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ORIGINAL RESEARCH

Dependence on subconcussive impacts of brain metabolism in collision sport athletes: an MR spectroscopic study

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Abstract

Long term neurological impairments due to repetitive head trauma are a growing concern for collision sport athletes. American Football has the highest rate of reported concussions among male high school athletes, a position held by soccer for female high school athletes. Recent research has shown that subconcussive events experienced by collision sport athletes can be a further significant source of accrued damage. Collision sport athletes experience hundreds of subconcussive events in a single season, and these largely go uninvestigated as they produce no overt clinical symptoms. Continued participation by these seemingly uninjured athletes is hypothesized to increase susceptibility to diagnoseable brain injury. This study paired magnetic resonance spectroscopy with head impact monitoring to quantify the relationship between metabolic changes and head acceleration event characteristics in high school-aged male football and female soccer collision sport athletes. During the period of exposure to subconcussive events, asymptomatic male (football) collision sport athletes exhibited statistically significant changes in concentrations of glutamate+glutamine (Glx) and total choline containing compounds (tCho) in dorsolateral prefrontal cortex, and female (soccer) collision sport athletes exhibited changes in glutamate+glutamine (Glx) in primary motor cortex. Neurometabolic alterations observed in football athletes during the second half of the season were found to be significantly associated with the average acceleration per head acceleration events, being best predicted by the accumulation of events exceeding 50 g. These marked deviations in neurometabolism, in the absence of overt symptoms, raise concern about the neural health of adolescent collision-sport athletes and suggest limiting exposure to head acceleration events may help to ameliorate the risk of subsequent cognitive impairment.

Keywords MR spectroscopy · Concussion · Subconcussive injury · Football · Soccer

Introduction

Recent interest in the diagnosis and prognosis of mild traumatic brain injury (mTBI) and concussion in collision sports has grown, driven by a number of disturbing trends related to the short- and long-term health of participating athletes (Schwarz 2011; Belson 2015; Auerbach and Waggoner 2nd 2016). Until recently, the standard of clinical care for a diagnosis of concussion would allow athletes to return to play as soon as they became symptom free (McCrory et al. 2013). Athletes with a history of diagnosed concussions often continued participation in practices and games, leading to greater risk of accumulating structural and functional brain alterations (Guskiewicz et al. 2005; Baugh et al. 2012). Long term neurological impairments associated with mTBI include early onset of Alzheimer's disease, dementia, depression, and even chronic traumatic encephalopathy (McKee et al. 2009;

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Gavett et al. 2011; McCrory et al. 2013). Among male athletes American Football has the highest annual rate of reported concussions, a position held by soccer in female athletes (Gessel et al. 2007; Zuckerman et al. 2015). Extra care and systematic evaluation is required in case of high school athletes before they return to play, because they are more neurologically vulnerable than a collegiate population (Field et al. 2003).

Critically, a growing body of research has now demonstrated that asymptomatic athletes are also at the risk of functional and structural brain damage (Bazarian et al. 2014; Davenport et al. 2014; McAllister et al. 2014; Talavage et al. 2014, 2015; Reynolds et al. 2017; Slobounov et al. 2017). Such findings have raised concerns regarding the many subconcussive impacts experienced by collision-sport athletes. A subconcussive event involves a direct blow to the head or an indirect acceleration or whiplash movement due to an impact elsewhere on the body, but does not result in clinical symptoms (Bailes et al. 2013)—hereafter referred to as a head acceleration event (HAE).

Repetitive exposure to HAEs is linked to later life-long cognitive and functional impairment (McKee et al. 2009; Broglio et al. 2012; Lipton et al. 2013; Montenigro et al. 2017). Athletes experience hundreds of HAEs during each competition season (Broglio et al. 2011), but these events largely go uninvestigated as they produce no overt symptoms. Continued participation by these seemingly uninjured athletes is hypothesized to increase susceptibility to meaningful brain injury (Stern et al. 2011; Alosco et al. 2017), with animal models suggesting the increased likelihood of development of neurodegenerative disease (Tagge et al. 2018).

Detection and intervention to reduce the potential long-term consequences of exposure to HAEs is, therefore, a critical direction for the research community (Bailes et al. 2015). Conventional structural imaging techniques (computed tomography or T_1/T_2 weighted MRI) may not always detect the subtle physiologic changes or cellular injury caused by the accumulation of these events. Functional neuroimaging techniques like functional MRI (FMRI) and diffusion weighted imaging (DWI), however, play a significant role in elucidating the structural and physiologic changes caused by repetitive head trauma in the absence of overt cognitive symptoms. Several studies using these modalities have demonstrated changes in functional connectivity, working memory, cerebrovascular reactivity and white matter in asymptomatic collision sport athletes due to repeated HAE exposure (Bazarian et al. 2014; Davenport et al. 2014; Abbas et al. 2015a, b; Chun et al. 2015; Shenk et al. 2015; Svaldi et al. 2015; Talavage et al. 2015; Reynolds et al. 2017; Slobounov et al. 2017; Svaldi et al. 2017). Another important non-invasive tool that provides assessment of metabolite profiles and helps in elucidating altered neurometabolism is proton (^{1}H) magnetic resonance spectroscopy (MRS). MRS has been used as a diagnostic tool to investigate changes in physiology in common neuropathologies like multiple sclerosis, dementia, Parkinson's disease, and Alzheimer's disease (Gujar et al. 2005; Lin et al. 2005; Groger et al. 2014; Oz et al. 2014; Zhang et al. 2015). MRS also serves as an ideal biomarker for identifying early brain changes in mTBI, including concussion (Ricci et al. 1997; Friedman et al. 1998; Newsholme et al. 2003; Govindaraju et al. 2004; Shutter et al. 2004; Gasparovic et al. 2009; Henry et al. 2010; Vagnozzi et al. 2010; Yeo et al. 2011; Maudsley et al. 2015). We have previously used MRS to determine alterations in neurometabolite concentrations occur during periods of exposure to HAEs (Poole et al. 2014, 2015).

This study of high school-aged male football and female soccer collision sport athlete (CSA) and non-collision athlete (NCA) control populations seeks to evaluate whether MRS-detected changes exist and are associated with particular HAE characteristics. Observation in asymptomatic athletes of alterations in neurometabolism that are found to be associated with particular HAE mechanical loading profiles would be suggestive of preventable cellular injury. Such a finding would have broad implications for mitigation of the effects of subconcussive events, through which collision-based sports could be made safer for all participants.

Methods

Participants

Ninety (90) high school athletes participated in this study. Two pools comprise this sample, 63 athletes participating in collision sports (CSA) and 27 athletes participating in non-collision sports (NCA).

CSA 40 male football athletes (ages: 15–18 years, mean = 16.4) and 23 female soccer athletes (ages: 14–17 years, mean = 15.9), each a member of a high school junior varsity or varsity team, were recruited from two local high schools over two seasons of play (seven football and six soccer players participated in both the seasons, yielding a total of 76 observations for CSA).

NCA 14 male athletes (ages: 15–18 years, mean = 16.21) and 13 female athletes (ages: 14–18 years, mean = 16.07), each participating only in non-collision high school sports (e.g. cross-country, swimming, track and field, tennis, basketball, softball) at the junior varsity or varsity level, were recruited (from the same high schools as the CSA participants) to serve as a control for the stability of the measurement process.

Note that participants were not excluded due to history of concussion. Self-reports of prior history of concussion (and associated counts) were 16 of 40 male CSA (1 prior

concussion: *n* = *9*; 2 prior concussions: *n* = *4*; 3 prior concussions: *n* = *3*), 6 of 27 female CSA (1: *n* = *3*; 2: *n* = *1*; 3: *n* = *2*), 5 of 14 male NCA (1: *n* = *3*; 2: *n* = *1*; 4: *n* = *1*), and 4 of 13 female NCA (1: *n* = *3*; 3: *n* = *1*).

None of the athletes were diagnosed with a concussion by their athletic trainer or team physician during the period of study.

Imaging schedule

The CSA pool underwent five imaging sessions: one prior to participation in collision activities (*Pre*), one each during the first (*In1*) and second (*In2*) halves of the competition season, and two following the cessation of collision activities at intervals of 4–8 weeks (*Post1*) and 20–24 weeks (*Post2*). Athletes were physically active during the period prior to collision activities (i.e., *Pre*) and the transition to *In1* was marked by the onset of practices involving collisions (i.e., tackling for football, heading for soccer). Note that data from *Post2* were not used in statistical analyses for our female CSA (soccer) cohort, because a simple survey of activity (conducted prior to each imaging session) revealed most of these athletes had been participating for at least the preceding month in club soccer without associated monitoring of HAE exposure.

NCA participants underwent two MRI scanning sessions (*Test*, *Re-Test*), 5 to 18 weeks apart within their training/competition seasons, maintaining comparable levels of physical activity at both sessions.

Head acceleration event (HAE) monitoring

Soccer For all 23 participants, HAEs were monitored during all practices and games using the xPatch (X2 Biosystems; Seattle, WA). The xPatch sensor was attached to each athlete's head, behind the right ear, using an adhesive patch applied subsequent to cleaning of the area with rubbing alcohol. On an as-needed basis, a spray adhesive (Cavilon™) was applied. Data from the sensors were downloaded after each practice and game, using the Head Impact Monitoring System software (X2 Biosystems; Seattle, WA). Sensors recorded HAEs having Peak Translational Accelerations (PTA) greater than 10 g as separate events. The software provided Peak Translational Acceleration (PTA) and Peak Angular Acceleration (PAA) for each recorded event. Low-acceleration events—those having PTA < 20 g (McCuen et al. 2015)—and events outside the valid time window of a practice or a game were discarded. Monitoring was conducted over two seasons of play, yielding 29 observations (six players participating in both seasons).

Football For one of the two seasons included in this analysis, 24 football athletes had HAEs monitored during all practices and games using the xPatch. During the other season, the commercially-available Vector mouthguard-based telemetry system (i1 Biometrics) was used to monitor HAEs; however, this device failed to reliably capture video-documented impacts, so no HAE data from this season were included in subsequent analyses.

Non-collision athletes Due to substantial anticipated difference in exposure to subconcussive trauma relative to CSA, NCA participants were not monitored for HAEs during training or competition.

MRS data acquisition

All imaging sessions were conducted at the Purdue University MRI Facility (West Lafayette, IN), using a 3 T General Electric Signa HDx (Waukesha, WI) with a 16-channel brain array (Nova Medical; Wilmington, MA). Single-voxel MR spectra were acquired using the PRESS (Point RESolved Spectroscopy) pulse sequence (TR/TE = 1,500/30 ms, 128 averages, $2.0 \times 2.0 \times 2.0$ cm^3). A high-resolution T_1-weighted anatomical scan was acquired for registration and tissue segmentation purposes using 3D spoiled gradient recalled echo (SPGR) sequence (TR/TE = 5.7/1.976 ms, flip angle = 73°, 1 mm isotropic resolution).

Spectra were obtained specifically from the left dorsolateral prefrontal cortex (DLPFC) and dominant primary motor cortex (M1), with voxels placed as shown in Fig. 1—see also Poole et al. (2014). DLPFC was monitored due to its role in working memory (Curtis and D'Esposito 2003) and our previous findings of altered functional activity (Breedlove et al. 2012, 2014; Talavage et al. 2014) and cerebrovascular reactivity (Svaldi et al. 2016) in this region for asymptomatic collision sport athletes. M1 has been documented to exhibit neurometabolic alterations subsequent to traumatic brain injury (Henry et al. 2010, 2011; De Beaumont et al. 2012).

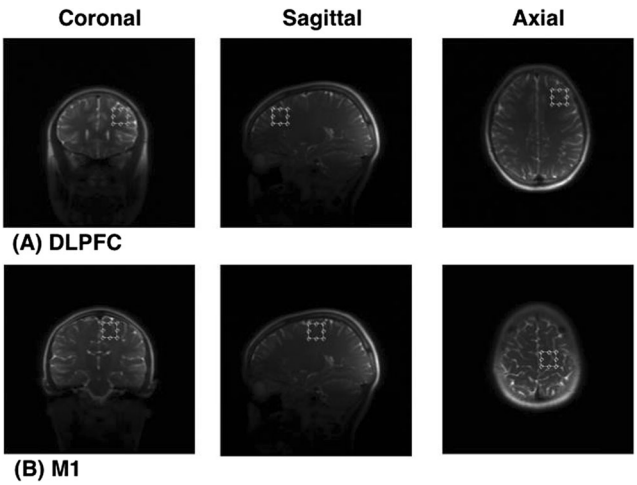

Fig. 1 Three planar views of MRS voxel placement in (**A**) DLPFC and (**B**) M1

Data processing

Evaluation of HAEs HAE metrics were determined for the 53 monitored CSA up to the date of each imaging session (*In1*, *In2* and *Post1*). First, the (to-date) cumulative PTA (***cPTA*** $_{Th,j,i}$) for *i-th* athlete at the *j-th* session (*In1–2*, *Post1*) was obtained by summing the PTA of each of those N_j HAEs which exceeded the threshold (***Th***) experienced by the corresponding athlete from the beginning of the season until either the day of assessment (for *In1*and *In2*) or through the end of the season (*Post1*):

$$cPTA_{Th,j,i} = \sum_{k=1}^{N_j} PTA_{k,i} \cdot u\left(PTA_{k,i} - Th\right)$$

where

$$u(x) = \begin{cases} 1 & if\ x > 0 \\ 0 & if\ x \leq 0 \end{cases}$$

While a similar procedure was used to compute cumulative PAA (cPAA), only cPTA was considered for further analysis. This decision was made given that cPTA and cPAA were found to be highly correlated (95% CI of sample correlation [0.995; 0.998]), and prior work has demonstrated that measurement of PTA is more accurate (Cummiskey et al. 2017).

Next, the (to-date) cumulative number of head acceleration events ***cHAE*** $_{Th,j,i}$ was obtained by counting each of the N_j head acceleration events exceeding the targeted threshold (*Th*), as above:

$$cHAE_{Th,j,i} = \sum_{k=1}^{N_j} u\left(PTA_{k,i} - Th\right)$$

Finally, the average PTA (***aPTA*** $_{Th,j,i}$) as experienced by the *i-th* athlete from the beginning of the season until the date of the *j-th* imaging session for a given threshold level *Th* was calculated as

$$aPTA_{Th,j,i} = \frac{cPTA_{Th,j,i}}{cHAE_{Th,j,i}}$$

To assess whether a clear threshold existed below which HAEs did not meaningfully contribute to changes in metabolite concentrations, the cPTA and aPTA were computed for three threshold levels: 20 g, 50 g and 70 g (approximately corresponding to 20th, 75th and 90th percentile of all impacts recorded that exceeded 10 g).

MR spectroscopy analysis Tissue water reference concentrations as reported by TARQUIN (Wilson et al. 2011) were used. Since the spectroscopic voxels contained fractions of CSF, grey matter and white matter, the values from TARQUIN were corrected for partial volume effects and for metabolite and water T_1 and T_2 relaxation effects—using AFNI (Cox 1996) and FSL (Smith 2002; Jenkinson et al. 2012)—following the procedure described in Poole et al. (2014). Our metabolites of interest include myo-inositol (Ins), an osmolyte involved in glial cell growth; total N-acetyl aspartate and N-acetylaspartylglutamate containing compounds (tNAA), a biomarker of neuronal integrity; total creatine-containing compounds (tCr), key in energy metabolism; total choline-containing compounds (tCho), a measure of inflammation, demyelination, and membrane turnover or repair; and glutamate and glutamine (Glx), a neurotransmitter and its precursor that reflect synaptic activity (Gujar et al. 2005; Zhu and Barker 2011; Poole et al. 2014; Wilde et al. 2015). A typical MRS spectrum of the metabolites of interest is shown in Fig. 2.

The placement of spectroscopic voxels in follow-up scans was accomplished by attempting to match the reconstructed voxel location from *Pre/Test* sessions. Since multiple operators were conducting the MRI sessions, any imaging session with inconsistent voxel placement resulting into voxel overlap of less than 30% with the *Pre/Test* session (within same subject) was discarded. The metabolite values from the surviving imaging sessions, with Cramer-Rao lower bounds less than 25% standard deviation as reported by TARQUIN, were included into the statistical analysis. Data from one female NCA were discarded for poor spectra, as detected by visual inspection. The percentage of data points excluded for each population due to these criteria is summarized in Table 1.

Statistical analysis Analyses were conducted in R. To account for missing values (e.g., lost after quality checks or athletes absent from an imaging session) the means of 50 imputations from Amelia II (Honaker et al. 2011) were used to complete the dataset. Because some groups did not satisfy normality (Shapiro-Wilk test; $p < 0.05$) and sphericity (Mauchly's Test; $p < 0.05$) conditions, non-parametric tests were conducted in lieu of ANOVA.

First, the stability of MRS measures across sessions was evaluated on the *Test* and *Re-Test* sessions from the NCA, using a Wilcoxon signed rank test followed by false discovery rate (FDR; Benjamini and Hochberg (1995)) correction. Any pairwise comparison was considered significant if $p_{FDR} < 0.05$. All metabolite concentrations were found to be stable across the two sessions in NCA. In contrast with Poole et al. (2014), the total concentrations of tCr were also found to be stable across sessions in both NCA and CSA. Therefore, metabolite concentrations and metabolite ratios to [tCr] from the NCA *Test* and *Re-Test* sessions were pooled for subsequent comparison to the CSA.

Neurometabolite concentrations and ratios for the CSA pool were evaluated for dependence on session by a Friedman Test (the non-parametric version of a one-way repeated measures ANOVA), conducted separately for

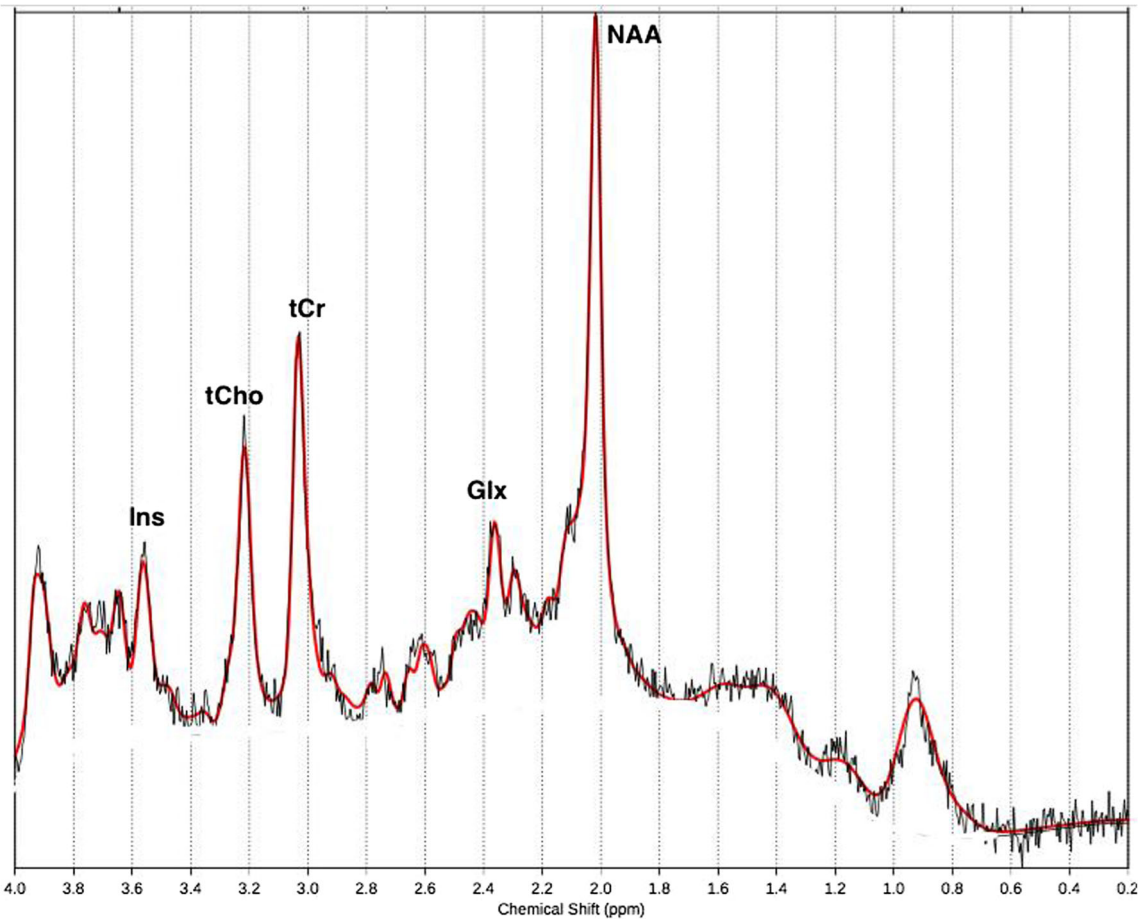

Fig. 2 Typical ^{1}H MRS spectrum (NAA = N-acetyl aspartate; Glx = glutamate and glutamine; tCr = creatine-containing compounds; tCho = choline-containing compounds; Ins = myo-inositol)

each metabolite concentration and ratio. Those metabolites and ratios that exhibited significant changes ($p < 0.05$) were further analyzed by pairwise comparisons among all sessions using the Wilcoxon signed rank test, followed by FDR correction.

Those metabolites/sessions in the male and female CSA pools found to be significantly different from the corresponding *Pre* session were further analyzed with linear regression models to examine the association between metabolite concentrations and *aPTA*, at multiple threshold levels. Regressions were performed iteratively by removing each data point, and at each iteration the goodness-of-fit (R^2) was computed and an F-test was used to compare the fit to a constant model. We report here the mean R^2 and p-value associated with the mean F-statistic of all iterations.

While not necessarily expected to differ at the group level—not all CSA experienced equivalent histories of HAE exposure—the NCA and (asymptomatic) CSA pools were compared at each session, using a Wilcoxon rank sum test followed by FDR correction. Note that only within-gender comparisons were made between NCA and CSA.

Results

HAE monitoring Summary HAE statistics are reported in Tables 2, 3 and 4 for male and female CSA. Table 2 reports the 25th, 50th, 75th and 90th percentiles of PTA of all HAEs experienced by the male and female CSA pools. Tables 3 and 4 report the median $cPTA_{Th,\ j,\ i}$ and $aPTA_{Th,\ j,\ i}$ for male and female CSA pools, respectively, at $Th = 20$ g, 50 g and 70 g at the time of each follow-up session (*In1*, *In2*, and *Post1*). Figure 3 depicts the distribution of $cPTA_{50g,\ In2,\ i}$ experienced by male and female CSA. Female CSA experienced statistically significant lower $cPTA_{50g,\ In2,\ i}$ than male CSA ($p = 0.002$; Wilcoxon rank sum test).

MR spectroscopy Summary statistics documenting stability of the assessed metabolites across sessions in the male and female NCA pools are listed in Tables 5 and 6. Comparable measures for session-dependence in male and female CSA pools are presented in Tables 7 and 8.

Male and female CSA pools exhibited alterations in metabolite concentration and/or metabolite ratio across sessions. In M1, the female CSA (i.e., soccer) pool exhibited a statistically

Table 1 Percentage of data points excluded for each of the two regions of interest (ROIs) in the male and female CSA pools, based on CRLB criteria, insufficient voxel overlap and missed imaging sessions

CSA Group	*ROI*	*Metabolite*	*Sessions*				
			Pre	In1	In2	Post1	Post2
Male	DLPFC	Ins	8.51	23.40	21.28	17.02	29.79
		tNAA	6.38	21.28	17.02	14.89	29.79
		tCho	6.38	19.15	17.02	14.89	27.66
		tCr	6.38	19.15	17.02	14.89	27.66
		Glx	6.38	19.15	17.02	14.89	27.66
	M1	Ins	14.89	6.38	10.64	17.02	25.53
		tNAA	14.89	6.38	10.64	19.15	21.28
		tCho	14.89	6.38	10.64	12.77	23.40
		tCr	14.89	6.38	10.64	12.77	21.28
		Glx	14.89	6.38	10.64	12.77	21.28
Female	DLPFC	Ins	6.90	6.90	17.24	10.34	13.79
		tNAA	6.90	6.90	17.24	10.34	13.79
		tCho	6.90	6.90	13.79	10.34	10.34
		tCr	6.90	6.90	13.79	10.34	13.79
		Glx	6.90	6.90	13.79	10.34	13.79
	M1	Ins	3.45	10.34	17.24	6.90	20.69
		tNAA	3.45	10.34	10.34	6.90	17.24
		tCho	0.00	6.90	6.90	6.90	17.24
		tCr	3.45	6.90	6.90	6.90	17.24
		Glx	3.45	6.90	10.34	6.90	17.24

significant (Friedman $\chi^2 = 11.36$, $p = 0.009$) increase in absolute concentration of M1 Glx at *Post1* relative to *Pre*. (Fig. 4A). This change in absolute concentration was accompanied by a statistically significant (Friedman $\chi^2 = 10.99$, $p = 0.012$) increase in the ratio [Glx]:[tCr] at *In2* and *Post1* relative to *Pre*. (Fig. 4B). In DLPFC, the male CSA (i.e., football) pool exhibited a statistically significant (Friedman $\chi^2 = 9.6$, $p = 0.047$) decrease in DLPFC [Glx] at *In2*, relative to each of *Pre*, *In1* and *Post2* (Fig. 5A). This group also exhibited a statistically significant (Friedman $\chi^2 = 15.013$, $p = 0.005$) increase in the ratio of DLPFC [tCho] to DLPFC [tCr] (i.e., [tCho]:[tCr]) at *In2* relative to each of *Pre*, *In1* and *Post2* (Fig. 5B).

Table 2 Magnitudes of peak linear acceleration (in g) corresponding to fixed points in the distribution of observed head acceleration events (HAEs)—exceeding 10 g—for male (football) and female (soccer) collision-sport athletes (CSA)

CSA Group	25th Percentile	50th Percentile	75th Percentile	90th Percentile
Male (football)	25.1	33.0	47.0	67.0
Female (Soccer)	23.9	30.0	43.3	62.2

Regression analysis Changes across session within CSA that did not result in group-level differences with the NCA stability measurements motivated assessment whether the across-session changes for CSA were primarily confined to a sub-population of athletes who had experienced greater levels of mechanical loading. Statistical results of the regression analyses are presented in Table 9. Nearly all conducted regressions evidenced their best goodness-of-fit (R^2) with *aPTA* when optimized for $Th = 50$ g (Fig. 6A). At this threshold, the regression for male CSA athletes at *In2* of DLPFC [tCho]:[tCr] against HAE measures achieved a mean F-statistic across iterations corresponding to an uncorrected significance level of $p < 0.05$ (Fig. 6B). No other regressions resulted in a mean F-statistic having $p < 0.05$.

Discussion

We have investigated metabolic changes in two CSA pools (male football, female soccer) both *(1)* across time, within individual competition seasons; and *(2)* as a function of HAE exposure. Our main findings indicate the presence of session-dependent metabolic alterations in both groups of CSA, suggesting that CSA are combating cellular dysfunction in the absence of observable external symptoms. Further, there is evidence that the time-dependent changes in male football athletes are a function of exposure to the history of mechanical loading. The significantly lesser cumulative exposure to HAEs for female CSA cohort as compared to their male counterparts (see Fig. 3 and Table 3) presents a likely explanation for the lesser number of metabolic changes for the soccer athletes. These findings suggest that metabolic disturbance is most likely to occur after accumulation of some as-yet-undetermined threshold of mechanical loading to the head.

Evidence for metabolic disturbance

Two versions of metabolic disturbance were observed in this study. Male CSA exhibited changes in DLPFC associated with glutamate+glutamine (Glx) and with total choline containing compounds (tCho), whereas female CSA exhibited changes in M1 associated only with glutamate+glutamine (Glx). These findings are in contrast with stable measurements observed for all metabolites across *Test* and *Re-Test* sessions in male and female NCA.

Football athletes (i.e., male CSA) exhibited changes in DLPFC with the onset of, and sustained exposure to, collision events, with changes disappearing once exposure to collision events ceased. For both [Glx] and [tCho]:[tCr], Fig. 5 illustrates clear deviation during the second half of

Table 3 Median $cPTA_{Th,\,j,\,i}$ (in g) for male and female CSA at each follow-up session given minimum HAE threshold, *Th* = 20 g, 50 g and 70 g

CSA Group	*Median* $cPTA_{Th,\,j,\,i}$ (g)								
	In1			*In2*			*Post1*		
	20 g	50 g	70 g	20 g	50 g	70 g	20 g	50 g	70 g
Male	2639.0	939.9	561.6	7624.0	2929.0	1303.0	12,770.0	5169.0	2262.0
Female	1492.0	396.4	168.30	3273.0	1333.0	721.0	4137.0	1707.0	758.7

the season (*In2*) relative to *Pre*, with the deviation lessening at *Post1* and returning to baseline levels at *Post2*. Altered metabolite measures trended toward *Pre*-season levels 1–2 months after the season, with return to baseline occurring after approximately five months of reduced (e.g., in the case of wrestling) or non-existent exposure to HAEs. These findings suggest that the sustained exposure to repetitive trauma, caused by subconcussive blows to the head, can trigger transient metabolic disturbance, with gradual return to baseline levels (plausibly reflecting healing of cellular damage) once exposure ends. In particular, the decrease observed in DLPFC [Glx] at *In2* assessment could be associated with the alteration in excitatory synaptic activity, and increased Glx catabolism resulting from hypoglycemia (Schuhmann et al. 2003). This decreasing trend has been previously observed in both animal models (Schuhmann et al. 2003) and studies of concussed and mild TBI patients (Newsholme et al. 2003; Gasparovic et al. 2009; Henry et al. 2010; Yeo et al. 2011; Poole et al. 2014). Elevation of DLPFC [tCho]:[tCr] suggests ongoing processes of membrane turnover for potential repair of cells and inflammation (Ricci et al. 1997; Friedman et al. 1998; Brooks et al. 2000; Garnett et al. 2000a, b; Govindaraju et al. 2004; Shutter et al. 2004; Maudsley et al. 2015).

Soccer athletes (i.e., female CSA) exhibited changes only in primary motor cortex (M1). The increases in M1 [Glx] and M1 [Glx]:[tCr] at *In2* and *Post1* could be linked to alterations in excitatory synaptic activity in motor area and motor dysfunction. The increase in [Glx] could further be associated with a secondary response to injury (Bullock et al. 1998; Ruppel et al. 2001) which can cause excitotoxic accumulation. This last has been previously observed or postulated in patients with mild TBI or concussion (Ashwal et al. 2004; Yeo et al. 2011; Poole et al. 2014). Two possible factors that could have led to this increase in Glx in female CSA include a gender effect associated with the menstrual cycle, and a sport-related effect arising from the significantly lesser cumulative exposure to HAEs for the females, relative to their male counterparts. (See below for further discussion of these matters.) Note from Fig. 4 that the altered metabolite measures trended toward *Pre*-season levels approximately five months after the season, even with known (but uncharacterized) recent HAE exposure for many of the athletes.

In general, the neurochemical deviations observed in both groups of clinically asymptomatic CSA during and subsequent to accumulation of HAEs indicate the presence of an altered metabolic state. The combination of impaired neurotransmission and hypermetabolism (associated with [Glx]) with evidence of inflammation and increased membrane turnover (associated with [tCho]:[tCr]) arising from HAE exposure strongly argue for cellular injury to the brain preceding the overt cognitive symptoms typically observed in clinically-diagnosed concussion.

Table 4 Median $aPTA_{Th,\,j,\,i}$ (in g) for male and female CSA at each follow-up session given minimum HAE threshold, *Th* = 20 g, 50 g and 70 g

CSA Group	*Median* $aPTA_{Th,\,j,\,i}$*(g)*								
	In1			*In2*			*In1*		
	20 g	50 g	20 g	20 g	20 g	70 g	20 g	50 g	20 g
Male	41.1	70.4	90.7	39.1	70.6	90.6	39.0	71.0	91.0
Female	35.2	67.2	84.1	36.3	69.6	85.3	36.2	68.7	88.4

Prolonged neurometabolic recovery period supported by other biomarkers

These findings of neurometabolic alterations in the presence of HAEs are consistent with previous biomarker findings suggesting that repetitive head trauma can produce disturbances in brain physiology in the absence of diagnosable cognitive symptoms. The timeline of metabolic deviations in this study are similar to aberrations observed in cerebrovascular reactivity (CVR) in asymptomatic female soccer athletes (Svaldi et al. 2017), and all of resting-state FMRI (Abbas et al. 2015a, b), working memory task-based FMRI (Shenk et al. 2015), and diffusion weighted imaging (Chun et al. 2015; Jang et al. 2016) in asymptomatic football athletes. In all cases, deviations from pre-participation measurements (i.e., *Pre*) persisted for at least two months following the end of the season,

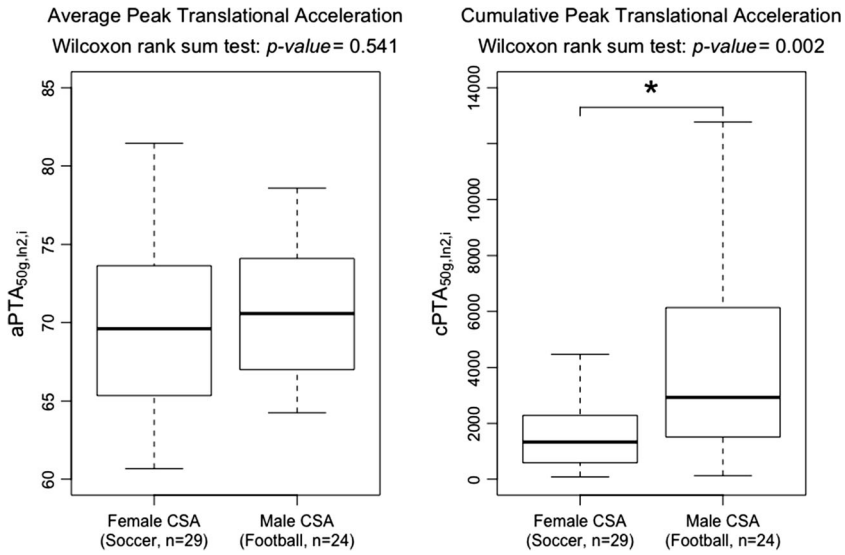

Fig. 3 Box-and-whisker plots comparing the $aPTA_{50g,In2,i}$ and $cPTA_{50g,In2,i}$ experienced by female CSA (Soccer) and male CSA (Football) pools at a threshold of $Th = 50$ g at follow-up session *In2*

sometimes being observed as late as five months after cessation of HAE exposure. This suggests that the asymptomatic CSA in this study are likely to have a transient injury from which it takes several months for natural repair processes to return physiologic health to baseline levels. The presence of a mismatch between injury accumulation and repair raises concerns for the neural health of all adolescent collision-sport athletes.

Dependence of metabolic disturbance on HAE exposure

Alterations in cellular signaling and inflammation observed in football athletes were correlated with the average acceleration per HAE. The deviant metabolite distributions of DLPFC [Glx] and [tCho]:[tCr] at *In2* exhibited negative and positive correlations, respectively, with the aPTA experienced by the football athletes during the second half of their competition season. These relationships with HAEs imply that greater mechanical stress may induce impaired neurotransmission, an acute state of hypermetabolism and increased membrane turnover and inflammation (Garnett et al. 2000b; Schuhmann et al. 2003). Of particular interest, the best modeling prediction was associated with an HAE threshold of 50 g, and is comparable to findings in the study of cerebrovascular reactivity in female soccer athletes (Svaldi et al. 2016). These results support the hypothesis that with accumulation of HAEs there is continual increment in neurometabolic alterations, which could ultimately exceed a threshold beyond which the metabolic disturbances never return to baseline levels.

Metabolic disturbances causing state of brain vulnerability

Considering a longer perspective, the absence of immediate symptoms could predispose athletes to alterations in brain physiology that appear later in life, or under particular circumstances (Stern et al. 2011; Alosco et al. 2017). The neural health of young athletes suffering from transient metabolic disturbances in the absence of diagnosable cognitive symptoms is therefore of concern, as these athletes are more likely to continue participation with associated accumulation of strain at the cellular level. The subsequent neurometabolic cascade of mild TBI follows a sequence of initial ionic fluxes, impaired neurotransmission and changes in glucose metabolism resulting in high energy demands and a period of metabolic crisis (Giza and

Table 5 Wilcoxon signed rank test statistic W and (p_{FDR}) assessing the across-session stability for each metabolite concentration, in each of two regions of interest (ROIs) in the male and female NCA pools

NCA Group	ROI	Metabolite – Concentration				
		Ins	tNAA	tCho	tCr	Glx
Male	DLPFC	69 (0.447)	68 (0.447)	69 (0.447)	60 (0.670)	36 (0.447)
	M1	58 (0.951)	80 (0.453)	54 (0.951)	46 (0.951)	45 (0.951)
Female	DLPFC	52 (0.566)	39 (1.000)	34 (0.917)	54 (0.566)	57 (0.566)
	M1	65 (0.106)	65 (0.106)	57 (0.220)	51 (0.380)	62 (0.129)

Table 6 Wilcoxon signed rank test statistic W and (p_{FDR}) assessing the across-session stability for each metabolite concentration ratio to [tCr], in each of two regions of interest (ROIs) in the male and female NCA pools

NCA Group	ROI	Metabolite – Ratio of Concentration to [tCr]			
		Ins	tNAA	tCho	Glx
Male	DLPFC	58 (0.808)	57 (0.808)	46 (0.808)	37 (0.808)
	M1	59 (0.903)	75 (0.690)	67 (0.782)	55 (0.903)
Female	DLPFC	44 (0.733)	25 (0.602)	17 (0.369)	44 (0.733)
	M1	55 (0.235)	59 (0.235)	45 (0.677)	69 (0.064)

Hovda 2001; DeLellis et al. 2009). The critical mismatch between supply and demand of energy during repeated exposure to HAEs could result in a positive feedback loop with negative consequences. In particular, increasing energy demands due to metabolic crisis could be exacerbated by progressively more impaired metabolite delivery, as suggested by changes in CVR (Svaldi et al. 2017). Such an escalation of the energy crisis could readily result in a prolonged recovery period from transient metabolic disturbances, similar to those observed in this study. Further, it would be expected that during the condition of energy crisis caused by repetitive head trauma, cellular metabolism would be stretched to its limits and cells could become more vulnerable. Overstimulation of the adolescent brain in this condition could cause long lasting effects on the complex sequence of neurochemical and anatomical events occurring during normal development (Klonoff et al. 1993; McKinlay et al. 2010). It is further feasible that young athletes suffering multiple instances of transient metabolic injury might never return to baseline levels, potentially leading to learning, memory, or cognition deficits. In addition, transient metabolic disturbances that lead to dysfunctional neurotransmission could increase the risk of diminished attention and cognitive performance, making adolescent collision-sport athletes susceptible to greater numbers and magnitudes of HAE, exacerbating the problem.

Reflections on the present study and future directions

The primary methodological limitation of this study was the imperfect voxel placement in longitudinal scans of the same athlete. Manual voxel placement by multiple MRI operators resulted in variable locations from measurement to measurement, leading to changes in the relative proportion of gray matter, white matter, and cerebrospinal fluid in the assessed voxel. Such errors would be expected to increase the variance across the measurements, but should not produce systematic biases in any specific metabolite concentration as a function of time. Future work will seek to eliminate this issue through use of automatic repositioning of MRS voxel—e.g., Hancu et al. (2005); Dou et al. (2015).

Another limitation of the present study is derived from the focus on specific anatomic locations, leaving a large portion of the brain unexamined. Incorporation of newer MR spectroscopic imaging (MRSI) techniques would permit investigation of changes over a larger region of the brain, providing spatial indifference (Maudsley et al. 2010; Ding et al. 2015), while also permitting separate examination of disturbances in white and gray matter (Chard et al. 2002; Zhu et al. 2006).

Additional factors potentially contributing to the differences observed between male (football) and female (soccer) CSA include the relatively modest sample sizes in this work, a possible gender effect given there was no control for the menstrual cycle stage at the time of imaging of female CSA, and a sport-related effect due to the significantly lesser cumulative exposure to HAEs for the females, relative to their male counterparts.

The modest population for which both MRS and HAE measurements were available was potentially exacerbated by the small number of teams from which athletes were recruited. Both football and soccer athletes were recruited from two local high school teams, wherein each team had different playing style (Martini et al. 2013), skill and athletic level of competition. A study involving more teams could sample a greater range of playing styles, and thus

Table 7 Friedman χ^2 score and (p-value) assessing the presence of a dependence on session for each metabolite concentration, in each of two regions of interest (ROIs) in the male and female CSA pools. Metabolites exhibiting significant dependence on session within the given CSA pool and ROI are marked in bold

CSA Group	*ROI*	Metabolite – Concentration				
		Ins	tNAA	tCho	tCr	Glx
Male	DLPFC	5.23 (0.264)	4.10 (0.392)	4.15 (0.385)	9.14 (0.058)	**9.60 (0.047)**
	M1	2.88 (0.578)	2.98 (0.561)	2.89 (0.576)	3.15 (0.533)	2.74 (0.602)
Female	DLPFC	1.26 (0.738)	3.41 (0.332)	3.99 (0.262)	3.91 (0.271)	2.71 (0.439)
	M1	4.24 (0.237)	0.02 (0.999)	2.54 (0.467)	3.00 (0.392)	**11.36 (0.009)**

Table 8 Friedman χ^2 score and (p-value) assessing the presence of a dependence on session for each metabolite concentration ratio to [tCr], in each of two regions of interest (ROIs) in the male and female CSA pools. Metabolite ratios exhibiting significant dependence on session within the given CSA pool and ROI are marked in bold

CSA Group	*ROI*	Metabolite – Ratio of Concentration to [tCr]			
		Ins	tNAA	tCho	Glx
Male	DLPFC	1.48 (0.830)	3.26 (0.54)	**15.01 (0.005)**	4.39 (0.356)
	M1	2.93 (0.570)	1.50 (0.827)	2.33 (0.675)	1.02 (0.910)
Female	DLPFC	1.68 (0.642)	5.40 (0.145)	2.05 (0.562)	2.21 (0.529)
	M1	2.34 (0.505)	1.26 (0.738)	1.10 (0.778)	**10.99 (0.012)**

could better quantify dose-response thresholds between HAE exposure and neurometabolic changes. Such a dose-response model will be critical in instituting exposure regulations that may best protect adolescent athletes from the long-term risks associated with repetitive head trauma. Further, an increase in the number of athletes studied would increase the chances of having participants who are diagnosed with a concussion, eventually enabling relative assessment of the severity of metabolic injury for asymptomatic athletes.

Some changes (or the absence thereof) in the female CSA neurometabolic concentrations could be affected by alterations occurring throughout the menstrual cycle. Previous studies have shown significant differences in metabolite concentrations in males and females (O'Gorman et al. 2011; Endres et al. 2016). These neurometabolic differences are not structural but are due to sex hormones between the two genders. In females the ovarian steroid hormones like estrogens and progesterone are widespread in brain and have modulating effect on the brain physiology, producing metabolite concentrations variations with the phase of the menstrual cycle. Significant changes in ratios of [tNAA], [tCho], [Ins] and [Glx] to [tCr] have been observed from the follicular to the luteal phase of menstrual cycle (Rasgon et al. 2001; Batra et al. 2008). Such changes could have affected the concentrations and ratios observed in this study, particularly given the modest sample sizes, and should be accounted for in future longitudinal studies.

The lesser cumulative HAE exposure for the female CSA—a consequence of appreciably fewer and lesser cumulative magnitude of HAEs per practice/game—does not necessarily guarantee safety or neural health. Rather, this lesser daily exposure has likely only put the female

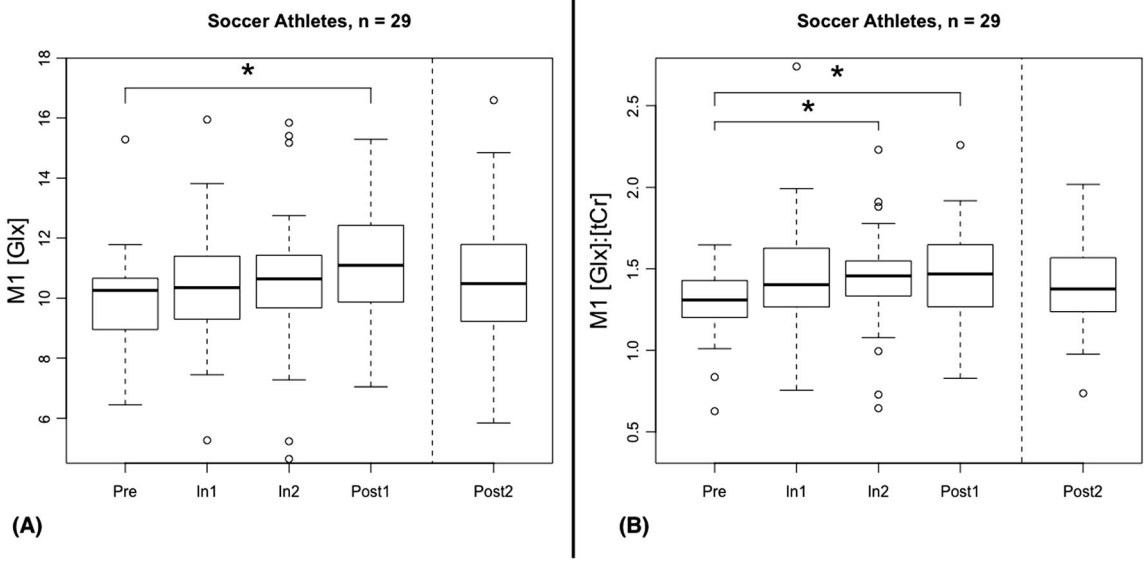

Fig. 4 Box-and-whisker plots of (**A**) M1 [Glx] and (**B**) M1 [Glx]:[tCr] for the total cohort of female (soccer) CSA ($n = 29$). Session-specific distributions found to be significantly deviant ($p_{FDR} < 0.05$, Wilcoxon signed rank test) by pairwise comparisons are marked by an asterisk. Note that *Post2*—while trending toward a return to levels at *Pre*—was not included in analyses due to unmonitored participation of many subjects in club soccer during the month preceding this session

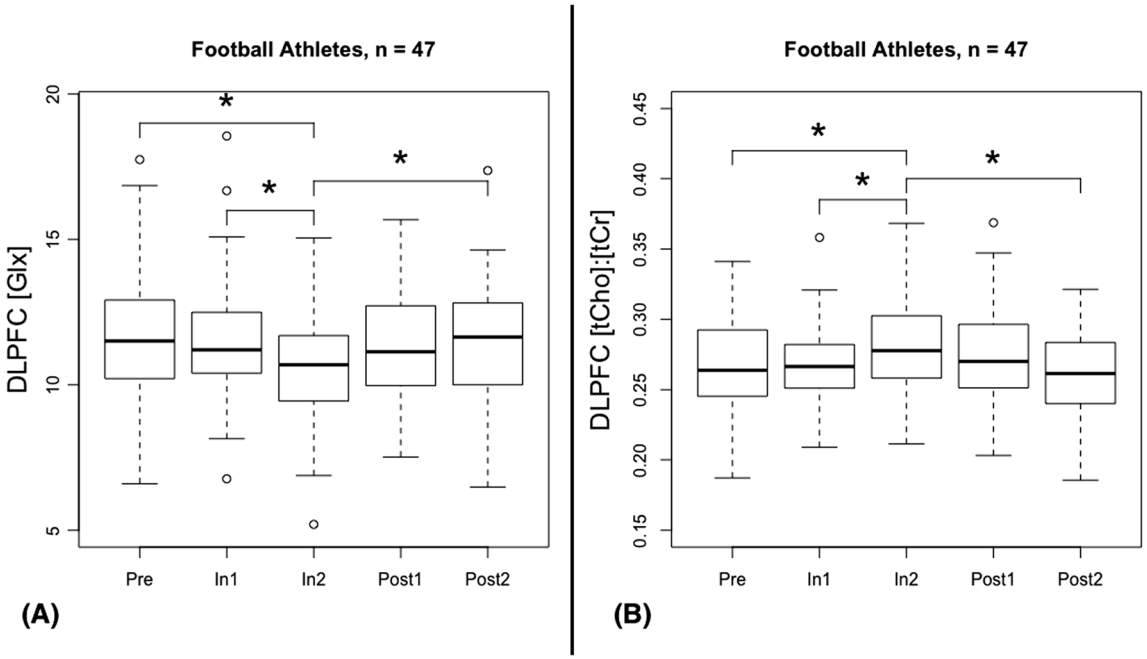

Fig. 5 Box-and-whisker plots of (**A**) DLPFC [Glx] and (**B**) DLPFC [tCho]:[tCr] for the total cohort of male (football) CSA ($n = 47$). Session-specific distributions found to be significantly deviant ($p_{FDR} < 0.05$, Wilcoxon signed rank test) by pairwise comparisons are marked by an asterisk

CSA at a reduced risk of physiologic changes and delayed crossing of the head impact exposure thresholds proposed to exist for cognitive and behavioral impairment (Montenigro et al. 2017), and white matter microstructural and cognitive abnormalities (Lipton et al. 2013). While both of these studies found that athletes did not exhibit significant changes in brain physiology while exposure remained below the identified thresholds, a constant baseline risk existed. Even a small number of additional HAE exposures could push an athlete above these thresholds, rapidly increasing the risk for later cognitive and behavioral impairment.

Conclusion

Asymptomatic male (football) and female (soccer) CSA, were found to exhibit statistically significant, albeit transient, neurometabolic disturbances in (male) dorsolateral prefrontal cortex and (female) motor cortex during a period of

Table 9 Mean R^2, mean F-statistic and associated p-value for iterative regression fits of neurometabolite concentrations that exhibited a significant difference from *Pre* (see Figs. 4–5) against average peak translational acceleration (*aPTA*) at three values of HAE threshold (*Th*). Boldface indicates regressions that (on average) achieved statistical significance ($p < 0.05$; uncorrected)

CSA Group	*ROI*	*Metabolite*	*Session*	*Th*	mean R^2	mean F (p-value)
Male	DLPFC	[tCho]:[tCr]	*In2*	20 g	0.043	0.958 (0.339)
				50 g	**0.198**	**5.218 (0.033)**
				70 g	0.015	0.360 (0.555)
		[Glx]	*In2*	20 g	0.035	0.779 (0.387)
				50 g	0.132	3.209 (0.088)
				70 g	0.037	0.803 (0.380)
Female	M1	[Glx]:[tCr]	*In2*	20 g	0.015	0.392 (0.538)
				50 g	0.031	0.801 (0.381)
				70 g	0.001	0.032 (0.860)
			Post1	20 g	0.061	1.632 (0.215)
				50 g	0.006	0.160 (0.692)
				70 g	0.014	0.350 (0.560)
		[Glx]	*Post1*	20 g	0.007	0.183 (0.673)
				50 g	0.100	2.753 (0.112)
				70 g	0.035	0.907 (0.352)

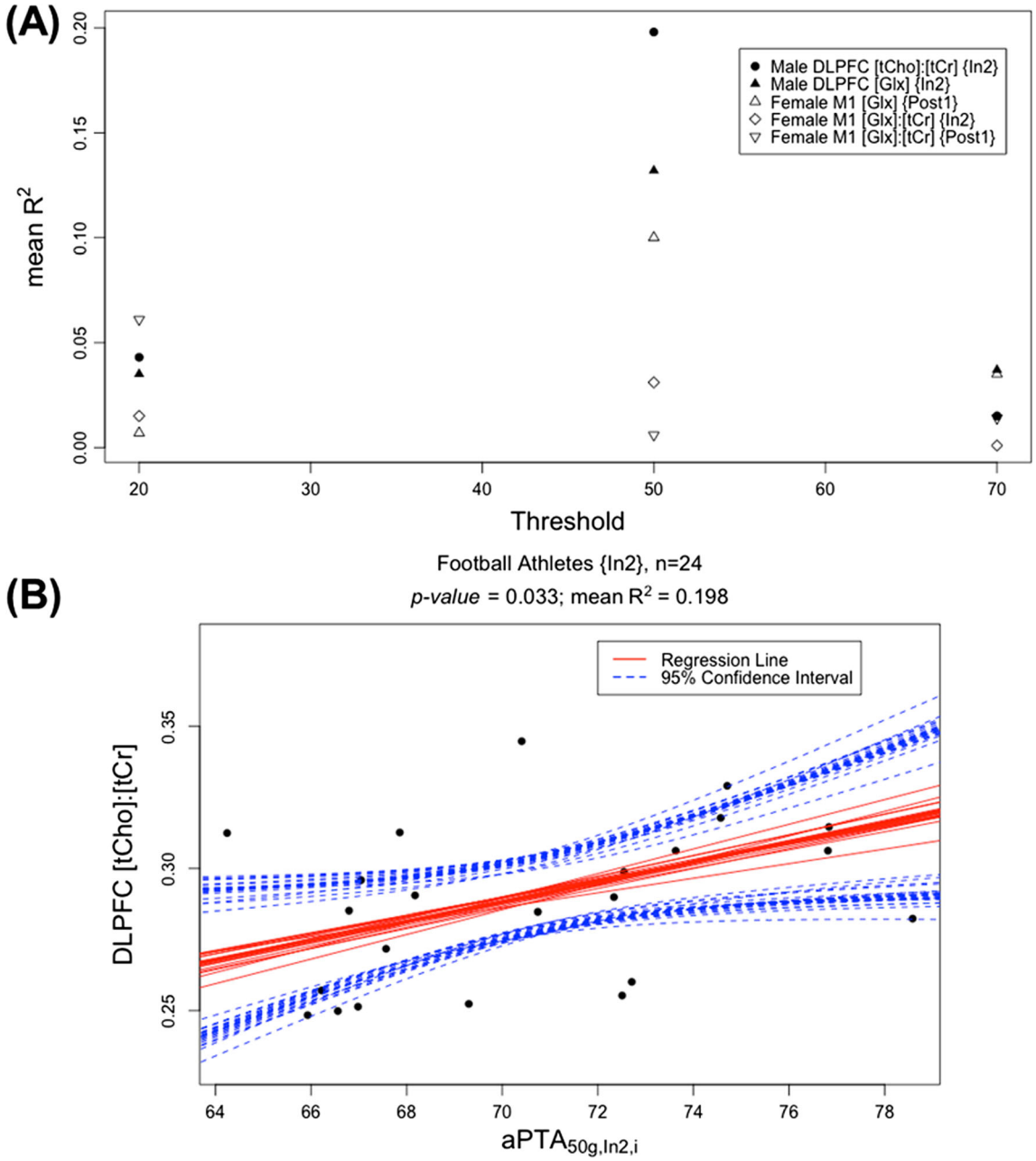

Fig. 6 Evaluation of regressions in male and female CSA groups plots for neurometabolite concentrations and ratios against *aPTA* across HAE thresholds. (**A**) Mean R^2 as a function of evaluated threshold value (*Th* = 20, 50 and 70 g) for all sessions found to significantly differ from their corresponding *Pre* measurement in Figs. 4 and 5. (**B**) The regression fit in male CSA (football) athletes for *In2* DLPFC [tCho]:[tCr] vs. *aPTA* at *Th* = 50 g—the maximal point in (**A**)—was the only fit to achieve a mean F-statistic that was associated with a *p*-value of less than 0.05 (*p* = 0.033; uncorrected)

appreciable exposure to head acceleration events. Extending previous work (Poole et al. 2014, 2015), neurometabolic alterations observed in football athletes during the second half of the season were found to be significantly associated with the average acceleration per HAE, being best predicted by the accumulation of events exceeding 50 g. While a smaller sample of female CSA (soccer) also exhibited significant changes in metabolite concentrations, these changes were not found to be well-linked to their significantly lesser HAE exposure. Marked deviations in neurometabolism, in the absence of overt symptoms, raise concern about the neural health of adolescent collision-sport athletes. These findings suggest that limiting HAE exposure and allowing adequate rest following the competition season are likely to be beneficial for the neural health of these athletes, and may help to ameliorate the risk of subsequent cognitive impairment.

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Compliance with ethical standards

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the Purdue Institutional Review Board and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all participants of 18 years and above, and parental consent and participant assent were obtained for all participants under the age of 18.

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