Neurometabolic Alterations in Retired NFL Athletes

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Scholarly Report submitted in partial fulfillment of the MD degree at Harvard Medical School

Date: 01/26/2017

Student Name: Jeffrey Kyle Cooper

Scholarly Report Title: Neurometabolic Alterations in Retired NFL Athletes

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TITLE: Neurometabolic Alterations in Retired NFL Athletes

Kyle Cooper, Alexander Lin, Huijun Liao, Ben Rowland, Sai Merugumala, Molly Charney, Martha Shenton, Robert Stern

PURPOSE: Repetitive brain trauma (RBT) places athletes at risk for chronic traumatic encephalopathy (CTE), a neurodegenerative disorder characterized by hyperphosphorylated tau protein. Not all those exposed to RBT will ultimately develop the disease, suggesting the importance of other factors such as the number of head impacts sustained throughout the career. American football athletes playing line positions are known to sustain more impacts per season than players in skill positions. We thus hypothesized neurometabolic differences reflective of traumatic brain injury between these groups of players compared to controls.

METHODS: Magnetic resonance spectroscopy (MRS) was used to acquire metabolite concentrations in retired National Football League players (n=69) and age-matched controls (n=17) who were retired professional athletes from non-contact sports. Single voxel point-resolved spectroscopy (PRESS; TE=35ms, TR=2s, 2x2x2 cm\(^3\)) and two-dimensional correlated spectroscopy (2D-COSY) was acquired from the posterior cingulate gyrus. Concentrations of N-acetylaspartate (NAA), glutamate (Glu), creatine (Cr), glutathione (GSH), choline-containing compounds (GPC+PCh), and myo-inositol (mI) were measured using an operator-independent time domain based fitting of linear combination models (LCModel). Subjects were organized by position according to average number of head impacts sustained per season. Offensive and defensive lineman formed a “high risk” group, while running backs, defensive backs, and linebackers made up a “moderate risk” group. Linear regression analysis was performed for each position and metabolite of interest, and repeated measures one-way ANOVA with Tukey’s test was used to assess for neurometabolite differences between groups.

RESULTS: A significantly higher concentration of Glu/Cr+PCr was observed in the high risk group compared to the moderate risk group (\(p=0.02\)), and concentrations of Glu/Cr+PCr by position played was positively correlated with average number of impacts sustained per season (\(p=0.007\)). There was also a significantly higher concentration of glutamate+glutamine (Glx-3) in the high risk group compared to both moderate risk (\(p=0.02\)) and controls (\(p=0.01\)). NFL players as a whole exhibited a significantly higher concentration of glutathione (GSH-1) (\(p=0.03\)) and a significantly lower concentration of choline containing compounds (GPC+PCh/Cr+PCr) compared to controls (\(p=0.008\)).

CONCLUSIONS: Our results support a model of glutamatergic excitotoxicity and a possible oxidative stress response with chronic exposure to RBT. The decrease in choline-containing compounds with RBT exposure may reflect oligodendrocyte pathology, though further work is needed to replicate these findings.
Table of Contents

1. Glossary of Abbreviations ...................................................................................... 4
2. Introduction ............................................................................................................. 5
3. Student Role .......................................................................................................... 6
4. Methodology .......................................................................................................... 7
5. Results ................................................................................................................... 8
6. Discussion ............................................................................................................. 9
7. Conclusions .......................................................................................................... 12
8. Acknowledgements ............................................................................................... 12
9. References ........................................................................................................... 13
10. Tables and Figures ............................................................................................... 17
**Glossary of Abbreviations**

CHII: Cumulative head impact index

Cho: Choline

COSY: Correlated spectroscopy

Cr: Creatine

CTE: Chronic traumatic encephalopathy

fMRI: Functional magnetic resonance imaging

Glu: Glutamate

Glx: Glutamate/glutamine

GPC: Glycerophosphocholine

GSH: Glutathione

mI: Myo-inositol

MRS: Magnetic resonance spectroscopy

MRI: Magnetic resonance imaging

mTBI: Mild traumatic brain injury

NAA: N-acetyl-aspartate

NFL: National football league

PCh: Phosphocholine

PCr: Phosphocreatine

Phe: Phenylalanine

Thr: Threonine

PET: Positron emission tomography

PRESS: Point resolved spectroscopy

RBT: Repetitive brain trauma

TBI: Traumatic brain injury

TE: Echo time

TR: Repetition time
Section 1: Introduction

Repetitive brain trauma (RBT) occurs among athletes participating in contact sports and has been extensively linked to deleterious effects on overall neurologic health.(1–3) An estimated 300,000 sports-related traumatic brain injuries occur each year,(4) and many of these injuries stem from participation in tackle football, which predisposes to concussive and repetitive sub-concussive head impacts. Unlike a concussion, which produces clinical symptoms that tend to resolve within a week,(5) sub-concussive impacts may produce mild neurologic injury without immediately apparent clinical signs or symptoms.(6,7) Despite the lack of overt clinical manifestations, there is growing concern that the cumulative burden of sub-concussive blows may contribute to future neurologic dysfunction.(6) Studies of athletes participating in contact sports have documented a negative impact on cognitive measures for those exposed to RBT without concussion compared to non-contact controls.(8–10) Moreover, alterations in neurophysiology and white matter integrity has been demonstrated in non-concussed RBT subjects.(11,12)

There is also growing evidence that athletes exposed to RBT are at risk for developing chronic traumatic encephalopathy (CTE), a progressive neurodegenerative disorder associated with accumulation of p-tau neurofibrillary tangles. CTE is progressive and has a diverse clinical presentation including poor executive functioning, memory decline, Parkinsonism, speech and gait abnormalities(13), and mood disturbances.(14) Concussion is not a necessary condition for diagnosis of CTE, as 16% of pathologically diagnosed cases of the disease had no concussion history.(15) Moreover, despite the fact that all cases of pathologically diagnosed CTE have had RBT exposure, not all those exposed to RBT will ultimately develop CTE.(2,16) This emerging field of evidence raises the question of whether concussive blows and/or the cumulative burden of sub-concussive head impacts are major determinants of CTE risk and future neurologic decline.

Data from helmet impact accelerometer devices suggests that the burden of head impacts is not shared equally across all positions in tackle football.(17,18) Several studies have found that offensive and defensive line players tend to sustain a larger number of cumulative impacts over the course of a typical season compared to skill positions such as running and defensive backs.(19–21) In a recent summary of this data collected from helmet accelerometer studies, Montenigro and colleagues derived a weighted mean number of impacts per season for each
football position, (22) which enables the study of varying levels of sub-concussive head impact burden on clinical and radiologic outcomes.

This study utilizes proton magnetic resonance spectroscopy (1H-MRS or MRS) to assess the effect of cumulative RBT exposure on neurometabolites reflective of traumatic brain injury by stratification through position played among retired NFL players. MRS detection and quantification of n-acetyl-aspartate (NAA), choline (Cho), creatine (Cr), myo-inositol (mI), glutamate (Glu), and glutathione (GSH) produces validated biomarkers associated with neurometabolic alterations following traumatic brain injury. These metabolites are biomarkers of physiologic processes including excitatory neurotransmitter release, neuronal depolarization, changes in glucose metabolism, altered cerebral blood flow, impaired axonal function, and antioxidant activity. (23–25) Most prior studies utilizing MRS have assessed neurometabolic alterations that occur following acute to subacute traumatic brain injury. (26–28) Studies of MRS analyzing long-term outcomes in athletes participating in contact sports are limited though have been performed in boxers (29), soccer players (30), and ice hockey/college football players. (31) In addition, a recent pilot study demonstrated neurometabolic alterations in a small cohort that included former NFL players. (32) However, this is the largest study to date examining long-term outcomes using MRS among players with a history of participation in the NFL. We hypothesized the existence of observable neurometabolic alterations with increasing levels of exposure to sub-concussive impacts from playing tackle football. Our overarching goal is to identify putative MRS biomarkers that may ultimately be used to develop a diagnostic test for CTE and guide future therapeutics.

Section 2: Student Role

My role on this project primarily consisted of literature review, data analysis, and manuscript preparation. Most MRS scans were completed at the time I joined the project, though I did assist with approximately 10 scans. I then reviewed the literature for data on the number of head impacts sustained by position played in the NFL. Based on this literature review, I identified two preliminary “high risk” and “moderate risk” groups in the summer of 2014. I then did a preliminary statistical analysis using two-sided t-tests for differences in means. During the summer of 2016, I continued my analysis with additional study subjects added and conducted
ANOVA for differences between these groups. In addition, I wrote the present manuscript with suggestions for edits from Dr. Lin.

Section 3: Methods

This report is part of the larger “Diagnosing and Evaluating Traumatic Encephalopathy Using Clinical Tests” (DETECT) study funded by the National Institutes of Health (Principal Investigator Dr. Robert Stern from the Boston University Center for Traumatic Encephalopathy). Research participants were sourced from the DETECT study for this analysis. The protocol was approved by the institutional review board of each participating institution and each research participant gave written informed consent. These participants were former NFL football players and meet the following inclusion criteria: age 30-59 years, played a minimum of one season in the NFL, English as a primary language, weight <350 pounds, and no metallic implants preventing MRS scanning. Research participants underwent neurological, neuropsychological, and psychiatric evaluations as part of the existing BU CSTE study. The final study group consisted of 69 former NFL players (mean age 54.35 ± 8.12) and 17 controls (mean age 58.53 ± 7.22) who were professional athletes from non-contact sports without a history of head injury. MRS scans were acquired on a Siemens 3T Verio using a 32 channel head coil from the posterior cingulate gyrus using single voxel point-resolved spectroscopy (PRESS; TE=35ms, TR=2s, 3x3x3 cm^3 voxel). The following validated biomarkers of traumatic brain injury were measured using single voxel proton MRS (1D MRS) and two dimensional COrrelatedSpectroscoY (2D COSY) using an operator-independent time domain based fitting of linear combination models (LCModel): N-acetylaspartate (NAA, neuronal marker), phosphorylated choline compounds (GPC+PCh, membrane marker), creatine (Cr+PCr, energy marker), glutathione (GSH, antioxidant), myo-inositol (mI, glial marker), and glutamate (Glu, excitotoxicity marker). All data was post-processed and partial volume corrected.

Based on a recent study by Montenigro et al. that derived a weighted mean number of impacts per season by position played in tackle football, two groups were identified that differed in the total average number of impacts sustained per season. Defensive lineman (871 mean impacts per season) and offensive lineman (728) formed a “high risk” group. Running backs (412), defensive backs (417), and linebackers (685) formed a “moderate risk” group.
The posterior cingulate gyrus was chosen as the region of interest for this analysis, as it is a remarkably homogeneous area of the brain and enables superior reproducibility compared to scanning other brain regions.\textsuperscript{(33)} In addition, prior studies of positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have found abnormalities in the PCG.\textsuperscript{(34,35)}

All data were stored in a common database and statistical analysis was conducted using GraphPad Prism 7 software. Risk groups were compared for differences in demographic information using means and \textit{two-sided t tests} for continuous variables. \textit{Repeated measures one-way ANOVA} with Tukey’s test was conducted to assess for differences in each metabolite of interest in the posterior cingulate gyrus with correction for multiple comparisons. \textit{Bartlett’s test} was used to test for homogeneity of variances. Mean metabolite levels were also calculated for each position of play, and mean impacts per season as reported by Montenigro et al. in a summary of head accelerometer data was used to perform a linear regression analysis for each metabolite of interest. A $p$ value $<0.05$ was considered statistically significant.

\textbf{Section 4: Results}

The mean number of total football years played and NFL years played was 18.28 ± 3.47 and 7.86 ± 2.67, respectively. There were no significant differences between groups with respect to age or years of football played, as shown in Table 1. Athletes played in the following positions during their career in the NFL: offensive line (n=24), defensive line (n=10), running backs (n=4), defensive backs (n=17), and linebackers (n=12). Because the total Cr concentration (creatine + phosphocreatine) did not differ significantly between groups (one-way ANOVA $p$ value=0.63), it was used to normalize data between subjects.

Significant differences were observed in concentration of Glu/Cr+PCr between the high risk (1.51 ± 0.12) and moderate risk (1.43 ± 0.13) groups ($p$=0.02). However, these groups did not differ significantly in Glu/Cr+PCr from controls (1.50 ± 0.14). In addition, there was a significant decrease in concentration of choline-containing compounds (GPC+PCh/Cr+PCr) for the moderate risk (0.19 ± 0.02) compared to the control (0.21 ± 0.02) group ($p$=0.008), as shown in Figure 1. A group of all NFL players also exhibited a lower mean concentration of choline-
containing compounds (0.19 ± 0.02) compared to controls (p=0.008). There were no significant differences between groups in concentrations of NAA/Cr+PCr, ml/Cr+PCr, or GSH/Cr+PCr, as shown in Table 2.

Mean neurometabolite levels of Glu/Cr+PCr for each position of play showed a positive correlation with mean impacts per season as reported by Montenigro et al. (p=0.0067, R²=0.9378), as shown in Figure 2. There were no other significant linear correlations between mean impacts per season and other metabolites of interest.

Analysis of 2D-COSY data revealed a significantly higher concentration of GSH-1 in NFL players (2.62 ± 0.05) compared to controls (2.59 ± 0.03, p=0.03). In addition, the high risk group exhibited a significantly higher concentration of Glx-3 (glutamate+glutamine) (2.31 ± 0.05) compared to both moderate risk (2.27 ± 0.05, p=0.02) and controls (2.26 ± 0.05, p=0.01). Mean concentration of Glx-3 was also significantly higher in the all NFL group (2.29 ± 0.05) compared to controls (2.26 ± 0.05, p=0.047), as shown in Table 2.

Section 5: Discussion

This study utilized magnetic resonance spectroscopy to study neurometabolic alterations in former NFL football players exposed to varying levels of repetitive brain trauma compared to a control group of athletes from non-contact sports. A significant increase in glutamate was observed in the high risk compared to the moderate risk group, and glutamate/glutamine (Glx-3) was observed to be significantly higher in the high risk group compared to both moderate risk and controls. In addition, mean levels of glutamate showed a significant positive correlation with increasing numbers of mean impacts per season by position played. Interestingly, this correlation did not hold when control subjects were included in the regression analysis, though this may be due to the comparatively smaller sample size of this group.

Glutamate is the primary excitatory neurotransmitter and is a validated MRS biomarker for excitotoxicity.(25) Evidence from both animal and human studies shows an indiscriminate release of excitatory amino acids including glutamate following traumatic brain injury.(36,37) Previous MRS studies have also shown characteristic glutamate/glutamine (Glx) alterations with head injury. One study of patients with acute traumatic brain injury illustrated elevations in Glx,
which was shown to predict poor outcomes.\textsuperscript{(38)} In addition, studies of the sub-acute stages of mTBI have shown Glx elevations in white matter and deep gray matter.\textsuperscript{(39,40)} Unfortunately, there are few studies assessing glutamate levels in the chronic stages of RBT. A recent study of five retired professional athletes with a history of RBT exposure showed a significant increase in Glx compared to controls, which is consistent with both our 1D MRS and 2D-COSY findings of a positive relationship between increasing levels of RBT exposure and glutamate levels.\textsuperscript{(32)} These results suggest that a more chronic excitotoxic response, distinct from the extracellular flux of glutamate with acute head injury, may develop with increasing exposure to RBT. This is particularly relevant to CTE and the process of neurodegeneration, as it is believed that excess glutamate release predisposes to neuronal cell death through increases in intracellular calcium.\textsuperscript{(41)}

The significant decrease in choline-containing compounds for moderate risk subjects and the overall finding of lower levels of these compounds for NFL athletes compared to non-contact controls was unexpected. Choline is a marker of increased membrane turnover and has been proposed as an indicator of diffuse axonal injury in cases of traumatic brain injury.\textsuperscript{(25)(42)} Increases in choline have been documented following acute traumatic brain injury\textsuperscript{(43)}, which likely occurs due to cell membrane injury. Further, choline increases in the sub-acute stages of traumatic brain injury have been hypothesized to occur as a result of glial activation.\textsuperscript{(44)} A recent study by Koerte and colleagues illustrated increased choline in retired soccer athletes with a history of exposure to repetitive sub-concussive head impacts compared to controls.\textsuperscript{(30)} The reason for our results differing so markedly from past studies is unclear. While a decrease in choline could be due to oligodendrocyte pathology, a more likely explanation for our results is age-related change. Choline has been shown to rise with age due to increasing membrane turnover.\textsuperscript{(45)} Our NFL subjects were of a younger average age than controls. While this difference did not reach significance ($p=0.08$), it may have contributed to these findings. Moreover, it should be noted that a significant difference in concentration of choline-containing compounds was not observed in our analysis of 2D-COSY data. The reason for the incongruence between these sets of results is also unclear though may have resulted from the imprecision of manually assigning cross-peaks during data processing.
A final significant finding in the present study is the observation of higher mean glutathione (GSH-1) concentration in NFL players compared to controls. Glutathione is an anti-oxidant and a known marker of neurologic oxidative stress and inflammation. Levels of glutathione have been shown to be elevated in the acute stages of traumatic brain injury.(46) In addition, a study of professional soccer players demonstrated a significant correlation between glutathione and lifetime exposure to repetitive sub-concussive head impacts.(30) Taken together, these results suggest a possible role for oxidative stress in the long-term pathology associated with RBT.

Interestingly, despite these players being at risk for CTE, a neurodegenerative disorder, we did not observe a decrease in the neuronal marker NAA with increasing exposure to repetitive brain trauma. In fact, the NFL players exhibited higher average levels of NAA compared to controls. Vagnozzi and colleagues have shown a decrease in NAA concentration in the acute stages following concussion with subsequent normalization over a period of months,(47,48) while Henry and colleagues have found that NAA/Cr levels may remain depressed compared to controls at six months after concussion.(27) However, in a study of the sub-acute stages of mTBI, Johnson and colleagues observed an increase in NAA/Cho and NAA/Cr that coincided with an increasing number of mild traumatic brain injuries, which was hypothesized to have occurred due to previous oxidative stress or injury yielding an adaptive effect of the neuron.(49) Such a protective mechanism may be the cause of our findings, as NFL athletes are known to sustain a number of concussive impacts throughout their career. Nevertheless, it remains unclear whether there is a detectable decline in NAA with later stages of neurodegeneration due to CTE.

There are several limitations to the current study. First, given that CTE is only diagnosable post-mortem on pathologic examination, it is not possible to ascertain the disease status of these NFL athletes. Therefore, the metabolic alterations observed between risk groups cannot be definitively attributed to changes of CTE and may indeed reflect other unknown pathophysiologic derangements due to RBT. The neurochemistry alterations detected in the present study also only apply to the posterior cingulate gyrus and will likely show some variation by brain regions, which may be differentially impacted by concussive versus sub-concussive impacts. While data for risk group stratification was obtained from Montenigro et al., this data may not be completely applicable to NFL players, as it was compiled from youth and collegiate football players. It may be reasonable to suspect that the burden of head impacts is similar between college and NFL
football, but better data is needed from NFL helmet accelerometer studies to more fully stratify risk. Further, position played served as a proxy for head impact severity in the current analysis, which is an imperfect metric. The ultimate measure of head impact burden put forth by Montenigro and colleagues is the cumulative head impact index (CHII), which was validated as a measure for detecting emotional and executive dysfunction and cognitive impairment. However, due to our lack of data on number of youth seasons played at each position, we were unable to calculate a CHII measure for each of the athletes in this study and instead relied on their summary of data by position played collected from helmet accelerometer studies. In addition, an ideal measure of head impact burden would incorporate time played per game and number of games played, which we also did not have at our disposal in this analysis. The present study stratified players by cumulative number of head impacts per season rather than by magnitude of head impacts, which has also been shown to vary with position played. This decision was made due to evidence suggesting that total number of head impacts may be the more important determinant of long-term neurologic decline, though more studies are needed to fully assess the differential impact of head impact number versus magnitude on neurometabolic alterations.

**Section 6: Conclusions**

Our results support a model of glutamatergic excitotoxicity and a possible oxidative stress response with chronic exposure to RBT. Future studies are needed to explore the utility of glutamate and glutathione as possible biomarkers for CTE and/or as therapeutic pathways to treatment of the disease. The decrease in choline-containing compounds with RBT exposure may have been influenced by the age difference between NFL athletes and controls though may also reflect oligodendrocyte pathology. Further work is needed to replicate these findings.

**Section 7: Acknowledgements**

Many thanks to Dr. Alexander Lin for his mentorship and guidance in conducting this research. This study was funded by support the National Institutes of Health. Portions of this study have
been presented in abstract form at the International Society for Magnetic Resonance in Medicine 24th Annual Meeting and Exhibition, May 2016.

References:


### Tables and Figures

**Table 1. Summary of study participant ages and years of football played**

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<th>Group</th>
<th>Controls</th>
<th>Moderate Risk</th>
<th>High Risk</th>
<th>Moderate vs High p value</th>
<th>Moderate vs Controls p value</th>
<th>High vs Controls p value</th>
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<td>Age</td>
<td>58.5 (7.2)</td>
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<td>7.3 (2.5)</td>
<td>8.4 (2.8)</td>
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There were no significant differences in age, total years of football played, or years played in the NFL between risk groups. Results are reported as mean (standard deviation). *P* values obtained using two-sided *t*-tests for differences between risk groups.

**Table 2. Mean ± standard deviation for neurometabolite levels of NFL players when grouped by risk according to position played versus controls for all 1D-MRS and 2D-COSY results**

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<th>MRS Metabolite</th>
<th>Controls n=17</th>
<th>All NFL n=69</th>
<th>Moderate Risk n=33</th>
<th>High Risk n=34</th>
<th>All NFL vs Controls p value</th>
<th>Moderate vs High p value</th>
<th>Moderate vs Controls p value</th>
<th>High vs Controls p value</th>
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<td>Cr+PCr</td>
<td>5.86 ± 0.40</td>
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<td>GSH/Cr+PCr</td>
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<td>0.43 ± 0.06</td>
<td>0.42 ± 0.06</td>
<td>0.44 ± 0.05</td>
<td>0.40</td>
<td>0.23</td>
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<td>1.50 ± 0.14</td>
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<td>mI/Cr+PCr</td>
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<tr>
<td>Thr</td>
<td>4.31 ± 0.03</td>
<td>4.33 ± 0.05</td>
<td>4.33 ± 0.04</td>
<td>4.33 ± 0.05</td>
<td>0.32</td>
<td>0.99</td>
<td>0.67</td>
<td>0.60</td>
</tr>
<tr>
<td>Phe</td>
<td>7.40 ± 0.04</td>
<td>7.41 ± 0.06</td>
<td>7.40 ± 0.05</td>
<td>7.41 ± 0.07</td>
<td>0.64</td>
<td>0.45</td>
<td>0.99</td>
<td>0.65</td>
</tr>
<tr>
<td>tCho-1</td>
<td>3.64 ± 0.04</td>
<td>3.64 ± 0.07</td>
<td>3.64 ± 0.07</td>
<td>3.64 ± 0.08</td>
<td>0.82</td>
<td>0.94</td>
<td>0.99</td>
<td>0.94</td>
</tr>
<tr>
<td>tCho-2</td>
<td>3.20 ± 0.01</td>
<td>3.20 ± 0.03</td>
<td>3.20 ± 0.03</td>
<td>3.20 ± 0.01</td>
<td>0.61</td>
<td>0.56</td>
<td>0.64</td>
<td>0.99</td>
</tr>
<tr>
<td>tCho-3</td>
<td>4.03 ± 0.04</td>
<td>4.04 ± 0.06</td>
<td>4.03 ± 0.06</td>
<td>4.05 ± 0.07</td>
<td>0.70</td>
<td>0.46</td>
<td>0.99</td>
<td>0.70</td>
</tr>
</tbody>
</table>

Cr+PCr, Creatine + Phosphocreatine; NAA, N-acetylaspartate; GSH, Glutathione; mI, Myo-inositol; GPC + PCh, Glycerophosphocholine + Phosphocholine; GSH, Glutathione; Glx, Glutamate+Glutamine; Thr, Threonine; Phe, Phenylalanine; tCho, Total Choline

*P* values obtained using repeated measures one-way ANOVA with Tukey’s test.
Figure 1. Mean values of glutamate (Glu/Cr+PCr) and choline-containing compounds (GPC+PCh/Cr+PCr) according to risk group. Lines represent 95% confidence intervals.
Figure 2. Correlations between glutamate (Glu/Cr+PCr) and position group mean impacts per season as reported by Montenigro et al.