thickness in the left posterior cingulate gyrus/precuneus as well as in the right occipital lobule relative to HC following corrections for multiple comparisons (Figure 1).

Longitudinal Analyses:

Ten of the 13 eligible MMA fighters and 10 of the 14 HC returned for follow-up visit. Follow-up ^{1}H-MRSI data for one MMA were lost secondary to acquisition issues. MMA showed

a significant decrease in WM concentration of NAA between the baseline and follow-up visits (t_8 = -2.90; p = 0.020; RM effect size: -1.02), with no differences in GM concentrations (p > 0.10). There were no significant differences across visits for HC for either WM or GM **Cre and Glx concentrations** (p > 0.10; RM effect size: -0.39 - 0.24). Supplemental analyses indicated that the significant difference in MMA WM NAA was only a statistical trend (t_6 = -1.99; p = 0.094) when the two participants with poor voxel overlap were eliminated. However, the effect size remained similar (RM effect size: -0.80) suggesting that this was a result of reduced power. **There were also no differences in Cho and mI for fighters (p > 0.10).**

There were no significant longitudinal differences between groups in terms of changes in total intracranial volume (p > 0.10). FreeSurfer results indicated that HC and MMA exhibited significantly different rates of change in cortical thickness within the left rostral middle and superior frontal gyrus, with HC SPC increasing from baseline and MMA decreasing (Figure 2). Repeated measure ANCOVAs indicated that there was a significant decrease in WM volume for the MMA group over the one year interval ($F_{1,8}$ = 11.02 p = 0.011; RM effect size = -1.40), with no changes in thalamic or hippocampal volumes (p > 0.10). In contrast, HC exhibited no changes in WM or thalamic volume (p > 0.10), with a non-significant finding of decreased hippocampal volume at their second visit ($F_{1,8}$ = 4.04 p =0 .079; RM effect size = -0.72). Effect sizes were variable for both MMA (RM effect size: -1.40 – 0.56) and HC (RM effect size: -0.72 – -0.06).

Two multiple regressions examined whether significant changes in WM NAA and volume (dependent variables) were related to self-reported number of fights, normalized days sparring and history of a post-baseline mTBI (independent variables) in MMA fighters. However, the overall model was not significant for predicting change in WM NAA levels or total volume (p > 0.10).

Effects of Acute Concussions

A total of 5 out of 10 MMA participants self-reported an acute mTBI during the course of the study. Acute events for 3 of the 5 participants were verified by a clinical neuropsychologist within 24 hours of injury and were followed with serial neuroimaging. Supplemental analyses were therefore performed comparing MMA participants with and without a self-reported history of acute mTBI during the one-year study period. There were no significant differences ($p > 0.10$) for changes (Follow-up - Baseline visit) in WM volume, WM NAA concentrations or relative cortical thinning within the left rostral middle and superior frontal gyrus for those with a history of acute mTBI (Figure 3).

Serial ^{1}H-MRS metabolite data (Figure 4A&B) and volume estimates (Figure 4C) are plotted for the three MMA fighters that were evaluated for acute mTBI while participating in this study. Due to the small N, these data were not evaluated quantitatively. The mean and two times the standard deviation of baseline (N=14) and longitudinal (N=10) HC data and MMA who were not scanned serially (N = 10) are also provided for an unbiased reference. A qualitative examination of the case study data indicates varying patterns of both metabolites and volume estimates from three days to approximately four months post-injury.

Discussion

Recently, there has been a dramatic sea change in public and scientific opinions regarding the consequences of mTBI. It was initially believed that concussion resulted in limited behavioral and no long-term neurological consequences,[44] with standard clinical neuroimaging methods (computed tomography scans; T_1- and T_2-weighted images) typically negative for the majority of concussed patients.[45] However, recent data suggest that the life-long effects of concussion may be more severe,[7] especially when concussions occur on a repetitive basis over extended periods of time (i.e., years). Currently, there is a large gap in knowledge regarding the more immediate consequences of concussion and repetitive head trauma. Our results indicate longitudinal changes in WM NAA concentrations, WM volume, and relative cortical thinning in a high-risk sample of actively competing MMA athletes. These changes occurred over the course of a single year, providing preliminary evidence that progressive brain changes may occur over a relatively short time period.

Not surprisingly,[46;47] MMA athletes in the current sample reported a higher lifetime incidence of mTBI, increased cognitive symptoms during past concussion recovery, and more somatic complaints relative to controls. These findings are consistent with previous results suggesting that a history of concussion is a risk factor for both prolonged recovery as well as for experiencing new concussions.[4] MMA fighters also performed worse on formal tests of memory and processing speed, consistent with previous work demonstrating a dose-dependent relationship between the number of previous concussions and baseline neurocognitive performance.[48] Although these cognitive domains are frequently implicated in studies of TBI,[49] the two groups also differed in years of education and estimated premorbid intelligence, both of

which contribute to formal cognitive testing[50] and serve as potential confounds for the current study.

Unlike our previous results,[21;25] we did not observe cross-sectional differences in either GM or WM metabolites between MMA and HC during the baseline assessment. These null findings may be secondary to sample characteristics (sub-acute versus more chronic injuries, single versus repetitive mTBI) or relative to the unique samples that were studied (e.g., MMA fighters). However, MMA fighters exhibited a significant decrease in WM NAA over the course of our one year follow-up period. NAA is an amino acid that is synthesized in the neuronal mitochondria[51] and is widely interpreted as a marker of GM and WM integrity.[52] An initial decrease and subsequent recovery of NAA has also been observed in rodent models of TBI in regions of brain without axonal injury or histological evidence of cell death.[22] Moreover, as reviewed in the introduction, a decrease in WM and GM NAA is one of the most widely reported finding across the spectrum of TBI[12] including the acute and sub-acute phases of concussion.[16-20] Changes in NAA correlate with initial Glasgow Coma Scale[53] and predicts long-term cognitive outcome following TBI.[54] Thus, current findings suggest that participation in high-risk combat athletics may result in changes in WM integrity, a supposition that can be further evaluated with diffusion tensor imaging.[55] However, longitudinal results from other non-diagnosed athletic samples have reported no changes in NAA concentration,[24] suggesting the need for replication in an independent sample.

MMA fighters also exhibited cortical thinning in the right occipital cortex, the left posterior cingulate cortex and precuneus relative to controls at baseline, which may be a result of previous history of concussions. The posterior cingulate gyrus/precuneus corresponds to the posterior hub of the default mode network (DMN),[56] exhibiting both high metabolic activity and

high connectivity with the rest of the brain.[57] The posterior hub of the DMN has also been found to exhibit differences in functional connectivity in previous studies of mild[58] and more severe TBI.[59] Moreover, longitudinal evidence of a differential rate of cortical thinning (increased for MMA fighters) was also observed in the left rostral middle and superior frontal gyrus over the course of one year, as well as a decrease in total WM volume. Previous work suggests that atrophy may occur only in the later stages of mTBI,[32;33] may be limited to patients with complicated mTBI,[31] or may be limited to chronically symptomatic patients.[30] In addition, cross-sectional differences in sub-cortical and ventricle volumes have been documented in professional fighters,[34] with an inverse relationship between fight exposure and subcortical volumes[35] and impulsivity and change in subcortical volume.[36] Others have reported decreased hippocampal volumes in American football athletes relative to controls, with a further reduction for individuals with a self-reported history of mTBI.[38] As evidenced by prior research,[33] the longitudinal design of the current study as well as the longer follow-up period may have increased the sensitivity for detecting subtle differences resulting from repeated exposure to traumatic events.

Current findings have several potential implications for participation in sports with a high likelihood of concussion. First, future studies aimed at improving the safety of participants are needed, such as recent data indicating that the use of protective boxing gloves and head padding can significantly reduce the impact force of simulated head strikes[60] as well as rule changes in American football that may decrease the incidence of concussion (i.e., kick-offs). Second, current and previous results highlight the critical need for objective biomarkers that indicate neuronal injury progression rather than reliance on subject self-report that may reflect non-specific symptomatology and/or symptom underreporting. The latter is a common problem in

athletes following mTBI, and it has been estimated that the failure to report concussion symptoms in high school football may be as high as 53%.[61] Others have shown that a second concussion occurring during the semi-acute stage of injury results in a further decrease in NAA and/or further delays the recovery of NAA to baseline levels.[19] Therefore, the identification of reliable longitudinal biomarkers related to repeated concussive and sub-concussive hits should ultimately produce physiological targets for the development of objective diagnostic (did this patient suffer a significant concussion?) as well as prognostic (can this patient safely return to play?) decisions. However, as illustrated by our serial neuroimaging data, identifying concussions by any single biomarker is likely to be challenging.

There are several limitations to the study that should be noted. First, the current study was based on a relatively small sample of MMA fighters as well as controls, which increases the likelihood of sampling bias and under-powered **results in between group designs.** Second, the amount of time between follow-up visits was different across the two groups, which may have potentially reduced the sensitivity of longitudinal data. To mitigate both of these limitations, the majority of our longitudinal analyses were based on within-subject comparisons, **and effect sizes are reported throughout the manuscript.** Third, 5/10 of the returning MMA fighters self-reported a concussion between the baseline and follow-up scans, with concussions for three of the fighters verified within 24 hours post-injury by the study team. Although there were no differences between MMA fighters with and without a concussive event at the follow-up visit, we cannot disambiguate whether longitudinal changes resulted from “diagnosable” events or repetitive trauma, or were due to some other factor. Finally, our control group was purposefully selected based on their levels of physical fitness and their history of non-participation in contact sports. The selection of this control group reduced the likelihood of potential exercise

confounds,[37] and reduced the likelihood of other confounding factors (e.g., history of performance enhancing drugs) present in a former contact-sport athletic control group.[38] **However, the current control sample included a subset of participants (6/14) with a history of head trauma, which may have contributed to null findings during group-wise metabolite comparisons.** Similarly, other factors unique to the MMA cohort (e.g., diet, more strenuous exercise routine, supplement usage, etc.) may have also contributed to observed cross-sectional differences.

In summary, there is a growing body of literature highlighting the possible deleterious effects of repetitive hits as well as concussions in athletes over a relatively short period of time.[11;55] Current results suggest that participation in high risk athletic events may alter basic WM neurochemistry (decreased NAA) as well as result in structural changes in both GM and WM. Future studies that include larger sample sizes are need to both replicate current effects as well as potentially delineate the effects of acute trauma.

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Table 1: Neuropsychological and Clinical Summary Measures

	MMA	**HC**	**Cohen's d**
Demographic	Mean (SD)	Mean (SD)	
Age	28.23 (4.94)	28.14 (5.08)	0.02
Education	13.85 (1.68)	15.79 (2.33)	-0.96
HQ	69.60 (52.01)	82.08 (52.96)	-0.24
Clinical			
Attention▲	52.36 (7.96)	54.00(5.17)	-0.25
Memory▲	39.15(8.90)	47.34(6.32)	-1.06
WM▲	52.92(9.34)	52.58(7.57)	0.04
PS▲	50.51(5.97)	56.57(6.97)	-0.93
EF▲	49.90 (7.95)	48.98(8.66)	0.11
WTAR	52.26 (10.02)	60.28(7.71)	-0.90
TOMM	53.65 (5.10)	51.21(6.96)	0.40
BESS	12.23(5.46)	12.64(4.01)	-0.09
Self Report-Emotional			
BDI-II	42.39 (4.78)	41.14 (4.25)	0.28
STAI-S	43.46 (9.73)	40.50 (6.20)	0.36
Self Report-Concussion History			
Lifetime mTBIs	2.23 (1.09)	0.43 (0.51)	2.11
Average RPSQ-Som	2.90 (2.12)	0.17 (0.41)	1.79
Average RPSQ-Cog	4.97 (7.01)	0.00 (0.00)	1.00
NBSI-Som	4.62 (5.11)	1.50 (2.14)	0.80
NBSI-Cog	2.62 (2.87)	1.07 (1.77)	0.65
NBSI-Emot	4.92 (4.50)	2.57 (2.87)	0.62
Self Report-Fight History			
Fights between visits	2.40 (1.51)	N/A	
Normalized Days of Sparring‡	0.26 (0.09)	N/A	
Normalized Days of Breaks‡	0.14 (0.10)	N/A	
Acute mTBI	5/10	N/A	
Days Baseline to Follow-up			
Imaging	385.9 (32.24)	169.3 (37.25)	
Neuropsych	384.3 (29.88)	169.3 (37.25)	

Abbreviations: HQ = handedness quotient; WM = working memory; PS = processing speed; EF = executive function; WTAR = Wechsler Test of Adult Reading; TOMM = Test of Memory Malingering; BDI-II = Beck Depression Inventory – Second Edition; STAI-S = State-Trait Anxiety Index – State; RPSQ & NBSI-Cog = Rivermead Post-Concussion Symptoms Questionnaire and Neurobehavioral Symptom Inventory cognitive complaints (Som = somatic complaints, Emot = emotional complaints); SD = standard deviation; N/A = not applicable

▲Means, standard deviations and effect sizes for neuropsychological indices reported following correction for WTAR as covariate. ₮Normalization presented as ratio of number of days reporting activity to number of days between baseline and follow-up visit.

Figure Legends

Figure 1: Figure 1 depicts baseline differences in cortical thickness between healthy controls (HC; blue) and mixed martial artists (MMA; red) in the right (Panel A) and left (Panel B) hemisphere. Box-and-whisker plots for regions showing differences in cortical thickness are presented in the adjacent columns.

Figure 2: Symmetrized percent change (SPC) values for cortical thickness over the course of the study (Follow-up – Baseline Visit) for healthy controls (HC; blue) and mixed martial artists (MMA; red). Box-and-whisker plots indicate increased thickness at follow-up scans for HC and decreased thickness for MMA fighters relative to their respective baseline evaluations.

Figure 3: Figure 3 depicts the potential effects of acute concussion over the one year follow-up period on significant longitudinal findings in mixed-martial artists (MMA). Scatter plots depicting **change scores** between the baseline and follow-up visits are presented for MMA fighters with (Pos Hx; black circles) or without (Neg Hx; light gray circles) a self-reported (N=2) or acutely verified (N=3) concussion. There were no significant differences **in change scores** for white matter (WM) N-acetylaspartate (NAA) **concentration in millimoles per kilogram of water (mmol/kg water; Panel A)**, in total WM volume (Panel B), or in cortical thickness in the left rostral middle and superior frontal gyrus (Panels C and D).

Figure 4: Serial ^{1}H-MRS and volumetric case study data for three mixed martial artists fighters with an acute concussion during the study follow-up period. Data from healthy controls at baseline (C_1; white diamonds) and 6-month follow-up (C_2; grey diamonds) and from MMA fighters (F; black diamonds; excluding data from serial cases) are presented as a reference point, with error bars equaling 2 times the standard deviation. The average

gray (GM) and white (WM) matter metabolite concentrations for N-acetylaspartate (NAA; Panels A and B, Row 1), glutamate/glutamine (Glx; Panels A and B, Row 2) and creatine (Cre; Panels A and B, Row 3) are presented in millimoles per kilogram of water (mmol/kg water). Panel C presents normalized total WM volume (Row 1), hippocampi volume (Hipc; Row 2) and thalami volume (Thal; Row 3). Serial data are shown at baseline (B), during the acute (A; within 72 hours), sub-acute (S; approximately 4 weeks-post), and early chronic (E; approximately 4 months-post) injury phase. One MMA fighter experienced two concussions during the course of the study, and each subject and injury time-point are uniquely labeled (S_1I_1 versus S_1I_2). Note that each graph is scaled differently.

Figure 1:

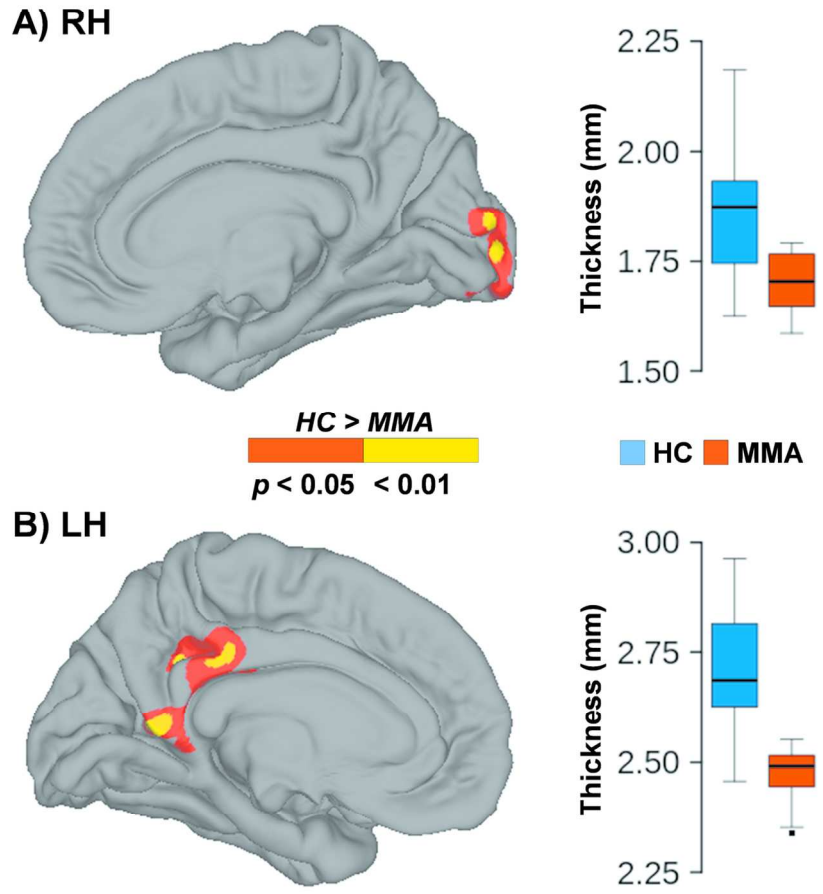


Figure 1: Figure 1 depicts surface representations of baseline differences in cortical thickness between healthy controls (HC; blue) and mixed martial artists (MMA; red) in the right (Panel A) and left (Panel B) hemisphere. Box-and-whisker plots for regions showing differences in cortical thickness are presented in the adjacent columns.
130x130mm (300 x 300 DPI)

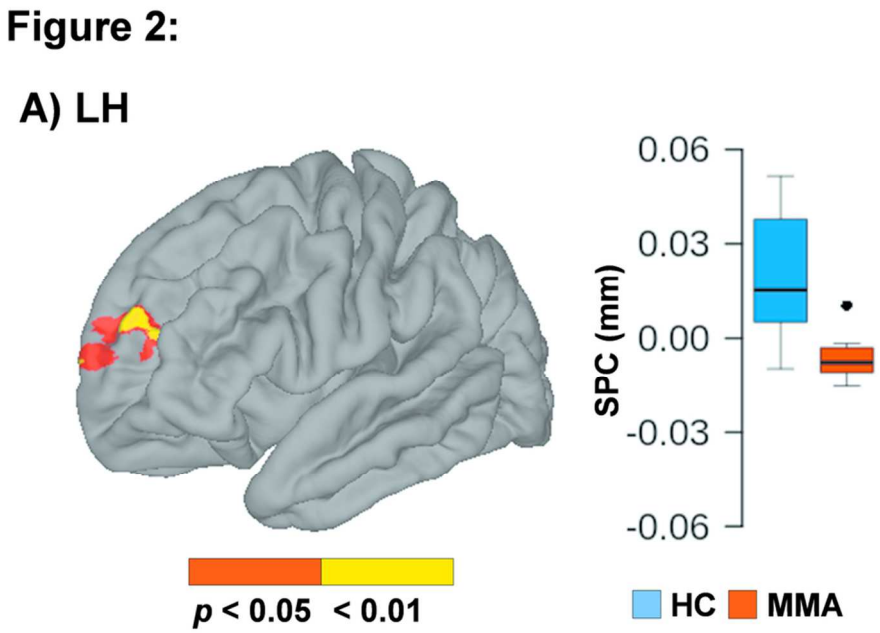


Figure 2: Symmetrized percent change (SPC) values for cortical thickness over the course of the study (Follow-up – Baseline Visit) for healthy controls (HC; blue) and mixed martial artists (MMA; red). Box-and-whisker plots indicate increased thickness at follow-up scans for HC and decreased thickness for MMA fighters relative to their respective baseline evaluations.
88x60mm (300 x 300 DPI)

Figure 3:

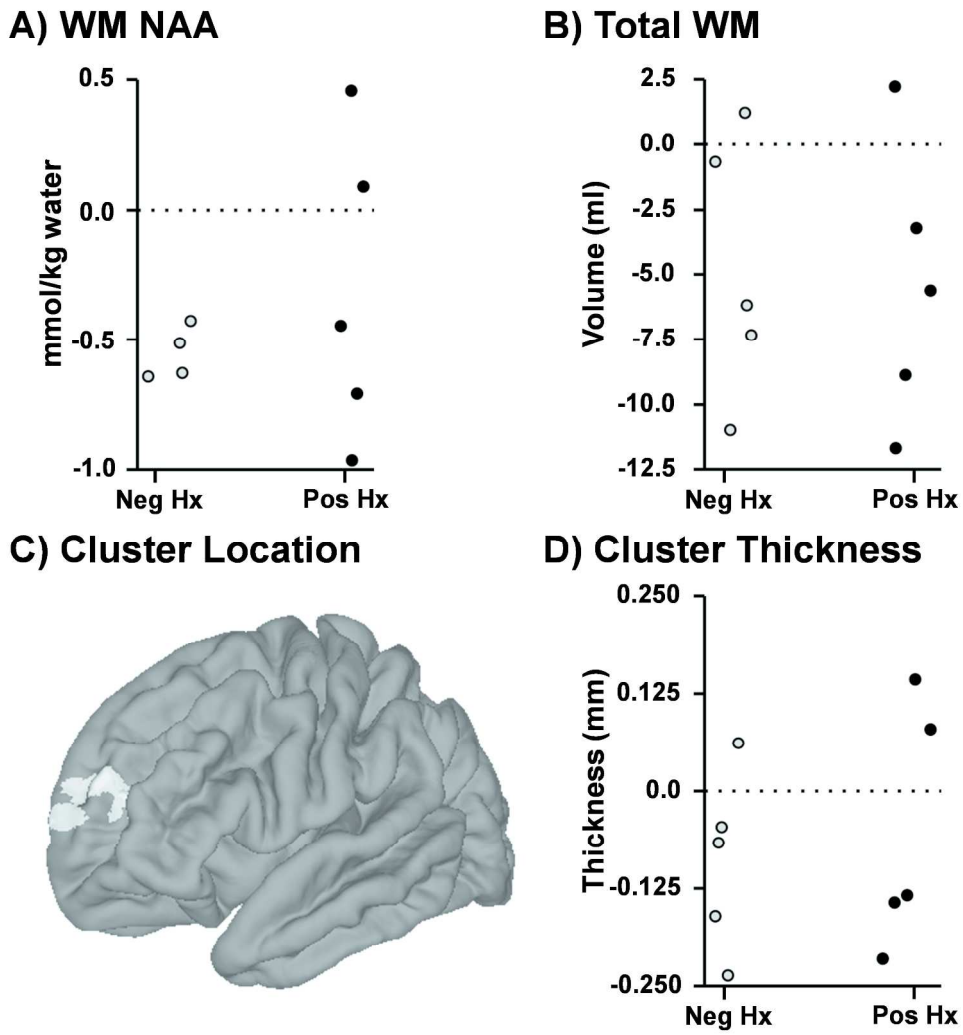


Figure 3: Figure 3 depicts the potential effects of acute concussion over the one year follow-up period on significant longitudinal findings in mixed-martial artists (MMA). Scatter plots depicting change scores between the baseline and follow-up visits are presented for MMA fighters with (Pos Hx; black circles) or without (Neg Hx; light gray circles) a self-reported (N=2) or acutely verified (N=3) concussion. There were no significant differences in change scores for white matter (WM) N-acetylaspartate (NAA) concentration in millimoles per kilogram of water (mmol/kg water; Panel A), in total WM volume (Panel B), or in cortical thickness in the left rostral middle and superior frontal gyrus (Panels C and D).

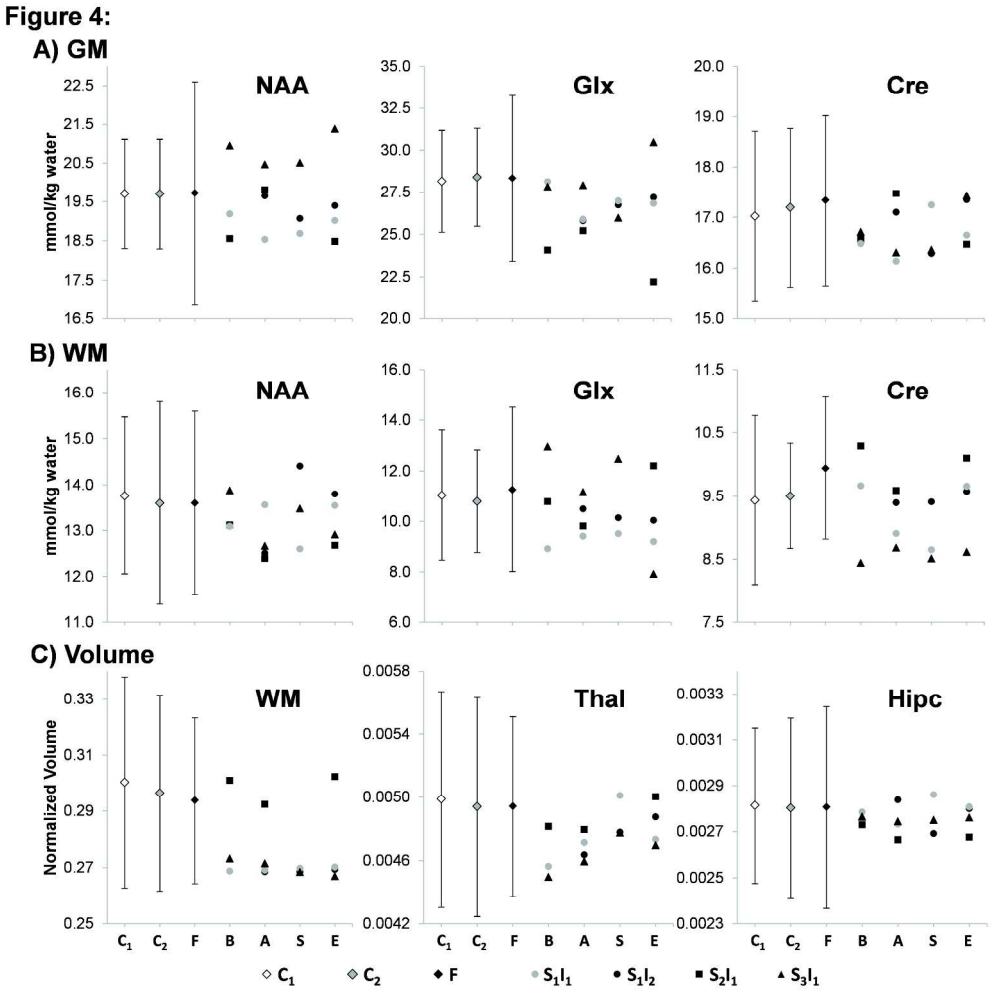


Figure 4: Serial 1H-MRS and volumetric case study data for three mixed martial artists fighters with an acute concussion during the study follow-up period. Data from healthy controls at baseline (C1; white diamonds) and 6-month follow-up (C2; grey diamonds) and from MMA fighters (F; black diamonds; excluding data from serial cases) are presented as a reference point, with error bars equaling 2 times the standard deviation. The average gray (GM) and white (WM) matter metabolite concentrations for N-acetylaspartate (NAA; Panels A and B, Row 1), glutamate/glutamine (Glx; Panels A and B, Row 2) and creatine (Cre; Panels A and B, Row 3) are presented in millimoles per kilogram of water (mmol/kg water). Panel C presents normalized total WM volume (Row 1), hippocampi volume (Hipc; Row 2) and thalami volume (Thal; Row 3). Serial data are shown at baseline (B), during the acute (A; within 72 hours), sub-acute (S; approximately 4 weeks-post), and early chronic (E; approximately 4 months-post) injury phase. One MMA fighter experienced two concussions during the course of the study, and each subject and injury time-point are uniquely labeled (S1I1 versus S1I2). Note that each graph is scaled differently.

Supplemental Table S1: Summary of Radiological Review Findings

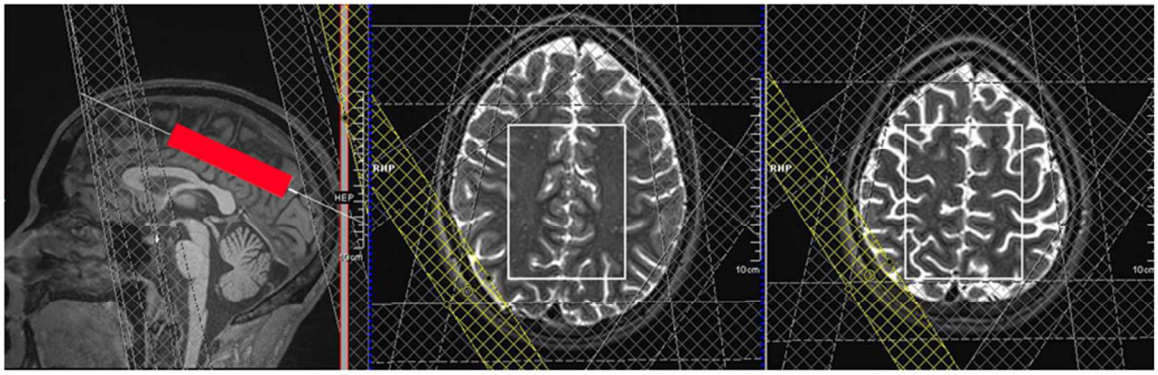
				Radiological Review - Baseline Visit			Radiological Review - Follow-up Visit	
Sex	Age	Group	HX of mTBI Prior to Study	Trauma-related Findings?	Review Summary	DX of mTBI During Study	Trauma-related Findings?	Review Summary
M	20	MMA	Yes	Yes	Tiny focal area of T2 hyperintensity in the posterior frontal subcortical white matter. Nonspecific. Probably tiny focus of microvascular ischemic change. Could also represent gliosis. Mild mucosal thickening of the paranasal sinuses - likely inflammatory/allergic.	Yes	Yes	Mucosal thickening of sinuses. Tiny focal area of bright T2 signal in the posterior white matter. Nonspecific.
M	22	MMA	Yes	Yes	5mm intraluminal defect in the left transverse sinus is probably an arachnoid granulation. Mucosal thickening of the paranasal sinuses - likely inflammatory/allergic. Tiny FLAIR hyperintensity in the posterior subthalamic area. Nonspecific. Small area of gliosis.	Yes	Yes	Tiny FLAIR hyperintensity in the posterior subthalamic area. Nonspecific. Unchanged. Focal gliosis?
M	24	MMA	Yes	Yes	Area of abnormal white matter in the anterior right periventicular white matter is better demonstrated with FLAIR/T2 sequences. Question gliosis? There seems to be accompanying slight asymmetry of venous structures.	No	Yes	Area of abnormal white matter in the anterior right periventicular white matter is better demonstrated with FLAIR/T2 sequences. Unchanged from previous scan. Question gliosis? There seems to be accompanying slight asymmetry of venous structures.
M	25	MMA	Yes	No	No abnormal findings.	Yes	Yes	Areas of abnormal white matter signal. (tiny left anterior subinsular area).
M	26	MMA	Yes	No	Mucosal thickening of the paranasal sinuses - likely inflammatory/allergic. 1.3 cm pineal gland cyst.	Yes	No	1.4 cm left paracentral pineal gland cyst. Mild mucosal thickening of the paranasal sinuses. Nonspecific signal heterogeneity of the pituitary.
M	26	MMA	Yes	No	No abnormal findings.	No	NA	
M	28	MMA	Yes	No	No abnormal findings.	No	Yes	Cavum septum pellicudum. 5 mm nasopharyngeal cyst (Thornwaldt cyst)
M	29	MMA	Yes	No	Right mastoid opacification. Question incomplete opacification, effusion, effects of chronic mastoiditis.	Yes	No	Partial right mastoid opacification. Question incomplete pneumatization, effusion, effects of chronic mastoiditis.

M	34	MMA	Yes	No	Tiny dark area between the anterior and posterior pituitary is most likely the normally occuring pars intermedia vs small cyst. Right frontal/parietal venous developmental anomaly. Right maxillary sinus mucous retention cysts.	No	No	Tiny dark area between the anterior and posterior pituitary is most likely the normally occuring pars intermedia vs small cyst. Right frontal/parietal venous developmental anomaly. Right maxillary sinus mucous retention cysts.
M	35	MMA	Yes	No	Right maxillary sinus mucous retention cyst.	No	NA	
M	35	MMA	Yes	No	Right ethmoid bright signal probably represent a mucous retention cyst. Maxillary sinus mucous retention cysts.	No	No	Areas of abnormal white matter signal (tiny foci, frontal subcortical white matter). Right ethmoid sinus mucous retention cyst.
F	31	MMA	Yes	No	No abnormal findings.	No	No	No abnormal findings.
F	32	MMA	Yes	Yes	Scattered foci of white matter bright signal. Nonspecific. Could represent microvascular ischemic change. Any clinical history of migraine headaches? Gliosis from prior injury?	No	NA	
M	24	HC	No	No	No abnormal findings.	No	No	No abnormal findings.
M	25	HC	Yes	No	No abnormal findings.	No	No	No abnormal findings.
M	26	HC	Yes	No	No abnormal findings.	No	NA	
M	27	HC	Yes	No	No abnormal findings.	No	No	No abnormal findings.
M	28	HC	No	No	Mucus retention cyst(s). (Right maxillary sinus)	No	NA	
M	29	HC	No	No	No abnormal findings.	No	No	No abnormal findings.
M	31	HC	No	Yes	Areas of abnormal white matter signal. Occasional foci mainly in the subcortical white matter. Nonspecific. In the appropriate clinical setting, could represent small gliotic foci associated with trauma. Other possibilities to consider include: microvascular ischemic change, migraine related change, vascular inflammatory changes, etc.	No	Yes	Areas of abnormal white matter signal. Occasional foci mainly in the subcortical white matter. Nonspecific. In the appropriate clinical setting, could represent small gliotic foci associated with trauma. Other possibilities to consider include: microvascular ischemic change, migraine related change, vascular inflammatory changes, etc.
M	32	HC	No	No	Developmental venous anomaly.	No	NA	
M	37	HC	Yes	No	Mucus retention cyst(s). (left maxillary sinus).	No	NA	
M	38	HC	No	No	Small bright area in the pituitary - likely a small cyst. Mild mucosal thickening of the	No	No	Images slightly degraded by motion artifact. No obvious abnormality.

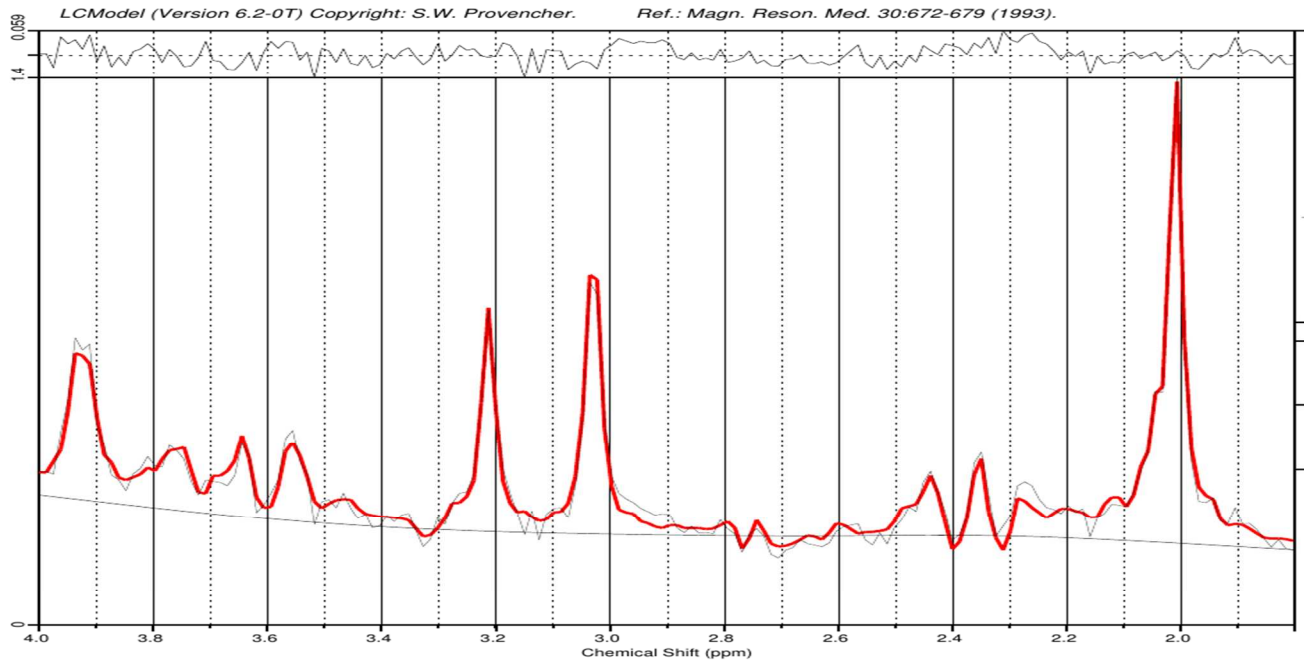
					maxillary sinuses.			
F	27	HC	No	No	Abnormal pituitary signal (hypointense, hyperintense, heterogeneous).	No	No	Abnormal pituitary signal (hypointense, hyperintense, heterogeneous).
F	28	HC	Yes	Yes	5 mm fluid signal in the posterior right periventricular white matter maybe a dilated perivascular CSF space vs benign neuroglial cyst. Right middle nasal concha bullosa.	No	Yes	4 mm fluid signal in the posterior right periventricular white matter maybe a dilated perivascular CSF space vs benign neuroglial cyst. Right middle nasal concha bullosa.

Supplemental Table 2: Table listing mean percentage of usable spectra per metabolite after Cramer-Rao filtering.

	MMA	HC
Metabolite	Mean % (SD)	Mean % (SD)
Cre	100.0 (0.0)	100.0 (0.0)
Glx	98.8 (2.06)	97.4 (2.62)
Cho	100.0 (0.0)	100.0 (0.0)
mI	98.5 (3.92)	98.0 (3.06)
NAA	100.0 (0.0)	100.0 (0.0)



Supplemental Figure S1: Representative ^{1}H-MRS volume of interest (solid white box) and saturation bands from a single subject. **The center axial image shows the location of the bottom of the volume. The right axial image indicates the top of the volume.**



Supplemental Figure S2: ^{1}H-MRS modeled spectra directly derived from LCmodel for a single subject. Red line is model fit over data (light gray line).