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A Magnetic Resonance Spectroscopy Investigation in Symptomatic Former NFL Players

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Ethical Approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Abstract

The long-term neurologic consequences of exposure to repetitive head impacts (RHI) are not well understood. This study used magnetic resonance spectroscopy (MRS) to examine later-life neurochemistry and its association with RHI and clinical function in former National Football

League (NFL) players. The sample included 77 symptomatic former NFL players and 23 asymptomatic individuals without a head trauma history. Participants completed cognitive, behavior, and mood measures. N-acetyl aspartate, glutamate/glutamine, choline, myo-inositol, creatine, and glutathione were measured in the posterior (PCG) and anterior (ACG) cingulate gyrus, and parietal white matter (PWM). A cumulative head impact index (CHII) estimated RHI. In former NFL players, a higher CHII correlated with lower PWM creatine (r=−0.23, p=0.02). Multivariate mixed-effect models examined neurochemical differences between the former NFL players and asymptomatic individuals without a history of head trauma. PWM N-acetyl aspartate was lower among the former NFL players (mean diff.=1.02, p=0.03). Between-group analyses are preliminary as groups were recruited based on symptomatic status. The ACG was the only region associated with clinical function, including positive correlations between glutamate (r=0.32, p=0.004), glutathione (r=0.29, p=0.02), and myo-inositol (r=0.26, p=0.01) with behavioral/mood symptoms. Other positive correlations between ACG neurochemistry and clinical function emerged (i.e., behavioral/mood symptoms, cognition), but the positive directionality was unexpected. All analyses controlled for age, body mass index, and education (for analyses examining clinical function). In this sample of symptomatic former NFL players, there was a direct effect between RHI and reduced cellular energy metabolism (i.e., lower creatine). MRS neurochemicals associated with neuroinflammation also correlated with behavioral/mood symptoms.

Keywords

Chronic traumatic encephalopathy; repetitive head impacts; magnetic resonance spectroscopy; magnetic resonance imaging; tackle football

INTRODUCTION

Chronic traumatic encephalopathy (CTE) is a neurodegenerative disease found in individuals exposed to repetitive head impacts (RHI), particularly American football players (McKee et al. 2013; Mez et al. 2017). The neuropathology of CTE is well-defined (McKee et al. 2016; McKee et al. 2013; Mez et al. 2017). Our understanding on its clinical presentation has improved (Alosco, Mez, et al. 2018; Mez et al. 2017; Montenigro et al. 2014; Stern et al. 2013), but CTE cannot be diagnosed in life because validated biomarkers do not exist. Proton MR spectroscopy (MRS) may assist in the detection of CTE. MRS measures neurochemical markers of neuronal viability (N-acetyl aspartate; NAA), immunoexcitotoxicity (glutamate [Glu]/glutamine; Glx), axonal injury (choline; Cho), astrocytosis and microglial activation (myo-inositol; mI), energy metabolism (creatine; Cr), and neuroinflammation (glutathione; GSH). CTE is associated with widespread neurodegeneration with the diagnostic lesion being perivascular phosphorylated tau (p-tau) at the sulcal depths (McKee et al. 2016). Neuroinflammation, gliosis, axonal loss, and white matter disease accompany CTE (McKee et al. 2013). MRS has indeed been used to detect neurodegenerative diseases, like Alzheimer's disease (AD) (Graff-Radford & Kantarci 2013; Kantarci et al. 2007; Watanabe, Shiino, & Akiguchi 2012).

MRS studies in former contact sport athletes (e.g., former professional soccer players, boxers, rugby players) (Davie et al. 1995; Gardner et al. 2017; Koerte et al. 2015; Lin et al. 2015; Tremblay et al. 2013) link RHI with long-term neurochemical alterations, which may contribute to cognition (Koerte et al. 2015; Tremblay et al. 2013). The few studies examining RHI and later-life neurochemistry used small sample sizes and lacked robust metrics to estimate RHI. This study used MRS to examine neurochemical concentrations in symptomatic former National Football League (NFL) players. The relationship between RHI and neurochemistry was tested, as were the associations between neurochemistry and neuropsychological and neuropsychiatric functioning. We hypothesized that NAA and higher Glu, Glx, GSH, and Cho would correlate with RHI and be associated with worse cognitive and neuropsychiatric functioning.

MATERIALS AND METHODS

Participants

Participants were from the "Diagnosing and Evaluating Traumatic Encephalopathy using Clinical Tests" (DETECT) study. Participants included 96 former NFL players. Inclusion criteria included: male, aged 40–69, a minimum of two NFL seasons and a minimum of twelve years of organized football, and self-reported complaints of cognitive, and/or behavioral/mood symptoms at the time of study screening. A same-age comparison group (n=24), who were without a history of contact sport participation, service in the military, or self-reported TBI, and denied symptoms (at telephone screen) were recruited. Since the groups differed according to RHI history and symptom status, all analyses with this "control" group were considered preliminary. Exclusion criteria for all participants included MRI and/or lumbar puncture contraindications, presence of another central nervous system disease, primary language other than English, and/or history of a TBI within one year of study screening.

Participants completed a two- to three-day study visit that included: demographic, medical, and athletic history interview(s); neurological evaluation; neuropsychological testing; structured psychiatric interview; self-report behavior/mood measures; neuroimaging; among other exams not relevant to this study (Alosco, Jarnagin, et al. 2017; Alosco, Tripodis, et al. 2018; Stamm, Bourlas, et al. 2015; Stamm, Koerte, et al. 2015).

Standard Protocol Approvals, Registrations, and Patient Consents—Study protocols were approved by the Institutional Review Boards at Boston University Medical Campus and Brigham and Women's Hospital and were HIPAA compliant. All participants provided written informed consent.

Measures

Magnetic Resonance Spectroscopy—The MR scanner was a 3T Siemens Verio with a 32-channel head coil array. The MRI protocol consisted of a 3D T1-weighted sequence (MPRAGE [1×1×1mm^3, TR=1800ms, TE=3.36ms)]), and a 3D T2-weighted sequence (SPACE, [1×1×1mm^3, TR=3200ms, TE=456ms]). Single voxel point-resolved spectroscopy (PRESS; TE=35ms, TR=2s, 2×2×2cm^3) measured NAA, mI, Cr, Cho, Glu, Glx, and GSH

concentrations in the anterior (ACG) and posterior cingulate gyrus (PCG), and the left parietal white matter (PWM) (Figure 1). The ACG, PCG, and PWM are sensitive to neurochemical changes in AD (Fayed, Modrego, Rojas-Salinas, & Aguilar 2011; Graff-Radford & Kantarci 2013) and provide optimal spectral quality. The voxel was localized using anatomical landmarks from T1-weighted images. Each voxel underwent automated optimization (3D shimming, transmit gain, frequency adjustment, and water suppression). Screen shots of voxel location and spectra were examined. Manual shimming to a line width of <14 Hz of the full-width half maximum (FWHM) of the unsuppressed water spectrum was performed.

The single voxel MRS spectra were exported and raw data were processed by the study physicist (B.R.), who was blind to group membership, using singular value decomposition (SVD) based channel combination, spectral registration to correct for frequency drift, and residual water suppression using the Hankel SVD method (Rowland et al. 2017). The metabolites were fit using linear combination models (LCModel). Metabolites FWHM were <0.10 and signal to noise ratio was <50. To optimize data quality and reliability, only metabolites with Cramer-Rao lower bound (CRLB) <20% were analyzed; a majority had a CRLB <10%. Concentrations were partial-volume corrected by segmenting gray matter, white matter, and CSF within the voxel and correcting for the water concentration. To do so, the concentrations were multiplied by the following factor:

$$[(55556/35880) \times (1.000 \times \text{partial volume fraction of CSF}) + (0.779 \times \text{partial volume of gray matter}) + (0.645 \times \text{partial volume of white matter})]$$

This equation corrects for the assumed LCModel water concentration (35880mM) with the actual water concentration (55556mM), and then corrects for partial volume of each compartment of water, where CSF is assumed 100% water content, gray matter is 77.9%, and white matter is 64.5% (Ernst 1993).

Cumulative Head Impact Index—Exposure to RHI was estimated by the CHII (Montenigro et al. 2017). The CHII is based on self-reported football history (number of seasons, position[s] at each level played), and estimated head impact exposure frequencies from published studies using helmet-mounted accelerometers. The CHII was developed in former amateur football players because published helmet accelerometer studies at the professional level do not exist. Estimates of head impact frequencies from published college accelerometer studies were applied to the seasons and positions played in the NFL in the present sample. Higher CHII reflects greater exposure to RHI.

Clinical Measures—Neuropsychological tests evaluated attention, executive function, verbal and visual episodic memory, language, and visuospatial function. Semi-structured interviews and self-report measures of neuropsychiatric function (e.g., depression, apathy, aggression) were completed. All tests administered are described elsewhere (Alosco, Jarnagin, et al. 2017). Raw scores were transformed to standard scores using normative data calibrated for age, sex, and/or education. Principal component analysis resulted in composite scores for behavioral/mood, psychomotor speed/executive function, verbal memory, and

visual memory domains (Alosco, Jarnagin, et al. 2017). Higher scores on the behavioral/mood composite are worse, whereas lower scores on the other composites are worse.

Sample Size

The sample of 96 former NFL players and 24 asymptomatic individuals without a history of head trauma was reduced to 77 and 23, respectively, following exclusion of participants who did not complete MRI or whose structural MRI and/or MRS data acquisition was of inadequate quality due to motion artifact. Two additional former NFL players were excluded due to neuropsychological evidence of intentional symptom exaggeration. Table 1 presents sample characteristics. There were no differences between the 77 former NFL players compared to those excluded in terms of age, education, or years of football, or clinical test scores (p's>0.05). Of the 77 former NFL players, four former NFL players did not have MRS data for the ACG (n=73) due to early termination of the MRI protocol; of these four, one did not have PWM data (n=76). As mentioned above, only neurometabolites with a CRLB <20% were examined. This criterion affected the sample size for GSH (PCG: n=75 former NFL players, n=23 comparison group; ACG: n=66 former NFL players, n=21 comparison group; PWM: n=66 former NFL players, n=22 comparison group). Finally, for analyses examining the clinical factor composite scores as the outcome, the sample size of the former NFL players was decreased to 69 due to missing data on individual cognitive, behavior, and/or mood measures that make-up the composite scores; note that the sample size was further reduced and varied across neurochemicals and brain regions due to the above described exclusionary reasons. For the controls, the sample size of 23 was constant with the exception of PWM GSH (n=22) and ACG GSH (n=21).

Statistical Analysis

In the former NFL players, partial correlations controlling for age and body mass index (BMI) examined associations among the ACG, PCG, and PWM neurochemicals with the CHII and the clinical composite scores (i.e., behavioral/mood, psychomotor speed/executive function, verbal memory, and visual memory). Analyses examining clinical function controlled for education, along with age and BMI. Multivariate linear mixed-effects models examined differences between the former NFL players and the controls in NAA, mI, Cho, Cr, Glu, Glx, and GSH concentrations in the ACG, PCG, and PWM. Correlated outcomes can artificially decrease p-values (Sainani 2010). The multivariate linear mixed-effects models were thus performed to reduce Type I error because they account for correlations between groups, outcomes from the same participant, and between the same test for metabolites and brain regions. The models were adjusted for age and BMI. Race was not included as a covariate because there is not a large enough sample of African American controls (n=1) to provide an accurate estimation on the differential effects of race on the neurochemicals between the former NFL players and controls. The analyses of group differences are considered to be preliminary due to differences between the groups in symptomatic status at the time of recruitment.

For all analyses, bootstrap analysis was performed on 500 replicates to further control for Type I error and increase statistical power. All results presented are from the bootstrap

analyses that protect against Type I error inflation for each individual p-value. Significance level was an alpha of 0.05. SAS (SAS Institute Inc., v.9.4) was used to perform analyses.

RESULTS

Table 1 provides an overview of the sample characteristics. The principal component factor composite scores for the symptomatic former NFL players and controls are presented in Table 2.

Exposure to RHI and Neurochemical Concentrations

In the former NFL players, a higher CHII was associated with lower later-life concentrations of Cr in the PWM ($r=-0.23$, $p=0.02$) (Figure 2). The CHII was not associated with NAA, Glu, Cho, Cr, mI, or GSH in the PWM (ps>0.10). In the ACG and PCG, there were no significant correlations with all of the measured metabolites (ps>0.20).

Symptomatic Former NFL Players versus Asymptomatic Controls: Group Differences

A summary of the linear mixed-effect models is provided in Table 3. The former NFL players exhibited significantly lower PWM NAA levels compared to asymptomatic individuals without a history of head trauma (mean difference=1.02, 95% CI = 0.08, 2.31, $p=0.03$). No significant differences between the former NFL players and controls were found for the other neurochemicals in the PWM, ACG, or PCG (p's>0.10).

Neurochemistry and Clinical Function

Partial correlations examining the relationship between neurochemistry in the PWM, ACG, and PCG with behavior/mood, psychomotor speed/executive function, and verbal and visual memory in the former NFL players are presented in Table 4. The ACG was the only brain region associated with clinical function. In particular, there were statistically significant positive correlations between ACG concentrations of Glu, GSH, and mI with the behavioral/mood composite score (Figure 3). Higher concentrations of NAA in the ACG were also associated with higher scores (i.e., worse) on the behavioral/mood composite. There was a statistically significant positive effect between ACG Glx concentrations and psychomotor speed/executive function, as well as positive correlations between ACG Cr and Glu with visual memory (Table 4).

DISCUSSION

In this sample of symptomatic former NFL players, there was a direct relationship between greater exposure to RHI (as measured by the CHII) and lower Cr in the PWM. However, only NAA concentrations in the PWM were found to be different (i.e., lower) among the former NFL players compared to the "controls". In the former NFL players, higher ACG concentrations of neurochemicals that can reflect immunoexcitotoxicity (i.e., Glu) and neuroinflammation (i.e., GSH, mI) corresponded with greater behavioral/mood symptoms. ACG NAA correlated with behavioral/mood symptoms and other ACG neurochemicals were associated with psychomotor speed/executive function and visual memory; however, the directionality of these effects was opposite of expected. This study provides evidence for

reduced cellular energy metabolism as a long-term consequence of RHI. The findings additionally provide initial support for neuroinflammation, particularly in the ACG, as a possible contributor to behavioral/mood disturbances in symptomatic former NFL players.

Our previous research has shown the CHII to be associated with later-life cognitive and neuropsychiatric function in former amateur American football players (Montenigro et al. 2017), as well as with higher concentrations of plasma (Alosco, Tripodis, et al. 2017) and cerebrospinal fluid (CSF) (Alosco, Tripodis, et al. 2018) total tau, and greater burden of white matter hyperintensities (Alosco, Koerte, et al. 2018) in former NFL players from the DETECT study. The current study extends these findings by showing an inverse relationship between the CHII and PWM Cr in former NFL players from DETECT. Cr regulates cellular energy metabolism and production of ATP (Brewer & Wallimann 2000), thereby exposure to RHI may be associated with later-life reduced cellular energy metabolism. Although Cr has traditionally been thought to be constant, this may not be the case in setting of head trauma (Gasparovic et al. 2009). The association between the CHII and Cr was only observed in the PWM. The parietal lobe may be more susceptible to later life consequences of RHI, given the specific effects for this region in this sample and other studies of former NFL players that examined molecular and functional imaging (Coughlin et al. 2015; Ford, Giovanello, & Guskiewicz 2013).

The CHII was not associated with other neurochemicals and there were no differences in Cr between the former NFL players and “controls”. Instead, only lower PWM NAA was observed in the former NFL players. NAA is of particular interest because it is produced in neurons and transported down axons, and decreased NAA reflects reduced neuronal, axonal, and dendritic viability. It is thus a marker of neurodegeneration (Lin et al. 2012). Previous work shows acute NAA reductions in active contact sport athletes (Chamard et al. 2012) and decreased NAA in three former boxers with parkinsonism (Davie et al. 1995), when compared to controls and idiopathic Parkinson’s disease. Although, other research did not report differences in NAA between former professional soccer players and non-contact sport athletes (Koerte et al. 2015). Results from the group comparison(s) in the present study have methodological caveats that limit the ability to draw meaningful conclusions. The control group was small and not well matched to the former NFL players on factors such as race. Study eligibility criteria required the former NFL players to be symptomatic at the time of recruitment, whereas the comparison group must have been asymptomatic. The neurochemical alterations may therefore be a result of exposure to RHI or related to differences in symptom status.

Other notable findings included the associations between higher ACG Glu, GSH, and mI with greater behavioral/mood symptoms in the former NFL players. A previous MRS study in former professional soccer players showed that higher mI and GHS concentrations correlated with greater number of lifetime estimate of headings; higher GSH was also associated with worse executive function (Koerte et al. 2015). Glu is the most abundant excitatory neurotransmitter in the brain and excessive Glu can lead to excitotoxicity (Ramadan, Lin, & Stanwell 2013). GSH is an antioxidant that is activated as a compensatory response to oxidative stress and neuroinflammation (Duffy et al. 2014). Increased mI is considered to be a marker of astrocytosis and microglial activation (Fisher, Novak, &

Agranoff 2002). Based on retrospective informant reports, the clinical presentation of CTE includes a constellation of cognitive, behavioral, and mood disturbances (Alosco, Mez, et al. 2018; Mez et al. 2017; Montenigro et al. 2014; Stern et al. 2013). Behavioral/mood disturbances tend to begin at a young age compared to a later onset of cognitive impairments (Alosco, Mez, et al. 2018; Mez et al. 2017; Stern et al. 2013). While later onset cognitive impairment is typical with neurodegeneration, it is not clear if neuropsychiatric disturbances in CTE are due to idiopathic mental illness, CTE p-tau deposition, or other types of pathologies. There is accumulating evidence for neuroinflammation as a possible mechanism that exposure to RHI may lead to CTE (Alosco, Tripodis, et al. 2018; Bari et al. 2018; Cherry et al. 2017; Cherry et al. 2016; Coughlin et al. 2017). Here, associations between the CHII with ACG Glu, GSH, or mI did not emerge. A recent study from DETECT similarly did not find a direct effect between the CHII and CSF sTREM2 (a marker of microglial activation) (Alosco, Tripodis, et al. 2018). Brain alterations can begin in youth football (Bahrami et al. 2016), but the course of brain changes and their corresponding relation with clinical function is unknown. Glu may be elevated during active RHI (Bari et al. 2018) and decrease with neurodegenerative disease due to loss of neuronal density (Fayed et al. 2011; Lin, Shic, Enriquez, & Ross 2003). Longitudinal research is needed to clarify the relation between RHI and neuroinflammation and the corresponding clinical consequences.

The current study revealed some unexpected effects. For example, the positive directionality of effects for several of the ACG neurochemicals and clinical function was opposite of expected (i.e., higher NAA and worse behavior/mood, higher Glx and better psychomotor speed/executive function, as well as higher Cr and Glu and better visual memory). In general, the utility of MRS neurochemicals in the setting of RHI exposure is not well understood. Although we implemented methods to attenuate risk for Type I error, this remains a limitation due to the number of analyses performed. There was substantial variability in neurochemical levels across participants, potentially reflecting the heterogeneity of this sample in terms of pathological burden. Metabolite concentrations and their effects on clinical function may depend on disease stage and this is unknown because CTE cannot be diagnosed during life. Notably, the former NFL players only had worse scores on the behavioral/mood composite and not on the cognitive composites compared to the controls.

Additional methodological limitations include the modest sample size, limited RHI variability, and cross-sectional study design. The ACG was the only brain region where MRS neurochemical measures were associated with clinical function. The ACG is a hub of neural networks that modulate cognition, behavior, and mood (Lichenstein, Verstynen, & Forbes 2016). However, brain regions not examined in this study may be more sensitive to RHI and clinical function. The prefrontal cortex is an initial CTE target (McKee et al. 2016; McKee et al. 2013; Mez et al. 2017). Former university level ice hockey and football players have exhibited elevated prefrontal Cho (Tremblay et al. 2013) and prefrontal neurochemical changes in active high school football players have been observed (Bari et al. 2018). Finally, the neurochemicals examined in this study can be impacted by various etiologies. It is plausible that a competing neurodegenerative disease is present, which could explain the

conflicting findings of lower NAA in the former NFL players but no association between NAA and RHI.

In summary, this study found evidence for a direct relationship between greater RHI exposure and reduced later-life cellular energy metabolism in symptomatic former NFL players. Findings additionally suggest that the ACG and associated neuroinflammatory processes may have clinical implications among individuals exposed to RHI, particularly in terms of the development of behavioral and mood disturbances. Yet, the mechanisms that underpin RHI, CTE, and clinical dysfunction are unknown and likely complex, multifaceted, and evolve over time. Although CTE is defined by its unique p-tau deposition, many cases have other non-tau pathologies (e.g., neuroinflammation) that may have commenced during active RHI exposure and lead to neurodegeneration through interaction with other risk factors (e.g., genetics). Further research is needed to better understand the utility of MRS for the study of disease mechanisms in the setting of RHI and CTE.

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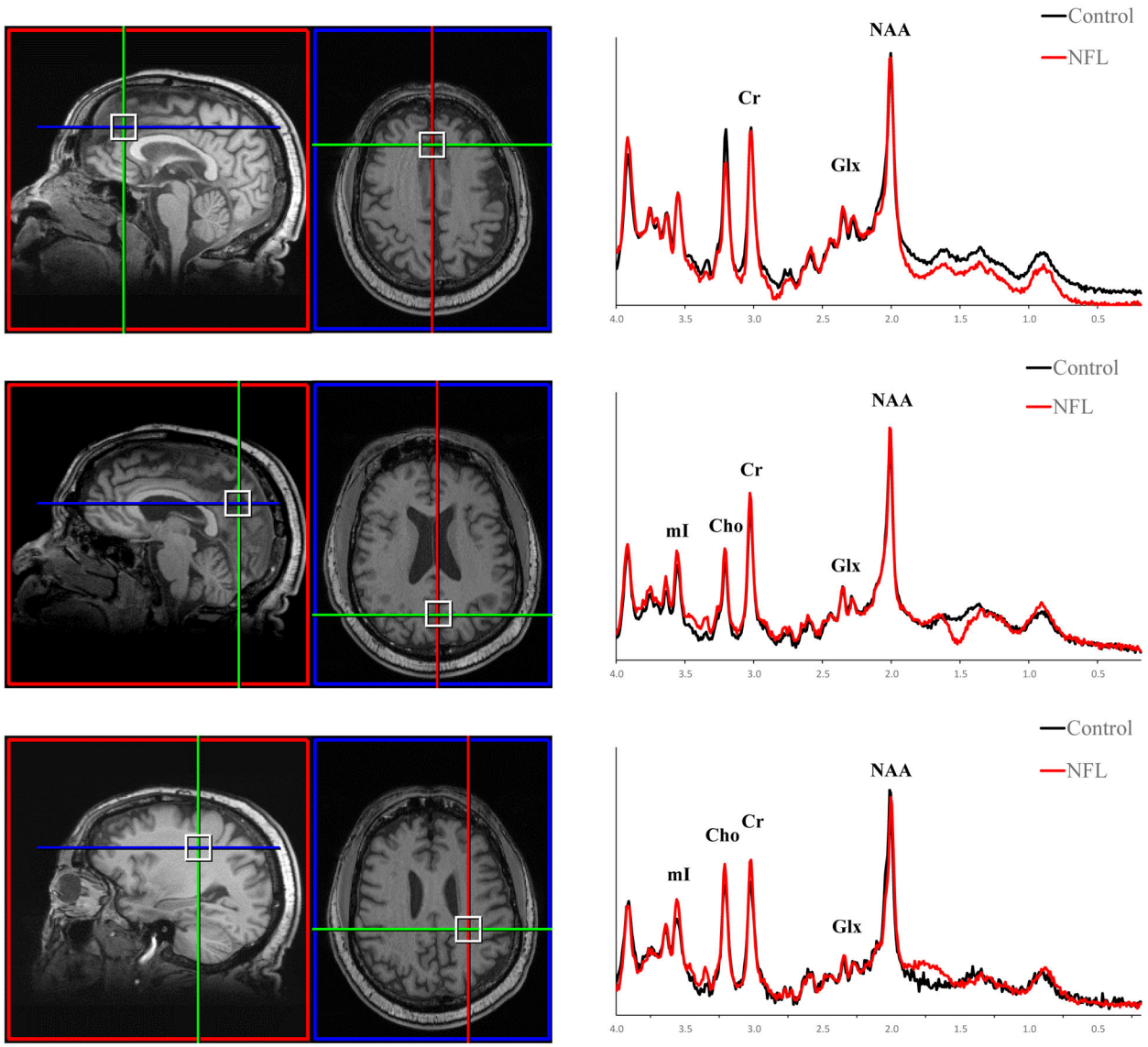

Fig. 1.

Representative Voxel Locations and Spectra. (A) Voxel location (sagittal and axial, respectively) and spectra for the anterior cingulate gyrus, (B) Voxel location (sagittal and axial, respectively) and spectra for the posterior cingulate gyrus, (C) Voxel location (sagittal and axial, respectively) and spectra for the parietal white matter. Former NFL players were required to be symptomatic and "Controls" were individuals who were asymptomatic and did not have a history of head trauma.

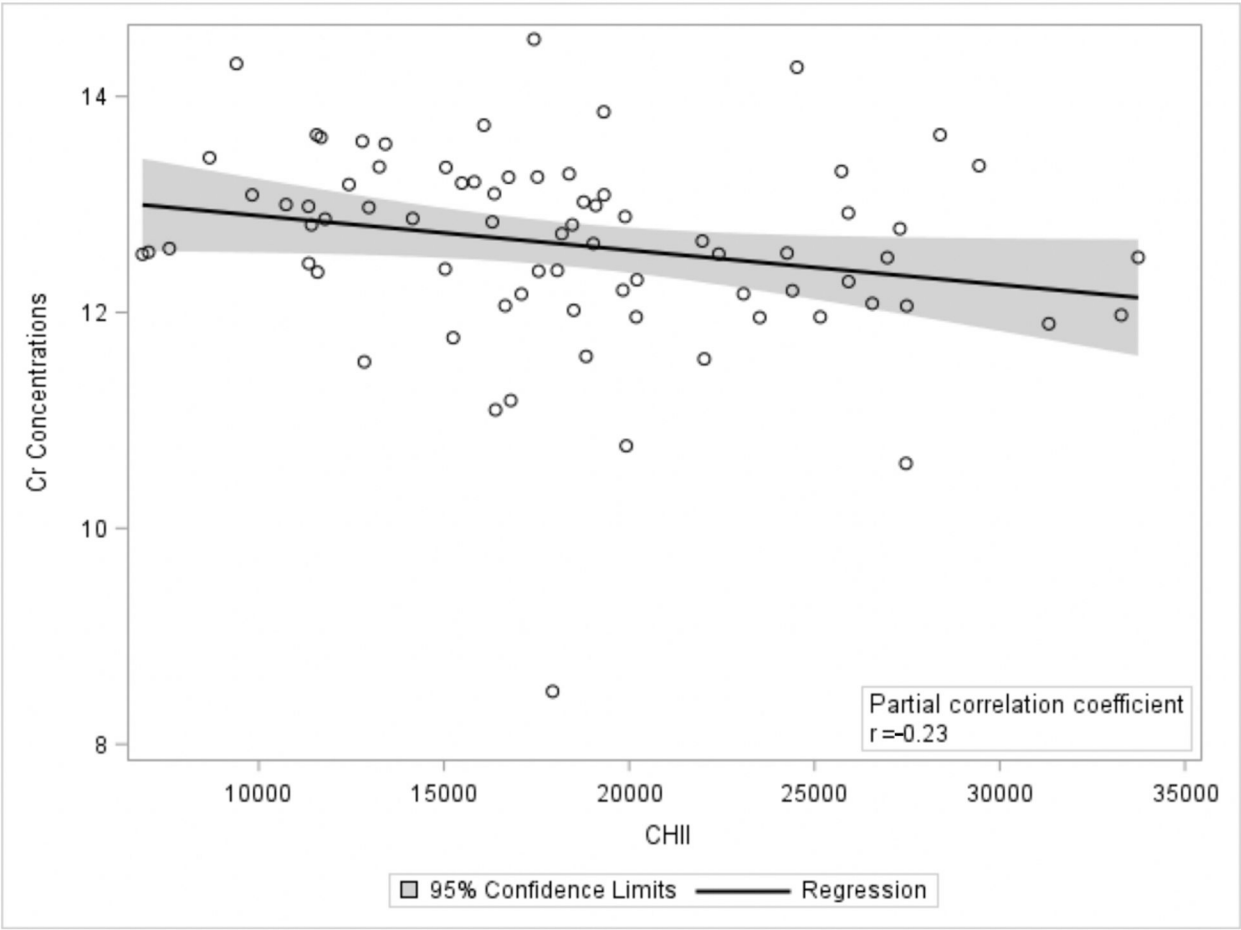

Fig 2.

Greater Exposure to Repetitive Head Impacts is Associated with Lower Later-life Parietal White Matter Creatine Concentrations. The scatter plot is of the residuals between the cumulative head impact index (CHII) and Creatine (Cr) after controlling for age and body mass index. X-axis represent each participant's CHII, with higher scores reflecting greater exposure to RHI. Y-axis values are Cr concentrations. Shaded region is the 95% confidence intervals. The results remain unchanged when the individual with very low Cr concentrations was removed from the analysis.

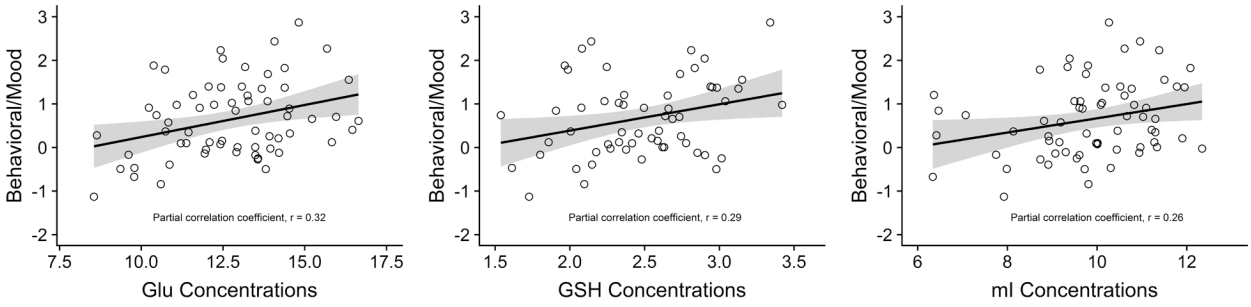

Fig 3.

Association between Anterior Cingulate Gyrus Neurochemistry and Behavioral/Mood Symptoms. The scatter plots are of the residuals between the anterior cingulate gyrus (ACG) glutamate (glu), glutathione (GSH), and myo-inositol (mI) concentrations and the behavioral/mood principal component composite score after controlling for age, body mass index, and education. Higher concentrations of Glu (A), GSH (B), and mI (C) were associated with higher (i.e., worse) behavioral/mood scores. The x-axis is the neurochemical concentrations and the y-axis is the behavioral/mood composite scores. Shaded region represents 95% CI.

Table 1.

Sample Characteristics

	Symptomatic Former NFL Players (n = 77)	Controls (n = 23)	p-value
Age, mean (SD) years	55.96 (7.77)	57.22 (6.89)	0.49
Education, mean (SD) years	16.45 (1.02)	17.30 (2.14)	0.08
African American, n (%)	30 (39.0)	1 (4.3)	0.002
Duration of football play, mean (SD) years	17.97 (3.59)	--	--
Years in the NFL, mean (SD)	7.76 (2.75)	--	--
Cumulative Head Impact Index, mean (SD)	19,881.71 (6540.99)	--	--
Primary Position Group, n (%)			
Offensive line	19 (24.7)	--	--
Running back	7 (9.1)	--	--
Tight end	4 (5.2)	--	--
Offensive skill	1 (1.3)	--	--
Defensive line	11 (14.3)	--	--
Linebacker	18 (23.4)	--	--
Defensive Back	17 (22.1)	--	--
Body mass index, mean (SD) kg/m^2	32.73 (4.89)	28.02 (3.90)	<0.001

The “control” group was required to be asymptomatic and have no history of head trauma at the time of recruitment. Independent samples t-tests tested for statistically significant group differences on age, education, and body mass index, whereas Fisher’s Exact Test (due to small cell sizes) was used for race.

Table 2.

Cognitive and Neuropsychiatric Test Performance

	Former NFL Players	Controls	P-value
Principle Component Factor Scores, mean (SD)			
Behavioral/Mood	0.35 (0.93)	−0.92 (0.46)	<0.001
Psychomotor Speed/Executive Function	−0.03 (0.84)	0.27 (0.68)	0.75
Verbal Memory	−0.08 (0.89)	0.35 (1.22)	0.05
Visual Memory	0.01 (0.90)	0.29 (0.74)	0.28

The “control” group was required to be asymptomatic and have no history of head trauma at the time of recruitment. Neuropsychological tests evaluated attention, executive function, verbal and visual episodic memory, language, and visuospatial function. Semi-structured interviews and self-report measures of neuropsychiatric function (e.g., depression, apathy, aggression) were completed. Raw scores were transformed to standard scores using normative data calibrated for age, sex, and/or education. Principal component analysis resulted in composite scores for behavioral/mood, psychomotor speed/executive function, verbal memory, and visual memory domains. The sample size of the former NFL players was decreased to 69 due to missing data on individual cognitive, behavior, and/or mood measures that make-up the composite scores. The sample size was further reduced and varied across the neurochemicals and brain regions due to other participant exclusionary criterions described in the text. A multivariate analysis of covariance (ANCOVA) controlling for age, body mass index, and years of education was performed to test for group differences on each clinical factor composite score.

Table 3.

Summary of Linear Mixed-Effects Models Examining Differences in Neurochemical Concentrations Between the Former NFL Players and Controls

Neurometabolites	ACG			PCG			PWM		
	Mean Diff	95% CI	P	Mean Diff	95% CI	P	Mean Diff	95% CI	P
Creatine	0.16	−0.64, 0.70	0.66	0.04	−0.74, 0.60	0.93	0.31	−0.14, 1.09	0.28
Glutamate_Glutamine	−0.95	−2.26, 0.12	0.10	−0.47	−1.64, 0.46	0.37	−0.03	−0.06, 1.17	0.90
Glutamate	−0.33	−1.25, 0.44	0.43	−0.14	−1.04, 0.62	0.78	0.14	−0.53, 1.39	0.80
Choline	0.17	−0.10, 0.39	0.26	0.09	−0.12, 0.23	0.36	0.26	−0.09, 0.52	0.08
Glutathione	−0.09	−0.32, 0.16	0.47	−0.04	−0.25, 0.15	0.65	0.11	−0.14, 0.39	0.38
Myo-inositol	0.24	−0.50, 0.95	0.56	0.32	−0.34, 0.91	0.32	0.12	−0.57, 0.81	0.81
N-acetyl aspartate	−0.27	−1.55, 0.92	0.60	−0.52	−1.57, 0.35	0.26	1.02	0.08, 2.31	**0.03**

The “control” group was required to be asymptomatic and have no history of head trauma at the time of recruitment. Analyses adjusted for age and body mass index. Mean diff = mean difference in neurochemical concentration between former NFL players and controls (former NFL-control). Bootstrap analysis was performed on 500 replicates to further control for Type I error and increase statistical power. ACG = anterior cingulate gyrus; PCG = posterior cingulate gyrus; and PWM = left parietal lobe white matter.

Table 4.

Partial Correlations between Neurochemistry and Clinical Factor Composite Scores in Symptomatic Former NFL Players

	Psychomotor Speed/Exec. Func.			Verbal Memory			Visual Memory			Behavior/Mood		
	ACG	**PCG**	**PWM**	**ACG**	**PCG**	**PWM**	**ACG**	**PCG**	**PWM**	**ACG**	**PCG**	**PWM**
Cr	.09(.31)	−.06(.74)	.02(0.79)	−.15(.28)	−.23(.07)	−.07(.57)	**.27(.04)**	.07(.59)	−.14(.50)	.19(.11)	.07(.57)	−.10(.38)
Glx	**.24(.02)**	.05(.63)	−.09(0.54)	−.002(.96)	−.06(.48)	.13(.37)	.25(.05)	−.06(.72)	.01(.96)	.22(.05)	.11(.30)	−.06(.71)
Glu	.18(.14)	−.0002(.86)	−.04(0.72)	−.01(.96)	−.12(.25)	.03(.94)	**.26(.02)**	−.03(.92)	−.10(.45)	**.32(.004)**	.04(.64)	−.001(.98)
Cho	.10(.38)	.001(.85)	.18(0.14)	.07(.58)	.01(.98)	.19(.20)	.22(.08)	.11(.38)	.04(.82)	.23(.05)	−.002(.98)	−.12(.34)
GSH	.16(.26)	−.14(.32)	−.15(0.37)	−.07(.68)	.02(.82)	.09(.34)	.11(.44)	−.02(.82)	−.06(.62)	**.29(.02)**	.15(.28)	−.13(.19)
mI	.08(.42)	−.06(.61)	−.02(0.95)	−.18(.16)	−.25(0.06)	−.24(.07)	.16(.14)	−.05(.66)	−.11(.41)	**.26(.01)**	.08(.47)	−.15(.27)
NAA	.14(.20)	.07(.52)	.13(0.34)	.08(.46)	−.09(.37)	.18(.09)	.21(.11)	−.01(.96)	−.21(.22)	**.42(<.001)**	.17(.10)	.05(.71)

Partial correlations controlling for age, body mass index, and education. P-value is in parenthesis and bolded are significant at the less than 0.05 alpha level. The clinical factor composites were derived using principal component analysis. Bootstrap analysis was performed on 500 replicates to further control for Type I error and increase statistical power. ACG = anterior cingulate gyrus; PCG = posterior cingulate gyrus; PWM = parietal white matter; Cr = creatine; Glx = glutamate/glutamine; Glu = glutamate; Cho = choline; GSH = Glutathione; mI = myo-Inositol; and NAA = N-acetyl aspartate.