**Monitoring long term effects of Mild Traumatic Brain Injury with Magnetic
Resonance Spectroscopy: A Pilot Study**

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Abstract

This pilot study explores the metabolic changes associated with persistent post-concussion syndrome (PCS) after mild traumatic brain injury (mTBI; >12 months post-injury) using magnetic resonance spectroscopy (MRS). We hypothesised that those mTBI participants with PCS will have larger metabolic differences than those without. Data was collected from mTBI participants with PCS, mTBI participants without PCS and non-head injured participants (all groups: n=8). MRS metabolite profiles within the dorsolateral prefrontal cortex showed a reduced Creatine/Choline ratio in mTBI patients compared to control participants. This data provides initial evidence for residual metabolic changes in chronic mTBI patients, but there was no conclusive relationship between these metabolic changes and PCS symptom report. Creatine is involved in maintaining energy levels in cells with high or fluctuating energy demand, suggesting that there may be some residual energy impairment in chronic mTBI.

Keywords: Magnetic Resonance Spectroscopy, mild Traumatic Brain Injury, persistent Post-Concussion Syndrome, Cognitive tasks, Creatine

Introduction

Head injury induces a cascade of metabolic changes which result in a generalised cellular energy crisis, and can lead to cell death or disruption in neural connectivity [1,2]. It is thought that these mechanisms may underlie the symptoms (collectively known as post-concussion syndrome or PCS) observed after mild traumatic brain injury (mTBI), with significant metabolic alterations occurring even in the absence of these symptoms [1]. Magnetic resonance spectroscopy (MRS, [3]) can be used to measure metabolite concentrations in vivo and investigate whether subtle differences caused by this metabolic cascade are still evident in the long-term after injury. Creatine (Cr) is particularly suited for investigation of the energy metabolism after head injury.

Impairment of the creatine/phosphocreatine (Cr/PCr) system can lead to a deterioration in energy metabolism resulting in symptoms similar to those seen in PCS such as predisposition to migraines [4], mental retardation, and other neurological symptoms [5].

There have been variable observations of Creatine concentration in acute mTBI, with higher concentrations generally seen in white matter [6,7], and no difference [8] in gray matter or mixed areas. This is despite the more consistent detection of lower levels of N-Acetyl Aspartate (NAA [9]) and higher levels of Choline [7], and some initial evidence of reduction in glutamine and glutamate in gray matter [3,7].. There is a paucity of studies on creatine after head injury, and its concentration is conventionally thought to be stable in healthy individuals. However, reduced creatine concentration has been observed in clinical populations such as those with cancer [10] and stroke [11].

The present pilot study aims to investigate the long-term effect of mTBI (>1 year post-injury) on metabolite concentrations in the dorsolateral prefrontal cortex (DLPFC, a

gray matter area involved in cognitive functions) in those reporting persistent PCS and those without on-going PCS. We conducted 3T ^1H -MRS at short echo time, in conjunction with cognitive testing in 16 patients with mTBI (n=8 for those with/without PCS), and 8 non-head injured controls. Only a few studies [8,9,12] have considered mTBI participants beyond the acute stage, and none have examined the association of PCS and metabolite concentrations in the long term. We hypothesised that mTBI patients with persistent PCS would show greater metabolic changes (in particular in metabolites related to energy processing) than both mTBI patients without PCS and non-head injured controls.

Methods

Recruitment

Participants were recruited from a database created by previous studies [13,14], with mTBI diagnosis according to ICD-10 criteria [15]. PCS diagnosis was based on Mittenberg and Strauman's modified DSM-IV criteria (which does not include cognitive testing) [16], and was achieved in the same way for control participants as mTBI participants (with the exception that controls had no “history of head trauma”). Inclusion criteria were that injury occurred at least one year previously, and exclusion criteria were report of litigation, major invasive head injury, chronic pain, or other neurological conditions. None of the participants had visible lesions using standard structural MRI. 24 participants were included in this study, divided into three groups containing 8 participants each: *mTBI+PCS* (participants who suffered an mTBI and have persistent PCS); *mTBI-PCS* (participants with mTBI but no PCS); *Control* (participants with no history of brain injury and no PCS). Group demographics and

questionnaire data are shown in Table 1. The study protocol was given a favourable opinion by the University of Surrey Ethics Committee, and informed consent was obtained from every participant.

Questionnaires and Cognitive Testing

The extent of PCS was measured through the Rivermead Post-Concussion symptoms Questionnaire (RPQ) and Rivermead Post-Concussion symptoms Questionnaire for Controls (RPQ-C; [17]). Other questionnaires assessed IQ (National Adult Reading Test, NART), everyday cognitive failures (Cognitive Failures Questionnaire, CFQ), daytime sleepiness (Epworth Sleepiness Scale, ESS), overall sleep quality (Pittsburgh Sleep Quality Index, PSQI), anxiety and depression (Hospital Anxiety and Depression Scale, HADS). In addition, working memory (n-Back: 0, 1, 2, 3-Back) and information processing speed (PVSAT [Paced Visual Serial Addition Task]: 1, 1.5, 2, 2.5 seconds) tasks were performed and error rates collected.

MRS Procedure

Single voxel MRS was performed over the right DLPFC (Fig. 1A) on a 3T Siemens' Trio MR Scanner, using a PRESS sequence at short echo time (1.5x1.5x1.5cm; TE=30ms; TR=1500ms; CP coil, bandwidth=2000Hz; 2048 data points). Both water-suppressed spectra (256 averages) and spectra without water suppression (16 averages) were acquired. Water unsuppressed spectra were used for eddy current correction and as an internal reference in order to measure metabolites relative to voxel water concentration. DLPFC was selected as the region of interest as it is differentially effected by mTBI, with differences observed using electrophysiological and fMRI studies e.g. [18]. MRS was performed after sustained activation of DLPFC during the working memory and information processing speed tasks. The voxel was placed using

T1-weighted axial and coronal structural scans and anatomical landmarks, in the area of DLPFC as reported in an fMRI n-Back meta-analysis [19]. When placing the voxel, care was taken so that the voxel contained no CSF, and extra-voxel lipid saturation bands were used. Each spectrum was assessed to ensure good quality (e.g. poor water signal suppression, subject motion or insufficient magnetic field shimming) and absence of artefacts. The spectra obtained were processed using LC Model [20], using the default settings of water scaling, to obtain metabolite concentrations (total Creatine (Cr), total Choline (Cho), total NAA (NAA), Myoinositol (mIns) and Glutamate/Glutamine (GLX), all CRLB<15%), relative to water. Metabolite ratios were also calculated in order to reduce individual variability: NAA/Cho and Cr/Cho.

Statistics

A series of one-way ANOVAs, with post-hoc Bonferroni-corrected comparisons, were performed on the participant demographics and questionnaires. The exception was gender, which was tested using chi-square. The cognitive tests were analysed using a mixed-model ANOVA with factor *difficulty level* (3-Back, 2-Back, 1-Back, 0-Back or 1s, 1.5s, 2s, 2.5s) and between-subjects factor of *group* (Control, mTBI-PCS, mTBI+PCS).

Metabolite concentrations were analysed using a univariate ANOVA with factor *group* (mTBI+PCS, mTBI-PCS, Control), and Bonferroni-adjusted post-hoc comparisons.

Following on from this, univariate ANOVAs with factor *mTBI* (mTBI [mTBI+PCS/mTBI-PCS]; Control) and *PCS* (PCS; No PCS [mTBI-PCS/Control]) were carried out. Associations between metabolites, participant demographics and behavioural data were explored across groups (N=24) using Pearson's correlations

where the data were normally distributed and Spearman's Rho where the assumption of normality was violated.

Results

Questionnaires and Cognitive Testing

Significant group differences (Table 1) were seen in post-concussion symptoms (RPQ; $F(2, 21)=31.3$, $p<0.001$), cognitive failures (CFQ; $F(2, 21)=7.6$, $p<0.005$), and sleep quality (PSQI; $F(2, 21)=6.1$, $p<0.001$). Comparisons revealed greater symptoms in the mTBI+PCS group compared to mTBI-PCS (RPQ: mean difference (m.d.)=13.5, $p<0.001$; PSQI: m.d.=4.1, $p<0.01$) or the Control group (RPQ: m.d.=21.4, $p<0.001$; CFQ: m.d.=27.6, $p<0.005$). No significant differences were observed for gender ($p=0.5$), age ($p=0.2$) or IQ ($p=0.1$). There was no significant correlation between IQ and any metabolite concentration, but both Creatine and Choline concentration tended to increase with increasing age across all participants (Cr: $r(24)=0.6$, $p=0.005$; Cho: $r(24)=0.6$, $p=0.003$) and in mTBI participants alone (Cr: $r(16)=0.8$, $p=0.001$; Cho: $r(16)=0.6$, $p=0.03$).

----Insert Table 1 around here----

For the cognitive tests, there was a main effect of *difficulty level* (n-Back: $F(3, 52)=21.9$, $p<0.001$; PVSAT: $F(3, 41)=31.7$, $p<0.001$), but no main effect of *group* (n-Back: $F(2, 20)=0.6$, $p=0.6$; PVSAT: $F(2, 21)=0.2$, $p=0.8$) and no interaction between *group* and *difficulty level*.

MRS Data

A significant group difference Cr/Cho ratio was observed (Figure 1B, $F(2, 21)=2.8$, $p=0.04$), but no difference in total Creatine concentration ($F(2, 21)=0.4$, $p=0.3$). Further

analysis revealed that the participants with mTBI (mTBI+PCS/mTBI-PCS) had a smaller Cr/Cho ratio than the control participants ($F(1, 22)=5.5, p=0.01$), whereas there was only a trend for smaller Cr/Cho ratio in participants with PCS (mTBI+PCS) compared to those without PCS ($F(1, 22)=2.3, p=0.07$). Across participants, there was also a trend for greater PCS symptom report (RPQ) in those with lower Cr/Cho ratio ($r(24)=-0.4, p=0.07$). However, this was not seen in mTBI participants alone ($r(16)=-0.07, p=0.8$).

----Insert Fig. 1 around here----

There were no group differences for any other metabolites (NAA ($F(2, 21)=0.6, p=0.6$); NAA/Cho ratio ($F(2, 21)=1.0, p=0.4$); Cho ($F(2, 21)=1.5, p=0.3$); mIns ($F(2, 21)=0.01, p=1$); GLX ($F(2, 21)=0.1, p=0.9$)). However, higher error rate in the hardest PVSAT condition (1s) correlated with lower NAA/Cho ratio ($r(24)=-0.5, p=0.03$) and total Choline concentration ($r(24)=0.5, p=0.02$) across all participants. These correlations were not seen in mTBI participants alone (NAA/Cho ratio: $r(16)=0.3, p=0.2$; Cho: $r(16)=0.4, p=0.2$).

Discussion

The present pilot study aimed to explore the metabolic alterations in chronic mTBI and their relationship to reported PCS symptoms. It provides initial evidence for residual metabolic changes, as participants with chronic mTBI exhibited lower Cr/Cho ratio compared to non-head injured controls (Fig. 1B). However, the trend for lower Cr/Cho in those with PCS, along with a trend for correlation between PCS symptom report (RPQ) and Cr/Cho ratio is not currently enough to substantiate an association between

these metabolite profiles and PCS diagnosis. Nevertheless, these results suggest an intriguing avenue for future research.

Changes in brain metabolism in mTBI

This study was particularly focused on alterations in metabolites used in energy metabolism, such as Creatine, in chronic mTBI. Creatine is involved in energy buffering and transport [5], impairment of its function leads to symptoms similar to those seen in PCS, and supplementation of Creatine can improve symptoms after injury [21,22].

However, there was no difference in cognitive performance between groups in this study and no correlation between performance and Creatine.

Furthermore, it was only the Cr/Cho ratio that was significantly different between groups, with no significant change in either absolute Creatine or absolute Choline concentration. Therefore, we cannot be certain whether the low Cr/Cho ratio is due to low Creatine concentration, a high Choline concentration, or a combination of both of these. Indeed, higher Choline has been seen in previous studies in acute mTBI [23], and could be indicative of neural inflammation or demyelination.

The present study illustrates a metabolic difference between participants with mTBI and non head-injured controls, even a year after injury. However, it cannot definitively conclude that this difference is due to alterations in energy metabolism (reduced Creatine), and not inflammation or cell damage (increased Choline).

Association with PCS

The second aim of this experiment was to investigate whether lasting metabolite changes may be related to the severity of PCS symptoms. There was some suggestion that Cr/Cho ratio is reduced in those with PCS, and decreases in relation to greater RPQ symptom report. This offers some support for the hypothesis that those mTBI

participants with higher PCS symptoms will have greater metabolic alterations.

However, the correlation was only a trend ($p=0.07$), and no correlation was observed when looking at mTBI participants alone ($p=0.8$). As such, the present study does not provide substantial enough evidence for our initial hypothesis.

Association with Cognitive Performance

Lower NAA/Cho ratio correlated with poorer cognitive performance in the hardest PVSAT condition. A similar association has been reported for the PASAT (an auditory version of the same task) in moderate to severe TBI [24]. However, there was also a significant correlation between higher total Choline concentration and poorer cognitive performance in the hardest PVSAT condition. The observed NAA/Cho ratio correlation could therefore be purely due to increased Choline in those with worse cognitive performance, or a combination lower NAA and higher Choline concentration. The association of increased Choline and cognitive performance is intriguing, especially in conjunction with the reduced Cr/Cho ratio in mTBI participants.

Future Directions

This pilot study provides tentative evidence of sustained changes in brain metabolism over a year after the episode of mild head injury. However, a larger scale follow-up experiment is required to examine our hypothesis more thoroughly and substantiate the more tentative findings.

The variability inherent to mTBI may have masked subtle changes in both absolute Creatine or Choline concentration and cognitive performance. Participants taking part in this study had previously undertaken longer versions of the two cognitive tasks as part of a larger sample, with results showing that those with mTBI+PCS performed significantly worse on both tasks [14]. The lack of similar findings in this task could be

due to the smaller sample size, fewer trial repetitions in the shorter task used here, or the novel MRI environment. In this study, participants with mTBI+PCS reported higher subjective cognitive failures, PCS symptoms and sleep problems, suggesting that there are indeed some on-going behavioural problems.

In addition, Creatine and Choline concentration increased with greater age, as seen in previous research [25]. This potentially limits the interpretation of the results, as another factor (age) is shown to systematically alter the metabolites of interest. However, there is no significant effect of age between the groups, suggesting that the distributions of the ages within the groups are similar, despite numerically different group means.

Indeed, if we add age as a covariate to the analysis there is still a significant reduction in Cr/Cho in mTBI participants compared to controls ($F(2,21)=3.6, p=0.04$), suggesting that it is unlikely to be causing the observed results. Future analyses will need to age-match the groups in order to factor out this potential confound. Another potential limitation is that although corrections and modifications were made for CSF contamination, it was not possible to perform tissue segmentation to look at volume effects of gray and white matter. As metabolite concentrations differ between these two tissue types, this could confound the results.

If a larger scale study was able to find equivocal evidence of a reduction in Creatine concentration in chronic mTBI, then it would suggest that dietary supplementation of Creatine may benefit those with chronic mTBI. Previous studies have observed that Creatine supplementation immediately after brain injury improved cognitive function, relieved headaches, dizziness and fatigue, reduced cell damage and post-traumatic amnesia and improved personality and communication [21,22]. However, Creatine has

only been administered in the acute phase of TBI, as soon as possible after the injury; it would be interesting to see if the effect is similar in participants with persistent PCS.

Conclusion

In conclusion, this is the first experiment to investigate metabolite changes in chronic mTBI at least a year after injury. Our data suggests that patients with mTBI do exhibit some on-going metabolic changes, with a reduction in Cr/Cho ratio. However, evidence for the association of this metabolic change with PCS symptom report was not substantial enough to warrant a definitive conclusion. A follow-up study with a larger sample is needed to confirm and expand upon the findings from this pilot work.

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Table 1. Questionnaire and demographic data.

	Age	Gender M/F	TSI (yrs)	NART	RPQ	CFQ	HADS		ESS	PSQI
							Anxiety	Depression		
Control (n=8)	22.5±1.6	4/4	-	111.9±2.2	3.9±1.1	25.6±4.3	5.9±1.3	1.8±1.2	5.1±0.6	6.1±0.6
mTBI- PCS (n=8)	30.3±2.9	4/4	3.9±1.9	117.6±1.0	11.8±2.2	35.4±4.5	3.9±1.0	1.8±0.8	6.9±1.4	4.8±1.0
mTBI+PCS (n=8)	27.4±3.8	2/6	5.5±2.2	112.0±2.4	25.3±2.2	53.3±6.2	7.8±1.2	4.5±1.1	7.6±1.7	8.9±0.9

Results expressed as Mean±SEM. Questionnaire data is expressed as raw sum scores. Grey shading represents values significantly different from other groups. TSI: Time since Injury; NART: National Adult Reading Test; RPQ: Rivermead Post-Concussion Questionnaire (scale 0-64); CFQ: Cognitive Failures Questionnaire (Scale 0-100); HADS: Hospital Anxiety and Depression Scale (scale 0-21); ESS: Epworth Sleepiness Scale (scale 0-24); PSQI: Pittsburgh Sleep Quality Index (scale 0-21).

Figure Legends

Fig. 1 A: Example voxel placement at right dorsolateral prefrontal cortex (white square box) and MRS Spectra. Image on left is reversed so left side is right hemisphere. White area within box represents average BOLD response during n-Back task (All n-Back > Baseline) over all participants, masked for the right dorsolateral prefrontal cortex ($p < 0.05$, FWE). **B:** Average metabolite concentration relative to water for each group. Cr: Creatine; tNAA: Total NAA (NAA and NAAG); tCho: Total Choline; mIns: Myoinositol; GLX: Glutamate and Glutamine. Cr/tCho and tNAA/tCho refer to the concentration of Creatine and NAA relative to Choline. Error Bars are Standard Error of the Mean (SEM).

Figure 1