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MR Spectroscopic Evidence of Brain Injury in the Non-Diagnosed Collision Sport Athlete

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With growing evidence of long-term neurological damage in individuals enduring repetitive head trauma, it is critical to detect lower-level damage accumulation for the early diagnosis of injury in at-risk populations. Proton magnetic resonance spectroscopic scans of the dorsolateral prefrontal cortex and primary motor cortex were collected from high school American (gridiron) football athletes, prior to and during their competition seasons. Although no concussions were diagnosed, significant metabolic deviations from baseline and non-collision sport controls were revealed. Overall the findings indicate underlying biochemical changes, consequential to repetitive hits, which have previously gone unnoticed due to a lack of traditional neurological symptoms.

Estimates by the Centers for Disease Control indicate that at least 1.7 million Americans seek treatment for traumatic brain injury (TBI) each year (Faul, Xu, Wald, & Coronado, 2010), but these numbers grossly under-estimate the occurrence of TBI in part because the signs and symptoms are difficult to quantify (Buck, 2011). In the past, medical professionals and researchers alike have used the presence of observable symptoms to classify concussion, a form of mild TBI. One population in particular, high school athletes, must be monitored more closely as research indicates that they are more neurologically vulnerable than the collegiate population (Field, Collins, Lovell, & Maroon, 2003). There were approximately 800,000 reported concussions suffered by high school athletes between 2005 and 2010, with boys' football accounting for 47.8% of this number (Associations 2009–2010; Castile, Collins, McIlvain, & Comstock, 2011). While this classification is important for the prevention of further catastrophic damage, in particular that associated with second impact syndrome (Cantu, 1998), numerous studies have demonstrated that neurophysiological changes can accumulate due to sub-concussive blows as well (Bailes, Petraglia, Omalu, Nauman, & Talavage, 2013; Baugh et al., 2012; Erlanger, Kutner, Barth, & Barnes, 1999; Gavett, Stern, & McKee, 2011; McKee et al., 2009; Talavage et al., 2014). Taken together, our knowledge of concussions and the potential for sub-concussive damage argues that quantification of the lower levels of damage that may accumulate over time will allow us to better understand and eventually prevent long term consequences of repetitive head impacts.

Proton (^{1}H) magnetic resonance spectroscopy (MRS) offers a non-invasive, direct assessment of the underlying physiology that is independent of subject motivation and participation (van der Graaf, 2010). ^{1}H MRS has been particularly useful for elucidating altered metabolism in common neuropathologies and has been recommended as a diagnostic tool (Lin, Ross, Harris, & Wong, 2005; Mountford, Lean, Malycha, & Russell, 2006). Previously, ^{1}H MRS has been used to diagnose and track the recovery of metabolism within the particular diseased states of mild TBI (mTBI) and concussion (Brooks, Friedman, & Gasparovic, 2001; Vagnozzi et al., 2008; Yeo et al., 2011). This metabolic assessment has proven useful beyond the mere documentation of changes associated with mTBI and concussion: a recent study found certain metabolites take as long as 6 months to recover (Henry et al., 2011), suggesting a time-dependence for post-injury measurements and a need to monitor at-risk individuals more closely and for longer periods of time than are commonly recommended. Although longitudinal studies following impairment have been conducted, only one study to date has compared metabolic levels before and after injury (Johnson et al., 2012). Therefore, given the growing evidence that sub-concussive blows alter neurophysiology, it is possible that the alterations from the norm observed for mTBI and concussion may not be attributable to a single devastating blow, but rather are likely to be a reflection of previous impairment due to repetitive smaller head injuries.

Based on previous work, we hypothesized that high school football players would demonstrate significant differences in metabolite concentrations relative to non-collision sport controls, due to the high number and magnitude of head impacts regularly experienced in the sport (Breedlove et al., 2012; Broglio et al., 2009). Our metabolites of interest include: N-acetyl aspartate (NAA), a biomarker of neuronal integrity; glutamate and glutamine (Glx), a neurotransmitter and its precursor that reflect synaptic activity; creatine-containing compounds (tCr), key in energy metabolism; choline-containing compounds (tCho), markers of membrane turnover; and myo-inositol (Ins), an osmolyte involved in glial cell growth. Findings of changes in one or more of these metabolites in the absence of documented symptoms would strongly support the argument that the accumulation of sub-concussive blows can alter neurophysiology, potentially predisposing an individual to more severe consequences of subsequent neural trauma.

METHODS

Participants and Controls

Over two seasons of data collection, a total of 34 high school American football athletes (male; range = 15–18 years) were enrolled for longitudinal spectroscopic analysis over the course of their competition season. Football athletes were drawn from two local high school teams (Team 1 and Team 2). During each year of data collection, all football athletes underwent a baseline session obtained prior to the onset of contact practices (corresponding to 7–8 months after the previous competition season). They also received at least one follow-up session during their competitive season, with several receiving multiple follow-up scanning sessions at approximately regular intervals of 4–6 weeks. These sessions, which included computer-based ImPACT^{TM} (Immediate Post-Concussion Assessment and Cognitive Testing) neurocognitive testing (Collins et al., 1999; Lovell & Collins, 1998), as described elsewhere (Breedlove et al., 2014), were later categorized by the month of the season in which they were collected. All subjects participated in the football season without interruption or intervention. Although evaluations were independent of suspicion of injury, only players remaining in play after not being diagnosed as concussed were included in the current analysis.

Ten controls (male; range = 15–18 years) were also recruited for this study while they were actively participating in an interscholastic non-collision sport (e.g., swimming, track and field, tennis) and had no prior history of medically diagnosed head injury or sanctioned collision sports play. This non-collision athlete population was evaluated at an initial baseline session and in a single follow-up session at a time interval comparable to the football population. Note that both sessions took place during active competition or training schedules. Prior to participation in this study, all subjects aged 18 or over signed university institutional review board (IRB)–approved consent forms and those under the age of 18 provided assent, with consent provided by a legal guardian.

Magnetic Resonance Spectroscopy (MRS)

Neuroimaging sessions were performed using a General Electric (Waukesha, WI) 3.0 Tesla Signa HDx scanner and a 16-channel Nova Medical (Wilmington, MA) brain array. Single voxel MR

spectra were obtained from both the left dorsolateral prefrontal cortex (DLPFC) and dominant primary motor cortex (M1) using the PRESS (Point RESolved Spectroscopy) pulse sequence (TR = 1,500 msec, TE = 30 msec, 128 averages, 2.0 × 2.0 × 2.0 cm^3). These regions were selected as volumes of interest because they were observed to exhibit altered functional activity in asymptomatic populations (Breedlove et al., 2012; Talavage et al., 2014) and have been reported to exhibit long-term neural changes upon traumatic injury (De Beaumont, Tremblay, Poirier, Lassonde, & Théoret, 2012; Zhang et al., 2010). The first volume (DLPFC) was placed in the left hemisphere of all subjects, laterally in the anterior middle frontal gyrus, near Brodmann area 46 (Figure 1). The second (M1) was placed in the hemisphere contralateral to the subject's dominant hand, at the superior aspect of the pre-central gyrus, immediately anterior to the central sulcus and lateral of the midline (Figure 1).

While the total concentrations of creatine-containing compounds (tCr) and choline-containing compounds (tCho) were found stable across sessions in the non-collision sport control population, neither was stable across time in both football populations. This observation forbade their typical use as internal references. Instead, the tissue water-referenced concentrations reported from LCModel (Provencher, 1993) were utilized, after correcting for partial volume and relaxation effects. This procedure was similar to Gasparovic et al. (2009), with the exception that metabolic

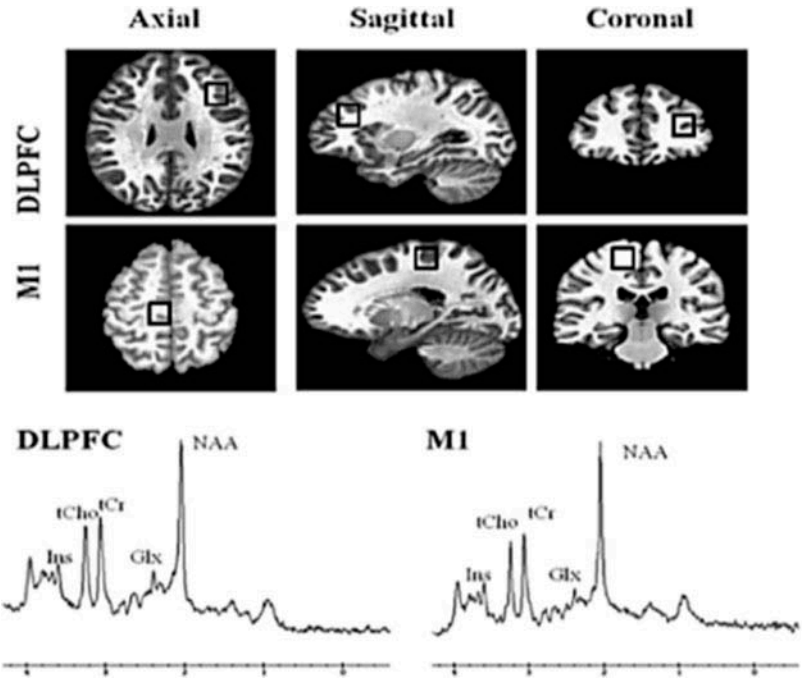

FIGURE 1 Anatomical MR images illustrating 1H MRS voxel placement and representative 1H MRS spectrum with labeled neural metabolites from the dorsolateral prefrontal cortex (DLPFC, 1st row/left) and primary motor cortex (M1, 2nd row/right).

relaxation factors, derived from the equation $R_{H2O\_met} = (e^{-TE/T_{2\_H2O\_met}}) \times \left(1 - e^{-TR/T_{1\_H2O\_met}}\right)$, were not assumed to be equal for both grey matter and white matter. The T_1 and T_2 relaxation times necessary to calculate these relaxation factors for the two tissue components were taken from Posse et al. (2007). Equation 2 from Gasparovic et al. was adjusted accordingly to reflect this change in metabolite concentration [M]:

$$[\mathrm{M}] = \frac{[M]_{LCM} \times \left(f_{GM} \times R_{H2o\_GM} + f_{WM} \times R_{H2o\_WM} + f_{CSF} \times R_{H2o\_CSF}\right)}{\left(f_{GM} \times R_{met\_GM}\right) + \left(f_{WM} \times R_{met\_WM}\right)}.$$

Using AFNI (Cox, 1996), the high resolution anatomical collected at each scan was realigned to the 3-dimensional localizer used for voxel placement, then segmented by FSL (http://www.fmrib.ox.ac.uk.ezp-prod1.hul.harvard.edu/fsl) into three tissue classes (cerebrospinal fluid, grey matter, and white matter). Masks were then applied to voxels in the original localizer space, yielding the voxel percent contributions to be used in the aforementioned procedure.

Due to the nature and scope of this longitudinal study, and the potential for inconsistent voxel placement with the multiple operators conducting magnetic resonance imaging (MRI) sessions, the practice of voxel reconstruction was also used to exclude spectra with inconsistent placement (discarded had <15% overlap) in regards to the baseline data collection. The addition of these criteria resulted in useful datasets from 25 high school football athletes (Team 1: 14) and 9 non-collision sport controls. Further, only LCModel fitting values with Cramer-Rao lower bounds less than 20% standard deviation were included in the analyses. This criterion also served as the rationale for the consideration of the combined glutamate and glutamine (Glx) signal, over the less-reliable individual components themselves.

Statistical Analyses

Possible metabolic and cognitive changes within each population were assessed using the MIXED Procedure in Statistical Analysis System (Version 9.3; SAS Institute Inc., Cary, NC), which assumed compound symmetry and accounted for missing observations in the context of a repeated measure model. Monthly differences from baseline were determined using Tukey-adjusted LSMEANS and differences from controls were determined using non-parametric Kruskal-Wallis tests of baseline concentrations and their follow-up measures, expressed as a percentage of baseline. In-season measures were assessed by comparing the non-collision trend to the trends of the football players at the first, second and third month follow-ups. Only observed p-values $<$ .05 were considered significant.

RESULTS

Cognitive Testing

No significant test–retest differences were observed in ImPACT^{TM} cognitive testing scores for the non-collision and (overall) football cohorts over the follow-up sessions. Differences between individual football teams were, however, observed at the first month, with Team 1 having lower

TABLE 1
ImPACT™ Cognitive Testing Composite Scores for Initial and Follow-Up Sessions for All Groups of Athletes

ImPACT™ Composite	*Group*	*Baseline*	*Follow-Up/Month 1*	*Month 2*	*Month 3*
Verbal Memory	Controls	91.78 (5.95)	93.78 (6.92)		
	Team 1	84.58 (9.92)	86.14 (10.95)	88.7 (10.09)	87.0 (9.38)
	Team 2	85.08 (11.86)	93.86 (6.44)	88.89 (11.85)	91.8 (7.92)
Visual Memory	Controls	81.44 (9.62)	89.22 (11.91)		
	Team 1	76.25 (9.45)	**67.29 (11.83)***	76.6 (12.38)	78.4 (11.01)
	Team 2	76.92 (13.12)	78.43 (16.92)	80.56 (14.13)	83.6 (9.24)
Visual-Motor	Controls	42.86 (6.05)	44.87 (7.27)		
	Team 1	40.69 (6.71	41.74 (7.24)	42.17 (8.57)	33.36 (12.33)
	Team 2	41.95 (6.1)	39.82 (7.33)	43.21 (7.91)	44.37 (8.1)
Reaction Time	Controls	0.54 (0.1)	0.53 (0.08)		
	Team 1	0.61 (0.21)	0.61 (0.1)	0.56 (0.08)	0.58 (0.06)
	Team 2	0.54 (0.09)	0.59 (0.1)	0.52 (0.07)	0.48 (0.08)
Impulse Control	Controls	7.67 (8.25)	9.33 (8.94)		
	Team 1	7.0 (3.33)	7.14 (4.3)	7.6 (4.01)	7.0 (3.54)
	Team 2	8.67 (5.18)	**5.29 (5.65)***	8.44 (6.75)	7.4 (5.13)
Symptom Score	Controls	3.11 (03.69)	**1.89 (2.09)***		
	Team 1	2.08 (2.07)	4.57 (3.95)	4.6 (3.92)	1.6 (1.82)
	Team 2	3.0 (4.29)	5.86 (5.01)	2.44 (3.94)	5.4 (8.53)

Note. Statistically significant ($p < .05$) group differences in changes relative to baseline (Kruskal-Wallis non-parametric tests for each time point) are indicated in bold and with an asterisk (*).

visual memory composite scores than controls (Kruskal-Wallis $X^2 = 9.302$, $p = .0096$). Also at the first month, non-collision controls exhibited a reduction in reported symptom scores that was not observed in the football athletes ($X^2 = 6.03$, $p = .049$). An increase in Impulse Control composite score was also observed in the controls and (for month 1) in Team 1, but Team 2 did not exhibit this decrease in performance ($X^2 = 7.11$, $p = .029$). Finally, additional non-significant decreasing trends in composite score performance were observed in the follow-up tests of the football athletes, contrasting with a trend of improving performance in non-collision controls (see Table 1).

Dorsolateral Prefrontal Cortex

No significant changes were observed in any of the DLPFC metabolites within the non-collision population (Table 2). Also, no changes in metabolites were observed over time or relative to controls for Team 1 in the DLPFC.

Within football Team 2, however, total creatine-containing compounds (tCr; see Figure 2A) in the DLPFC were observed to significantly decrease over the season ($F(3, 16) = 7.45$, $p = .002$). The concentration was significantly lower than baseline at the first ($t(16) = 3.98$, $p = .044$), second ($t(16) = 3.98$, $p = .005$), and third ($t(16) = 3.62$, $p = .011$) months of contact practices and games. These (negative) deviations from baseline tCr were significantly different from the

TABLE 2
Metabolite Concentration Means (and Standard Deviations) for the Non-Collision Sport Controls (n = 9) per Region of Interest (No Significant Time Effect Is Reported for This Population)

ROI	*Metabolite*	*Baseline*	*Follow-Up*	F_{month}	$Pr_{Mixed} > \|t\|$
DLPFC	NAA	12.62 (1.4)	13.05 (2.05)	0.38	0.56
	Glx	12.12 (1.8)	13.54 (2.64)	1.93	0.21
	tCr	8.26 (0.7)	9.56 (1.81)	2.62	0.15
	tCho	2.11 (0.31)	2.28 (0.41)	1.17	0.31
	Ins	5.34 (0.76)	5.68 (0.63)	0.71	0.43
	%GM	37.8% (5.8%)	37.1% (4.7%)	0.00	0.99
	%WM	43.7% (4.8%)	45.% (14.6%)	0.04	0.85
M1	NAA	11.95 (0.56)	12.83 (1.24)	3.50	0.10
	Glx	9.83 (1.91)	9.82 (1.24)	0.00	0.98
	tCr	8.18 (.46)	8.28 (0.62)	0.34	0.58
	tCho	1.88 (.25)	1.97 (0.24)	2.69	0.14
	Ins	4.95 (.53)	4.9 (0.51)	0.05	0.82
	%GM	33.1% (11.2%)	32.6% (10%)	0.01	0.92
	%WM	47.7% (12.8%)	51.3% (10.9%)	0.38	0.55

Note. DLPFC = dorsolateral prefrontal cortex; ROI = region of interest.

test–retest variation in the controls, for each of the first ($X^2 = 4.27$, $p = .039$), second ($X^2 = 5.84$, $p = .016$), and third months ($X^2 = 5.91$, $p = .015$).

Team 2 exhibited significantly higher concentrations of inositol (Ins) than controls at the time of baseline scan ($X^2 = 6.09$, $p = .0496$), and subsequently experienced a significant change in this concentration by month ($F(3,16) = 6.84$, $p = .004$). These significantly lower concentrations were observed at the first ($t(16) = 3.36$, $p = .0325$), second ($t(16) = 3.08$, $p = .033$) and third ($t(16) = 3.72$, $p = .009$) months of the season (Figure 2B). Further, these changes from baseline were significantly different at the first ($X^2 = 4.27$, $p = .039$) and third ($X^2 = 4.84$, $p = .028$) months from the test–retest variation of the controls.

Primary Motor Cortex

Within M1, there were no significant metabolic changes between baseline and follow-up scans for the non-collision population.

Both teams exhibited significantly higher concentrations of M1 glutamate and glutamine (Glx) than controls at the time of baseline ($X^2 = 7.11$, $p = .029$). Significant deviations from baseline were subsequently observed in this concentration (Figure 2C) for both Team 1 ($F(3, 23) = 3.74$, $p = 0.027$) and Team 2 ($F(3, 17) = 15.16$, $p < .0001$). Team 1 exhibited low concentrations relative to baseline at the time of the first month ($t(21) = 3.19$, $p = .021$) with corresponding changes from baseline that were significantly different from the test–retest variation in the controls ($X^2 = 6.19$, $p = 0013$). These deviations recovered to non-significant levels ($t(21) = 2.16$, $p = .166$) beginning with the second month. Team 2 also exhibited a depressed concentration of Glx in M1 at the first month ($t(17) = 5.16$, $p = 0.0004$), but, unlike Team 1, this deviation persisted at the second ($t(17) = 5.93$, $p < .0001$) and third ($t(17) = 3.88$, $p = .006$). As in DLPFC, the

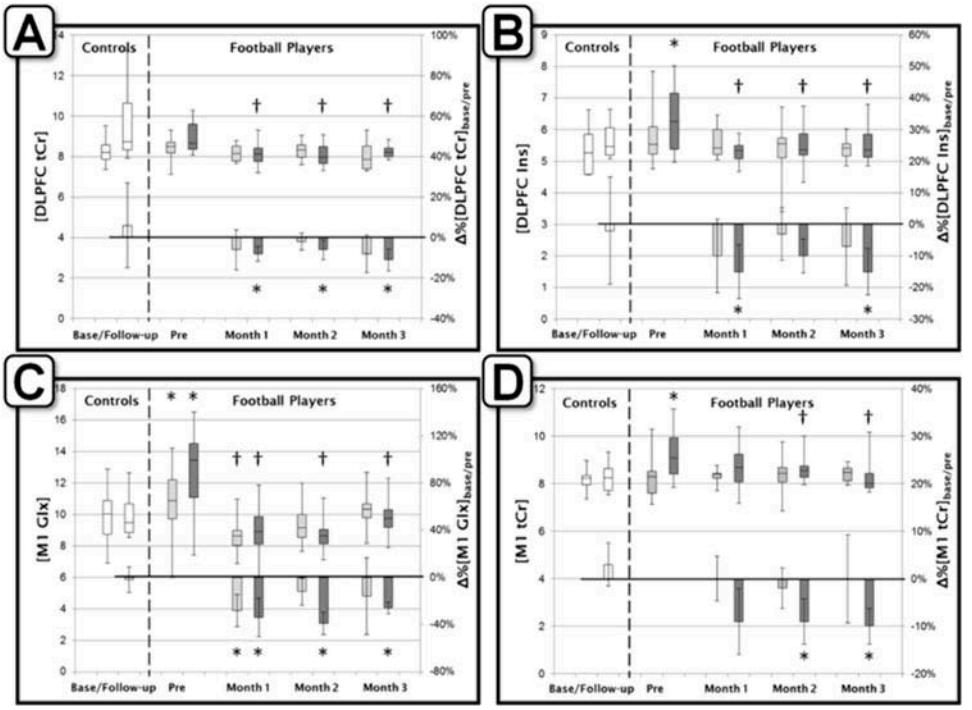

FIGURE 2 Metabolic concentrations (left axis) and changes in concentrations at each follow-up scan, expressed as a difference in percentage of baseline scan (right axis) for (A) DLPFC total creatine, (B) DLPFC inositol, (C) M1 glutamate+glutamine, and (D) M1 total creatine. Error bars represent ±1 standard deviation. Statistically significant ($p < .05$) differences are indicated relative to non-collision sport Control (*) or collision-sport Pre-Season (†) measures.

changes associated with these depressions were significantly different than the test–retest variation in the controls, for each of the first ($X^2 = 4.08$, $p = .043$), second ($X^2 = 8.75$, $p = .003$), and third months ($X^2 = 4.08$, $p = .043$).

Total creatine-containing compounds (tCr) in M1 were found to be significantly different for Team 2, relative to non-collision sport controls. Team 2 had higher concentrations of tCr ($X^2 = 6.78$, $p = .034$) at their baseline, and lower concentrations during the season ($F(3, 17) = 6.21$, $p = .005$), but these changes were not observed until the second ($t(17) = 3.39$, $p = .017$) and third ($t(17) = 3.54$, $p = .012$) months. Changes in tCr were significantly different from the test–retest variation of the controls, at both the second ($X^2 = 4.31$, $p = .038$) and third months ($X^2 = 7.35$, $p = .007$).

Finally, the concentration of total choline-containing compounds (tCho) in M1 for Team 2 exhibited a decrease during the first month ($t(17) = 3.13$, $p = .028$).

Summary statistics for all assessed metabolites, across sessions per group, may be found in Table 3.

TABLE 3
Metabolite Concentration Means (and Standard Deviations) for the Initial and Monthly Follow-Up Scans of Football Players, by Team

ROI	*Group*	*Metabolite*	*Baseline*	*Month 1*	*Month 2*	*Month 3*	F_{month}	$Pr_{Mixed} > \|t\|$
DLPFC	Team 1	N	14	6	10	6	$\mathbf{F_{3,\,18}}$	
		NAA	12.6 (0.68)	11.88 (0.87)	12.11 (0.55)	12.14 (0.96)	2.5	0.092
		Glx	12.13 (2.19)	10.43 (2.6)	10.84 (1.63)	10.39 (1.18)	1.89	0.167
		tCr	8.43 (0.55)	8.18 (0.47)	8.32 (0.49)	8.05 (0.82)	0.75	0.537
		tCho	2.04 (0.22)	1.96 (0.15)	2.07 (0.19)	1.85 (0.24)	1.41	0.271
		Ins	5.73 (0.85)	5.61 (0.58)	5.4 (0.87)	5.4 (0.4)	0.97	0.429
		GM	31.9% (6.8%)	35.1% (6.8%)	31.4% (5%)	33.% (3.6%)	1.10	0.375
		WM	55.6% (9.6%)	50.3% (11.8%)	55.6% (10.4%)	51.6% (9.5%)	1.91	0.164
	Team 2	N	11	6	8	7	$\mathbf{F_{3,\,17}}$	
		NAA	13.02 (1.21)	12.05 (1)	12.22 (0.79)	12.5 (1.32)	2.30	0.114
		Glx	11.88 (1.01)	9.97 (2.06)	10.52 (1.54)	10.66 (2.0)	2.77	0.074
		tCr*	8.85 (0.73)	8.06 (0.73)	7.97 (0.46)	8.17 (0.25)	**7.35**	**0.002***
		tCho	2.03 (0.12)	1.85 (0.13)	1.87 (0.18)	1.99 (0.26)	2.75	0.075
		Ins*	6.41 (0.98)	5.38 (0.41)	5.66 (0.63)	5.66 (0.62)	**7.45**	**0.002***
		GM	32.1% (6.1%)	28.1% (7.%)	27.4% (6.3%)	32.3% (3.7%)	1.23	0.329
		WM	51.2% (9.5%)	57.6% (8.2%)	60.6% (9.%)	55.5% (4.9%)	2.23	0.122
M1	Team 1	N	14	7	12	6	$\mathbf{F_{3,\,21}}$	
		NAA	11.96 (1.42)	12.17 (0.39)	12.36 (0.97)	12.3 (0.55)	0.69	0.568
		Glx*	11.1 (2.94)	8.58 (1.15)	9.5 (1.44)	10.19 (1.24)	**3.740**	**0.0269***
		tCr	8.28 (0.81)	8.33 (0.33)	8.43 (0.71)	8.43 (0.39)	0.63	0.606
		tCho	1.92 (0.27)	1.96 (0.2)	1.96 (0.2)	1.79 (0.12)	0.03	0.993
		Ins	5.5 (0.75)	5.52 (0.38)	5.38 (0.65)	5.61 (0.38)	0.38	0.769
		GM	37.1% (5.7%)	38.2% (2.8%)	35.9% (8.9%)	33.7% (7.2%)	0.87	0.471
		WM	41.1% (10.4%)	40.1% (5.9%)	45.6% (11.9%)	49.3% (12.1%)	1.87	0.166
	Team 2	N	11	7	8	7	$\mathbf{F_{3,\,17}}$	
		NAA	12.81 (1.24)	11.8 (1.32)	12.38 (0.83)	11.74 (1.64)	2.83	0.053
		Glx*	12.85 (2.64)	8.78 (1.62)	8.5 (0.73)	9.64 (1.02)	**15.16**	**<0.001***
		tCr*	9.22 (1.03)	8.7 (1.04)	8.66 (0.64)	8.34 (0.89)	**6.21**	**0.0048***
		tCho*	1.99 (0.19)	1.79 (0.11)	1.89 (0.08)	1.92 (0.12)	**3.37**	**0.0429***
		Ins	5.91 (0.94)	5.2 (0.42)	5.22 (0.63)	5.18 (0.49)	2.72	0.070
		GM	36.6% (7.1%)	38.9% (5.8%)	34.6% (7.9%)	38.% (4.6%)	0.98	0.424
		WM	41.4% (11.3%)	38% (5.5%)	43.3% (10.5%)	38.8% (5.3%)	1.16	0.352

Note. Statistically significant ($p < .05$) changes in metabolite concentrations over the season (SAS Mixed Procedure) are indicated in bold and with an asterisk (*). DLPFC = dorsolateral prefrontal cortex; ROI = region of interest.

DISCUSSION

Our main findings suggest that a cohort of prospectively studied high school American football athletes have experienced brain injury associated with their participation in the sport. We have observed changes in collision sport athletes in both neurocognitive performance and neurometabolism, specifically within the dorsolateral prefrontal cortex (DLPFC) and primary motor cortex (M1), that were not observed in non-collision sport high school athlete controls. Decreases in visual memory composite scores in ImPACT^{TM} are consistent with impaired efficiency of

working memory tasks, as previously observed in a similar population (Talavage et al., 2014). Observed reductions in total creatine (tCr) and dorsolateral prefrontal inositol (Ins) suggest energy crises and glial dysfunction (Faul et al., 2010; Xu et al., 2011), within one of the populations. Deviations in the glutamate and glutamine (Glx) concentration in primary motor cortex are consistent with alterations in cellular signaling (Newsholme, Procopio, Lima, Pithon-Curi, & Curi, 2003). In aggregate, these dramatic neurocognitive and metabolic deviations relative to baseline, found to be significantly different from non-collision sport controls, could reflect increased (likely pre-clinical) risk of changes in cognitive behavior and performance (Breedlove et al., 2014). It is quite possible that these American football players are combating cellular injury in the absence of externally observable symptoms.

Team-Specific Changes

A reported decrease in regional tCr, Ins in DLPFC, and tCho in M1 in one of the two studied American football teams suggests a risk of compromised executive function, working memory, and motor execution at the individual level. These three decreases are discussed below.

tCr decrease. The lack of stability in total creatine-containing compound concentrations within Team 2 supports a conjecture of limited energy sources for both the M1 and DLPFC regions and, since these changes have been observed in all levels of TBI (Gasparovic et al., 2009; Signoretti et al., 2010; Yeo et al., 2011), it challenges creatine use as the traditional internal reference in MRS study of brain injury.

Inositol decrease in DLPFC. The inositol decrease in DLPFC represents a unique finding as it contradicts several TBI studies documenting a delayed inositol increase (Ashwal et al., 2004b; Brooks et al., 2000; Henry et al., 2011), which has been associated with glial proliferation and the maintenance of cellular volume (Brand, Richter-Landsberg, & Leibfritz, 1993; Fisher, Novak, & Agranoff, 2002). This delayed inositol increase was possibly seen in Team 2 at the time of our pre-season scans. The initial in-season decrease could, however, demonstrate the opposite—damage to cells responsible for neuronal protection and neurotransmission recycling, that has been captured immediately following head injury in animal models (Schuhmann et al., 2003; Xu et al., 2011; Zhao, Ahram, Berman, Muizelaar, & Lyeth, 2003). These changes may also be due to the defensive mechanisms of reactive astrogliosis and osmolyte imbalance in the presence of reoccurring insult (Sofroniew, 2009), and have the potential to be masked in ratios where total creatine (tCr) is also decreased, as was here observed to be the case.

Choline decreases in M1. A brief decrease in choline in primary motor cortex was also not expected based on the prior clinical literature. Previous literature shows increased choline, which suggests increased membrane turnover and inflammation for chronically damaged individuals (Brooks et al., 2000). A brief reduction in tCho, however, has been observed immediately after injury in animal models, just prior to an increase and could be a result of the initial focal injuries (Schuhmann et al., 2003). Therefore, this finding is not unsupported and likely an indicator of actual damage in the collision sport athletes.

Collision Sport Changes

In addition to the changes noted above on a team-specific basis, a reduction was observed in available excitatory neurotransmitters and an increase in potential functional impairment within the primary motor cortex of both football populations. The finding of a decrease in M1 Glx as late as three months into the season (ref. Figure 2C) is consistent with literature; Glx reductions have been observed in the primary motor cortex of concussed athletes and grey matter of mild TBI patients (Gasparovic et al., 2009; Henry, Tremblay, Boulanger, Ellemberg, & Lassonde, 2010; Yeo et al., 2011). Found to be significantly different from controls, the decreased glutamate (the bulk of the Glx signal) exhibited by the football athletes is associated with decreased excitatory synaptic activity within the Betz cells found in the motor strip area, and potentially with motor dysfunction (De Beaumont et al., 2012). This impairment of the M1 region is also supported by previous discoveries of altered neurometabolism as measured by functional magnetic resonance imaging (fMRI) (Breedlove et al., 2012, 2014; Talavage et al., 2014). The potential for such impairment was seemingly alleviated in the first team, where decreased concentrations recovered toward normal levels within the season, but this "recovery" could also be Glx accumulation as a secondary response to sustained injury (e.g., Ashwal et al., 2004a; Yeo et al., 2011). This excitotoxic accumulation is also suggested within the observation of higher pre-season Glx in both football populations.

It is worth noting that the non-significant rise observed in NAA for the control population (see Table 2) is consistent with literature studying the effects of aerobic exercise (Erickson et al., 2012; Gonzales et al., 2013). The lack of a consistent increase in the overall football-playing population is intriguing and of future interest.

Implications of Team-Specific Findings

As noted above, in-season metabolic activity was *not* found to be consistent between the two participating football teams, suggesting some influence on neurometabolic outcomes of individual physiology, head collision history or skill level/technique. Team 1, among the lower performing teams in the state over these two seasons, exhibited an initial drop in M1 Glx with subsequent recovery, while other metabolites remained stable relative to pre-season concentrations. In contrast, Team 2, one of the highest ranked teams in the state, exhibited more sustained depression of tCr, M1 Glx, and DLPFC Ins. It is important to note that several factors may have contributed to observation of findings in the two football teams, including the unbalanced representation of the two teams for the included seasons of study, and a lack of complete head collision data for Team 2 for each of the two included seasons. We have, however, reported differences in head impact distributions across the teams in previous seasons (Breedlove et al., 2012).

Implications for Recovery From Injury

Coupling the metabolic and ImPACT^{TM} neurocognitive changes observed during Month 1, it appears these collision-sport athletes are in fact injured without being recognized as "concussed."

While non-collision athletes reported fewer symptoms, presumed to be among the neural benefits of continued exercise, the collision sport athletes exhibited both altered metabolism and cognition. During the period shortly after the onset of collision activities, both Team 1 and Team 2 exhibit a decrease in M1 Glx, as well as greater reporting of symptoms. Both of these trends were also previously observed by Henry et al. (2010). This change in apparent state seems to be ameliorated subsequent to the first month, which may be interpreted as evidence that football players exhibit damage with onset of collision activity, and require a 4–6-week period of bodily adjustment before compensatory mechanisms may repair or overcome the associated effects. It is of interest that Team 1 at Month 1 was the only team/month combination to demonstrate significantly worse cognitive re-testing than controls, specifically exhibiting higher (poorer) Impulse Control scores, and lower visual working memory—an observation consistent with Talavage et al. (2014). While Team 2 was not found to have continued cognitive impairment, its members still exhibited dramatic metabolic depression. As a consequence, it would appear possible for ImPACT^{TM} evaluations to permit return-to-play of athletes prior to actual completion of recovery from deviant metabolism. Further study is warranted to understand if the temporary deficit exhibited by the repair mechanisms (relative to the putative injury mechanisms) is a consequence of the initial two-a-day loading of typical collision sport practices, or if there is a universal lag between injury and repair, or a minimum threshold of injury before repair mechanisms are significantly engaged.

Limitations on Generalization of Findings

The main concern for generalization of results from this study is the observation that metabolite concentration patterns differed by football team. While some of the experimental sample issues noted above may have contributed to these differences, there are also concerns that, in the context of participation in collision sports, there may be meaningful contributions to neurological health from socioeconomic, performance, or competition level differences. Generalization of observations is further limited by the modest sample sizes obtained here for each population, and the lack of comparison of deviations from baseline measures with athletes who *have* been diagnosed as concussed. Comparison to concussed athletes would allow quantification of the severity of the apparently present injury in a context that is relevant to a clinician. Also, although the study incorporates an age- and gender-appropriate non-collision sport control population that adjusts for confounding factors such as environmental factors (Babb et al., 2004; Dechent, Pouwels, Wilken, Hanefeld, & Frahm, 1999; Tan et al., 1998) and exercise (Maddock, Casazza, Buonocore, & Tanase, 2011), such a population should be studied more closely in this age bracket as there are many other biological changes that may occur.

It is argued that these shortcomings are offset by the notable strength that each athlete herein serves as a self-reference, against which changes over time may be compared. The pre-season baseline measures of the experimental population allow for quantitative assessment of individual metabolic changes over the course of repetitive blows to the head, and facilitate comparison of metabolism with other assessments (e.g., hit histories, MRI, fMRI, and cognitive testing) in future analyses.

CONCLUSIONS

All of the reported observations reveal evidence of metabolic disturbance within two critical regions of interest, presumably as a consequence to repetitive sub-concussive events, in normal-appearing young collision sport athletes. The presented results suggest that previous research involving changes in fMRI is, in fact, revealing early stages of damage throughout the brain. While here reported for a modest high school football population, it is desirable that the study generate future interest and investigation, as the underlying mechanisms producing the hypothesized injuries are yet largely unknown, and damage at this pre-clinical stage remains to be studied in detail.

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REFERENCES

Ashwal, S., Holshouser, B., Tong, K., Serna, T., Osterdock, R., Gross, M., & Kido, D. (2004a). Proton MR spectroscopy detected glutamate/glutamine is increased in children with traumatic brain injury. *Journal of Neurotrauma*, *21*(11), 1539–1552.

Ashwal, S., Holshouser, B., Tong, K., Serna, T., Osterdock, R., Gross, M., & Kido, D. (2004b). Proton spectroscopy detected myoinositol in children with traumatic brain injury. *Pediatric Research*, *56*(4), 630–638.

Associations, N. F. o. S. H. S. 2009–2010. High school athletics participation survey. Retrieved from http://www.nfhs.org/content.aspx?id=3282&linkidentifier=id&itemid=3282

Babb, S. M., Ke, Y., Lange, N., Kaufman, M. J., Renshaw, P. F., & Cohen, B. M. (2004). Oral choline increases choline metabolites in human brain. *Psychiatry Research*, *130*(1), 1–9.

Bailes, J. E., Petraglia, A. L., Omalu, B. I., Nauman, E., & Talavage, T. (2013). Role of subconcussion in repetitive mild traumatic brain injury. *Journal of Neurosurgery*, *119*(5), 1235–1245.

Baugh, C. M., Stamm, J. M., Riley, D. O., Gavett, B. E., Shenton, M. E., Lin, A., . . . Stern, R. A. (2012). Chronic traumatic encephalopathy: Neurodegeneration following repetitive concussive and subconcussive brain trauma. *Brain Imaging and Behavior*, *6*(2), 244–254.

Brand, A., Richter-Landsberg, C., & Leibfritz, D. (1993). Multinuclear NMR studies on the energy metabolism of glial and neuronal cells. *Developmental Neuroscience*, *15*(3–5), 289–298.

Breedlove, E. L., Robinson, M., Talavage, T. M., Morigaki, K. E., Yoruk, U., O'Keefe, K., . . . Nauman, E. A. (2012). Biomechanical correlates of symptomatic and asymptomatic neurophysiological impairment in high school football. *Journal of Biomechanics*, *45*(7), 1265–1272.

Breedlove, K. M., Breedlove, E. L., Robinson, M., Poole, V. N., King, J. R., Rosenberger, P., . . . Nauman, E. A. (2014). Detecting neurocognitive & neurophysiological changes as a result of subconcussive blows in high school football athletes. *Athletic Training & Sports Health Care*, *6*(3), 119–127.

Broglio, S. P., Sosnoff, J. J., Shin, S., He, X., Alcaraz, C., & Zimmerman, J. (2009). Head impacts during high school football: A biomechanical assessment. *Journal of Athletic Training*, *44*(4), 342–349.

Brooks, W. M., Friedman, S. D., & Gasparovic, C. (2001). Magnetic resonance spectroscopy in traumatic brain injury. *Journal of Head Trauma Rehabilitation*, *16*(2), 149–164.

Brooks, W. M., Stidley, C. A., Petropoulos, H., Jung, R. E., Weers, D. C., Friedman, S. D., . . . Yeo, R. A. (2000). Metabolic and cognitive response to human traumatic brain injury: A quantitative proton magnetic resonance study. *Journal of Neurotrauma*, *17*(8), 629–640.

Buck, P. W. (2011). Mild traumatic brain injury: A silent epidemic in our practices. *Health & Social Work*, *36*(4), 299–302.

Cantu, R. C. (1998). Second-impact syndrome. *Clinics in Sports Medicine*, *17*(1), 37–44.

Castile, L., Collins, C. L., McIlvain, N. M., & Comstock, R. D. (2011). The epidemiology of new versus recurrent sports concussions among high school athletes, 2005–2010. *British Journal of Sports Medicine*, *46*(8), 603–610.

Collins, M. W., Grindel, S. H., Lovell, M. R., Dede, D. E., Moser, D. J., Phalin, B. R., . . . McKeag, D. B. (1999). Relationship between concussion and neuropsychological performance in college football players. *JAMA*, *282*(10), 964–970.

Cox, R. W. (1996). AFNI: Software for analysis and visualization of functional magnetic resonance neuroimages. *Computers and Biomedical Research*, *29*(3), 162–173.

De Beaumont, L., Tremblay, S., Poirier, J., Lassonde, M., & Théoret, H. (2012). Altered bidirectional plasticity and reduced implicit motor learning in concussed athletes. *Cerebral Cortex*, *22*(1), 112–121.

Dechent, P., Pouwels, P. J., Wilken, B., Hanefeld, F., & Frahm, J. (1999). Increase of total creatine in human brain after oral supplementation of creatine-monohydrate. *American Journal of Physiology*, *277*(3 Pt 2), R698–704.

Erickson, K. I., Weinstein, A. M., Sutton, B. P., Prakash, R. S., Voss, M. W., Chaddock, L., . . . Wojcicki, T. R. (2012). Beyond vascularization: Aerobic fitness is associated with N-acetylaspartate and working memory. *Brain and Behavior*, *2*(1), 32–41.

Erlanger, D. M., Kutner, K. C., Barth, J. T., & Barnes, R. (1999). Neuropsychology of sports-related head injury: Dementia Pugilistica to Post Concussion Syndrome. *Clinical Neuropsychology*, *13*(2), 193–209.

Faul, M., Xu, L., Wald, M., & Coronado, V. (2010). *Traumatic brain injury in the United States: Emergency department visits, hospitalizations and deaths 2002–2006*. Atlanta, GA: Centers for Disease Control and Prevention, National Center for Injury Prevention and Control, 2–70.

Field, M., Collins, M. W., Lovell, M. R., & Maroon, J. (2003). Does age play a role in recovery from sports-related concussion? A comparison of high school and collegiate athletes. *Journal of Pediatrics*, *142*(5), 546–553.

Fisher, S. K., Novak, J. E., & Agranoff, B. W. (2002). Inositol and higher inositol phosphates in neural tissues: Homeostasis, metabolism and functional significance. *Journal of Neurochemistry*, *82*(4), 736–754.

Gasparovic, C., Yeo, R., Mannell, M., Ling, J., Elgie, R., Phillips, J., . . . Mayer, A. R. (2009). Neurometabolite concentrations in gray and white matter in mild traumatic brain injury: An 1H-magnetic resonance spectroscopy study. *Journal of Neurotrauma*, *26*(10), 1635–1643.

Gavett, B. E., Stern, R. A., & McKee, A. C. (2011). Chronic traumatic encephalopathy: A potential late effect of sport-related concussive and subconcussive head trauma. *Clinics in Sports Medicine*, *30*(1), 179–188.

Gonzales, M. M., Tarumi, T., Kaur, S., Nualnim, N., Fallow, B. A., Pyron, M., . . . Haley, A. P. (2013). Aerobic fitness and the brain: Increased N-acetyl-aspartate and choline concentrations in endurance-trained middle-aged adults. *Brain Topography*, *26*(1), 126–134.

Henry, L. C., Tremblay, S., Boulanger, Y., Ellemberg, D., & Lassonde, M. (2010). Neurometabolic changes in the acute phase after sports concussions correlate with symptom severity. *Journal of Neurotrauma*, *27*(1), 65–76.

Henry, L. C., Tremblay, S., Leclerc, S., Khiat, A., Boulanger, Y., Ellemberg, D., & Lassonde, M. (2011). Metabolic changes in concussed American football players during the acute and chronic post-injury phases. *BMC Neurology*, *11*, 105.

Johnson, B., Gay, M., Zhang, K., Neuberger, T., Horovitz, S. G., Hallett, M., . . . Slobounov, S. (2012). The use of magnetic resonance spectroscopy in the subacute evaluation of athletes recovering from single and multiple mild traumatic brain injury. *Journal of Neurotrauma*, *29*(13), 2297–2304.

Lin, A., Ross, B. D., Harris, K., & Wong, W. (2005). Efficacy of proton magnetic resonance spectroscopy in neurological diagnosis and neurotherapeutic decision making. *NeuroRx*, *2*(2), 197–214.

Lovell, M. R., & Collins, M. W. (1998). Neuropsychological assessment of the college football player. *Journal of Head Trauma Rehabilitation*, *13*(2), 9–26.

Maddock, R. J., Casazza, G. A., Buonocore, M. H., & Tanase, C. (2011). Vigorous exercise increases brain lactate and Glx (glutamate+glutamine): A dynamic 1H-MRS study. *Neuroimage*, *57*(4), 1324–1330.

McKee, A. C., Cantu, R. C., Nowinski, C. J., Hedley-Whyte, E. T., Gavett, B. E., Budson, A. E., . . . Stern, R. A. (2009). Chronic traumatic encephalopathy in athletes: Progressive tauopathy after repetitive head injury. *Journal of Neuropathology & Experimental Neurology*, *68*(7), 709–735.

Mountford, C., Lean, C., Malycha, P., & Russell, P. (2006). Proton spectroscopy provides accurate pathology on biopsy and in vivo. *Journal of Magnetic Resonance Imaging*, *24*(3), 459–477.

Newsholme, P., Procopio, J., Lima, M. M., Pithon-Curi, T. C., & Curi, R. (2003). Glutamine and glutamate—Their central role in cell metabolism and function. *Cell Biochemistry and Function*, *21*(1), 1–9.

Posse, S., Otazo, R., Caprihan, A., Bustillo, J., Chen, H., Henry, P. G., . . . Alger, J. R. (2007). Proton echo-planar spectroscopic imaging of J-coupled resonances in human brain at 3 and 4 Tesla. *Magnetic Resonance in Medicine*, *58*(2), 236–244.

Provencher, S. W. (1993). Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magnetic Resonance in Medicine*, *30*(6), 672–679.

Schuhmann, M. U., Stiller, D., Skardelly, M., Bernarding, J., Klinge, P. M., Samii, A., . . . Brinker, T. (2003). Metabolic changes in the vicinity of brain contusions: A proton magnetic resonance spectroscopy and histology study. *Journal of Neurotrauma*, *20*(8), 725–743.

Signoretti, S., Di Pietro, V., Vagnozzi, R., Lazzarino, G., Amorini, A. M., Belli, A., . . . Tavazzi, B. (2010). Transient alterations of creatine, creatine phosphate, N-acetylaspartate and high-energy phosphates after mild traumatic brain injury in the rat. *Molecular and Cellular Biochemistry*, *333*(1–2), 269–277.

Sofroniew, M. V. (2009). Molecular dissection of reactive astrogliosis and glial scar formation. *Trends in Neuroscience*, *32*(12), 638–647.

Talavage, T. M., Nauman, E. A., Breedlove, E. L., Yoruk, U., Dye, A. E., Morigaki, K. E., . . . Leverenz, L. J. (2014). Functionally-detected cognitive impairment in high school football players without clinically-diagnosed concussion. *Journal of Neurotrauma*, *31*(4), 327–338.

Tan, J., Bluml, S., Hoang, T., Dubowitz, D., Mevenkamp, G., & Ross, B. (1998). Lack of effect of oral choline supplement on the concentrations of choline metabolites in human brain. *Magnetic Resonance in Medicine*, *39*(6), 1005–1010.

Vagnozzi, R., Signoretti, S., Tavazzi, B., Floris, R., Ludovici, A., Marziali, S., . . . Lazzarino, G. (2008). Temporal window of metabolic brain vulnerability to concussion: A pilot 1H-magnetic resonance spectroscopic study in concussed athletes—Part III. *Neurosurgery*, *62*(6), 1286–1295.

van der Graaf, M. (2010). In vivo magnetic resonance spectroscopy: Basic methodology and clinical applications. *European Biophysics Journal*, *39*(4), 527–540.

Xu, S., Zhuo, J., Racz, J., Shi, D., Roys, S., Fiskum, G., & Gullapalli, R. (2011). Early microstructural and metabolic changes following controlled cortical impact injury in rat: A magnetic resonance imaging and spectroscopy study. *Journal of Neurotrauma*, *28*(10), 2091–2102.

Yeo, R. A., Gasparovic, C., Merideth, F., Ruhl, D., Doezema, D., & Mayer, A. R. (2011). A longitudinal proton magnetic resonance spectroscopy study of mild traumatic brain injury. *Journal of Neurotrauma*, *28*(1), 1–11.

Zhang, K., Johnson, B., Pennell, D., Ray, W., Sebastianelli, W., & Slobounov, S. (2010). Are functional deficits in concussed individuals consistent with white matter structural alterations: Combined FMRI & DTI study. *Experimental Brain Research*, *204*(1), 57–70.

Zhao, X., Ahram, A., Berman, R. F., Muizelaar, J. P., & Lyeth, B. G. (2003). Early loss of astrocytes after experimental traumatic brain injury. *Glia*, *44*(2), 140–152.