Smaller Dentate Gyrus and CA2 and CA3 Volumes Are Associated with Kynurenine Metabolites in Collegiate Football Athletes

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Abstract

An imbalance in kynurenine pathway metabolism is hypothesized to be associated with dysregulated glutamatergic neurotransmission, which has been proposed as a mechanism underlying the hippocampal volume loss observed in a variety of neurological disorders. Pre-clinical models suggest that the CA2-3 and dentate gyrus hippocampal subfields are particularly susceptible to excitotoxicity after experimental traumatic brain injury. We tested the hypothesis that smaller hippocampal volumes in collegiate football athletes with ($n=25$) and without ($n=24$) a concussion history would be most evident in the dentate gyrus and CA2-3 subfields relative to nonfootball healthy controls ($n=27$). Further, we investigated whether the concentration of peripheral levels of kynurenine metabolites are altered in football athletes. Football athletes with and without a self-reported concussion history had smaller dentate gyrus ($p<0.05$, $p<0.10$) and CA2-3 volumes ($p's<0.05$) relative to healthy controls. Football athletes with and without a concussion history had a trend toward lower ($p<0.10$) and significantly lower ($p<0.05$) kynurenine levels compared with healthy controls, while athletes with a concussion history had greater levels of quinolinic acid compared with athletes without a concussion history ($p<0.05$). Finally, plasma levels of 3-hydroxykynurenine inversely correlated with bilateral hippocampal volumes in football athletes with a concussion history ($p<0.01$), and left hippocampal volume was correlated with the ratio of kynurenic acid to quinolinic acid in football athletes without a concussion history ($p<0.05$). Our results raise the possibility that abnormalities of the kynurenine metabolic pathway constitute a mechanism for hippocampal volume differences in the context of sports-related brain injury.

Key words: concussion; football; hippocampus; kynurenine pathway; mTBI

Introduction

WE HAVE PREVIOUSLY OBSERVED significantly smaller bilateral hippocampal gray matter volumes in American-football athletes compared with healthy controls (HC) regardless of concussion history.¹ The pathophysiological mechanism underpinning this reduction in gray matter volume remains unclear, however. Imbalances in kynurenine (KYN) pathway metabolism are hypothesized to be associated with dysregulated glutamatergic neurotransmission, which has been proposed as a mechanism behind neuropathological changes in various psychiatric and neurological disorders.^{2–4}

The KYN metabolic pathway is a major route for degradation of tryptophan in both the periphery and central nervous system (CNS). Various pro-inflammatory cytokines activate the enzyme indoleamine

2,3-dioxygenase (*IDO*), altering the metabolism of tryptophan to increase KYN release, and simultaneously enhancing the activity of the enzyme kynurenine-3-monooxygenase (*KMO*), shifting KYN metabolism away from kynurenic acid (KYNA) toward 3-hydroxykynurenine (3HK) and subsequently the terminal metabolite, quinolinic acid (QUIN).⁵ While 3HK is a potent free radical generator that promotes oxidative stress,⁶ microglia and macrophage-derived QUIN is an N-methyl-D-aspartate (NMDA) receptor agonist that can cause excitotoxicity at high concentrations.⁷ In contrast to these potentially neurotoxic metabolites, astrocyte-derived KYNA is pleiotropic, acting as an endogenous competitive antagonist of ionotropic excitatory amino acid receptors including the NMDA receptor as well as an $\alpha 7$ nicotinic receptor antagonist, an orphan G-protein-coupled receptor (GPR35) agonist, an aryl hydrocarbon receptor (AHR) agonist, and an enhancer of

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nerve growth factor (NGF) expression.⁸ Based in part on their contrasting effects on the NMDA receptor, KYNA has been hypothesized to exert neuroprotective effects while 3HK and QUIN have been hypothesized to be neurotoxic in the context of injury, neuroinflammatory diseases, and psychiatric diseases.²⁻⁴

The proposed neuromodulatory effects of KYN metabolites receive support from the pre-clinical and clinical literature. For instance, disturbances in the KYN pathway have been documented after severe injury to the CNS. In animals, increased levels of QUIN have been observed in the CNS after experimental spinal cord injury.⁹ Elevated levels of QUIN have also been observed in cerebral spinal fluid of children and adults after severe traumatic brain injury (TBI).^{10,11} In addition, one study found that the ratio of KYN to tryptophan, a proxy measure of *IDO* activity, was higher in the blood of patients with chronic brain injury compared with HC.¹² Interestingly, we previously reported a reduction in the ratio of KYNA to QUIN in major depressive disorder (MDD).^{2,3} Further, KYNA/QUIN was positively associated with hippocampal volume in the MDD subjects. Abnormalities in KYN pathway metabolites have also been documented in other neuroinflammatory disorders, such as Alzheimer disease.¹³ Here we explore the effects of repeated concussive and subconcussive head impacts on the KYN metabolic pathway and its relationship with hippocampal volume, which we previously reported to be reduced in football athletes with and without a history of concussion.¹

Work in animals suggests that specific subfields of the hippocampus are more sensitive to TBI. Multiple studies have found neuronal loss in the dentate gyrus (DG) after fluid percussion injury or controlled cortical impact in rodents.¹⁴⁻¹⁶ Moreover, this neuronal loss is often localized to the hilus of the DG.¹⁵⁻¹⁷ The DG shows the highest number of activated microglia after controlled cortical impact.¹⁸ Similarly, the cornu Ammonis (CA) 3 is also particularly susceptible to experimentally induced TBI.¹⁹ One study observed neuronal loss in the DG and CA3 after fluid percussion injury and a localized increase of microglia in these regions at 2 weeks post-injury.¹⁶ Whether exposure to collision sports and concussions is associated with lower volumes in specific hippocampal subfields has not been investigated.

The primary goal of this work was to investigate whether football players with and without a history of concussion have smaller hippocampal subfields than a group of control participants. Based on previous articles in the literature on animals demonstrating that the DG and CA2-3 are particularly sensitive to TBI,^{14-16,19} we hypothesized that bilateral DG and CA2-3 would be smaller in football athletes compared with HC regardless of concussion history. Second, we extend previous work reporting abnormal levels of KYN metabolites after severe TBI.^{10,12} We hypothesized that athletes with and without a history of concussion would have higher peripheral levels of putatively neurotoxic KYN metabolites and lower peripheral levels of putatively neuroprotective KYN metabolites. Finally, given our previous findings in MDD, we hypothesized that hippocampal volume would correlate inversely with peripheral concentrations of putative neurotoxic KYN metabolites but positively with concentrations of putative neuroprotective KYN measures in football athletes.

Methods

Participants

Male student-athletes from a National Collegiate Athletic Association Division I American-rules football program, noncollision collegiate sport athletes, and nonathletes were recruited for this

study. Exclusion criteria included a past or current diagnosis of mood/anxiety disorders or alcohol/substance abuse, based on participant self-report. Concussion history was based on self-reported incidents of concussion that were medically diagnosed by a clinician at the time of injury.

A total of 25 current or former football athletes with at least one self-reported incidence of a clinician-diagnosed concussion (Ath-mTBI) and 24 football athletes without a self-reported history of clinician-diagnosed concussion (Ath) were included for this study. One track and field athlete had a history of concussion and extensive football experience (9 years) and was included in the Ath-mTBI group. Complete resolution of symptoms from previous concussions as determined by clinicians trained in sports medicine was an additional inclusion criterion for athletes with a history of concussion. Twenty-seven male noncollision sport athletes and nonathletes with no self-reported history of concussion or football experience were enrolled as a HC group.

All participants provided written informed consent approved by a local Institutional Review Board. Additional demographic information is listed in Table 1. Behavioral data for this cohort are presented in a separate article investigating differences in cortical thickness and fractional anisotropy and are not reported here.²⁰

Magnetic resonance imaging (MRI)-parameters and subfield segmentation

MRI was performed using a GE Discovery MR750 3-Tesla whole body MRI scanner (General Electric Healthcare) and brain-dedicated receive-only 32-element coil array optimized for parallel imaging (Nova Medical, Inc.). T1-weighted anatomical images were collected using a parallelized magnetization-prepared rapid gradient-echo sequence (MPRAGE) with sensitivity encoding and the following parameters for all participants: FOV = 240 mm, 130 axial slices, slice thickness = 1.1 mm, image matrix = 256 × 256, voxel size 0.9375 × 0.9375 × 1.1 mm, TR/TE = 5/1.948 msec, acceleration factor R = 2 in the phase encoding direction, flip angle = 8 degrees, TI = 725 msec, sampling bandwidth = 31.25 kHz, scan time = 5:05 min.

Cortical reconstruction and volumetric segmentation was performed using the FreeSurfer image analysis suite version 5.1.²¹ The total subcortical gray matter volume from the subcortical segmentation was used in subsequent analyses. An automated hippocampal segmentation algorithm available in the FreeSurfer software was used to estimate subfield volumes using Bayesian inference and a probabilistic hippocampal atlas based on *in vivo* manual segmentations.²² This software estimates volumes of CA1, CA2-3, DG-CA4, pre-subiculum, subiculum, fimbria, hippocampal fissure, and a nonspecific hippocampal tail. The results for the smaller and less reliably segmented hippocampal fissure and fimbria and the nonspecific hippocampal tail subfield are not assessed individually here.²²

Blood collection and processing

Nonfasting afternoon blood was collected in two, 6 mL K2 ethylenediaminetetraacetic acid BD Vacutainer™ tubes (Becton, Dickinson and Company, Franklin Lakes, NJ). Blood was collected within 1 month of the scanning date, but on the same day of scanning for the majority of participants (see Results). Blood was centrifuged at 1300 g for 10 min at room temperature, and plasma was collected. Blood was then either aliquoted into microfuge tubes and stored at -80°C that day, or stored at 4°C overnight and then aliquoted into microfuge tubes and stored at -80°C the next morning (see Supplementary Information; see online supplementary material at ftp.liebertpub.com). Frozen plasma samples were shipped overnight on dry ice to Brains Online (www.brainsonline.org) for analysis of KYN metabolites. Concentrations of KYN, QUIN, 3HK, and KYNA in plasma samples were determined using

TABLE 1. DEMOGRAPHIC INFORMATION

Demographics	Mean (standard deviation)		
	HC	Ath	Ath-mTBI
Number of participants	27	24	25
Education (years)	13.70 (1.56)	13.08(1.25)	13.40 (1.26)
Age (years) ^a	21.89 (2.24)	20.22 (1.20)	21.0 (1.47)
Body mass index ^a	24.21 (4.99)	30.55 (4.48)	28.46 (3.75)
Subcortical gray matter Volume (mm ³)	61,557 (4615)	60,616 (3943)	60,413 (3867)
Years football experience	0	10.56 (2.73)	11.6 (3.79)
Diagnosed concussions	0	0	2.08 (1.0)
<i>Football position</i>			
Quarterback		1	0
Running back		2	4
Tight end		1	0
Wide receiver		3	7
Offensive line		3	1
Defensive tackle		4	1
Defensive end		2	1
Linebacker		4	6
Defensive back		4	5
<i>Other sport controls</i>			
Nonathlete	20		
Cross country/track	6		
Basketball	1		
<i>Health/medication on day of scan</i>			
Self-reported	0	0	3
ADHD			
Acetaminophen, antibiotic, Mucinex	0	1 ^b	0
Antibiotic	1 ^c	0	0
Adderall	0	0	1
Loratadine	1	0	0
Protriptyline	1 ^d	0	0
Antacid	1	0	0

HC, healthy control; Ath, athlete without concussion history; Ath-mTBI, athlete with concussion history; ADHD, attention deficit hyperactivity disorder.

^aIndicates significant group difference ($p < 0.05$). ^bThis participant not included in blood analyses. ^cAntibiotic taken on day of scanning session; participant returned for blood sample unmedicated. ^dReported for recurring headaches.

high performance liquid chromatography (HPLC) with tandem mass spectrometry (MS/MS) detection according to their standard protocols.

Blood samples were not collected from all participants. One Ath was excluded from blood metabolite analyses because of a sinus infection necessitating medication, and one Ath-mTBI was excluded from blood analyses because of a nonhead injury at the time of blood draw.

Statistics

All statistical analyses were performed in SYSTAT Version 13 (Systat Software, Inc., San Jose, CA). One-way analyses of variance were performed to determine whether age, education, subcortical gray matter volume, or body mass index (BMI) differed between groups. A two-tailed independent samples t test was conducted to determine whether football groups differed in the years of tackle football experience.

Multivariate analyses of covariance (MANCOVAs) were used to reduce the number of tests and control for false positives. Age was used as a covariate because of differences in age across groups and the developmental trajectory of hippocampal volume.²³ Separate MANCOVAs were performed for left and right hippocampal subfield volumes (subiculum, pre-subiculum, CA1, CA2-3, DG-CA4), with total subcortical gray matter included as an additional covariate.^{24,25} A MANCOVA, with age as a covariate, was performed to assess group differences in KYN pathway measures (KYN, QUIN, 3HK, KYNA, the ratio of KYNA to QUIN [KYNA/QUIN], and the ratio of KYNA to 3HK [KYNA/3HK]). Protected univariate analyses of covariance (ANCOVA) were performed after significant ($p < 0.05$) or trending toward significant ($p < 0.10$) multivariate group effects. Hypothesis-based *a priori* ANCOVAs were performed for left and right CA2-3 and DG-CA4 volumes. Two-tailed planned contrasts of Ath-mTBI versus Ath, and both football athlete groups versus HCs were performed after significant or trending toward significant ANCOVAs.

Finally, the association between hippocampal volume and KYN metabolites was assessed within each group using two-tailed Spearman rank correlation coefficients. To control for type I error, all subfield volumes from each hemisphere were summed into left and right total hippocampal volume and *a priori* correlations focused on the correlation between left and right total hippocampal volumes with each KYN metabolite.

Results

Demographics

Groups did not differ in the number of years of education $F(2,73) = 1.30$, $p = 0.28$ or subcortical gray matter volume $F(2,73) = 0.56$, $p = 0.57$ (Table 1). There was a significant main effect of group, however, on age $F(2,73) = 6.01$, $p = 0.004$, because Ath were significantly younger than HC ($p = 0.001$), and Ath-mTBI were younger than HC at a trend level ($p = 0.077$). There was no difference in age between football Ath and Ath-TBI ($p > 0.1$). Groups also differed in BMI $F(2,73) = 13.6$, $p < 0.001$, with football groups having higher BMI than HC ($p = 0.001$) but not differing from each other ($p = 0.10$). Football Ath and Ath-mTBI did not differ in the number of years of tackle football experience $t(46) = -1.08$, $p = 0.29$. The most recent concussion for athletes with a history occurred on average 9.96 months (standard deviation [SD] = 11.98; range 1–42 months) before scanning. The blood draw occurred on average 0.92 days before the scanning session (SD = 6.78, range 28 days before to 28 days after).

Hippocampal subfield analyses

The averages of hippocampal subfield volumes for each group can be found in Table 2. The multivariate effect of group on left subfield volumes was trending toward significance (Wilk Lambda = 0.79, $F(10,134) = 1.71$, $p = 0.084$). Confirming our *a priori* hypothesis, there was a significant univariate effect of group on left DG-CA4 volume $F(2,71) = 3.8$, $p = 0.027$, and on left CA2-3 volume $F(2,71) = 3.5$, $p = 0.036$ (Fig. 1). Planned contrasts revealed that Ath-mTBI had significantly smaller DG-CA4 than HC ($p = 0.009$; 8% smaller) and that Ath had a trend for smaller DG-CA4 than HC ($p = 0.056$, 6% smaller). There was no difference in left DG-CA4 volumes between athletes with and without a history of concussion ($p = 0.55$). Similarly, planned contrasts for the CA2-3 subfield revealed that both Ath and Ath-mTBI had significantly smaller volumes than HC ($p = 0.014$, 8% smaller and $p = 0.049$, 6% smaller, respectively). Again, there were no differences between Ath and Ath-mTBI ($p = 0.72$). Univariate analyses were performed for other left hemisphere subfields because the MANCOVA was

TABLE 2. MEAN, STANDARD DEVIATION, AND THE NUMBER OF DATA POINTS FOR HIPPOCAMPAL SUBFIELD VOLUMES, AND KYNURENINE METABOLITE CONCENTRATIONS

Hippocampal subfield	HC (n=27)	Ath (n=24)	Ath-mTBI (n=25)
DG-CA4			
Left ^a	577.2 (64.3)	542.2 (45.7)	530.0 (65.3)
Right	578.2 (64.5)	561.8 (45.3)	546.5 (75.6)
CA2-3			
Left ^a	1010.5 (110.8)	949.7 (88.4)	933.4 (113.3)
Right	1026.6 (126.7)	994.1 (95.3)	971.1 (138.2)
CA1			
Left	334.8 (40.6)	334.0 (43.7)	327.5 (38.1)
Right	341.3 (36.8)	337.7 (40.8)	338.3 (40.1)
Subiculum			
Left	670.9 (66.3)	644.9 (67.7)	632.2 (54.9)
Right	665.9 (70.2)	646.7 (55.2)	630.1 (64.4)
Presubiculum			
Left	524.4 (49.2)	499.9 (50.7)	493.4 (66.1)
Right	511.3 (61.6)	494.0 (50.0)	481.8 (41.5)
Total hippocampal volume ^b			
Left	3629.2 (324.0)	3458.8 (296.9)	3395.0 (319.6)
Right	3643.9 (339.4)	3566.0 (294.4)	3471.5 (347.0)
Metabolite	HC (n=21)	Ath (n=20)	Ath-mTBI (n=20)
KYN (μ M) ^a	1.9 (0.3)	1.7 (0.3)	1.7 (0.4)
3HK (nM)	31.2 (8.8)	33.2 (6.9)	36.3 (12.6)
QUIN (nM) ^c	334.1 (72.4)	304.5 (68.4)	368.5 (121.3)
KYNA (nM)	43.4 (14.2)	47.6 (11.3)	48.7 (20.2)
KYNA/3HK	1.4 (0.3)	1.5 (0.3)	1.4 (0.3)
KYNA/QUIN	0.13 (0.04)	0.16 (0.03)	0.14 (0.04)

HC, health control; Ath, athlete without concussion history; Ath-mTBI, athlete with concussion history; DG-CA, dentate gyrus-cornu Ammonis; KYN, kynurenine; 3HK, 3-hydroxykynurenine; QUIN, quinolinic acid; KYNA, kynurenic acid; KYNA/3HK, ratio of kynurenic acid to 3-hydroxykynurenine; KYNA/QUIN, ratio of kynurenic acid to quinolinic acid.

^a $p < 0.05$; ^btotal volume calculation includes volumes for the fimbria, hippocampal fissure, and the nonspecific hippocampal tail subfields; ^c $p < 0.10$.

trending toward significance. There was no significant univariate effect of group, however, for left CA1 $F(2,71) = 0.096$, $p = 0.91$, left pre-subiculum $F(2,71) = 1.48$, $p = 0.23$, or left subiculum $F(2,71) = 1.95$, $p = 0.15$.

There was no multivariate effect of group on right hippocampal subfield volumes (Wilk Lambda = 0.86, $F(10,134) = 1.06$, $p = 0.40$). Likewise, contrary to our hypothesis, there was no significant effect of group on right DG-CA4 volume, $F(2,71) = 1.0$, $p = 0.37$, or right CA2-3 volume, $F(2,71) = 0.73$, $p = 0.49$. Both the right CA2-3 and DG-CA4 subfield volumes, however, were 5% smaller in Ath-mTBI and 3% smaller in Ath compared with HC (Fig. 1).

KYN metabolites analyses

The total number of participants included in the metabolite analyses as well as the mean concentration and SD are presented in Table 2. The multivariate effect of group on KYN pathway measures showed a trend toward significance (Wilk Lambda = 0.68, $F(12,104) = 1.83$, $p = 0.052$). Specifically, there was a significant univariate effect of group on KYN $F(2,57) = 3.2$, $p = 0.048$ (Fig. 2). Planned contrasts showed that HC had a trend for higher levels of

KYN than Ath-mTBI ($p = 0.072$) and significantly higher levels than Ath ($p = 0.017$). Ath and Ath-mTBI did not differ in KYN concentration ($p = 0.43$).

The univariate effect of group on QUIN concentrations was trending toward significance $F(2,57) = 2.41$, $p = 0.099$ (Fig. 2). Planned contrasts demonstrated that HC did not differ from athletes with ($p = 0.24$) or without ($p = 0.36$) a history of concussion. Ath-mTBI had a significantly higher concentration of QUIN, however, compared with Ath ($p = 0.034$). There were no group differences in 3HK $F(2,57) = 1.26$, $p = 0.29$, KYNA $F(2,57) = 0.46$, $p = 0.63$, KYNA/3HK $F(2,57) = 0.49$, $p = 0.62$, or KYNA/QUIN $F(2,57) = 2.20$, $p = 0.12$.

A priori correlation analyses

The relationship between KYN metabolites and hippocampal volumes was assessed separately within each group. Spearman correlations for all *a priori* comparisons are listed in Supplementary Table 1 (see online supplementary material at ftp.liebertpub.com). In Ath-mTBI, 3HK concentration was significantly inversely correlated with both left total hippocampal volume ($\rho_{20} = -0.61$, $r = -0.57$, $p = 0.005$) and right total hippocampal volume ($\rho_{20} = -0.57$, $r = -0.60$, $p = 0.009$; Fig. 3A). Follow-up correlations of 3HK with each subfield separately confirmed that the strongest correlations were between 3HK and DG-CA4 and CA2-3 (Supplementary Table 2; see online supplementary material at ftp.liebertpub.com). In Ath, the ratio of KYNA over QUIN positively correlated with total left hippocampal volume ($\rho_{20} = 0.50$, $r = 0.32$, $p = 0.028$; Fig. 3B). Follow-up correlations showed that KYNA/QUIN was positively correlated with all left hemisphere hippocampal subfields and most strongly with the left DG-CA4 (Supplementary Table 2; see online supplementary material at ftp.liebertpub.com). There were no significant correlations between KYN metabolites and hippocampal volume in HC ($p > 0.10$; Supplementary Table 1; see online supplementary material at ftp.liebertpub.com).

Exploratory correlation analyses

Additional exploratory correlations were performed to determine the relationship between the primary findings of the current study and areas of thinner cortex (i.e., left ventromedial pre-frontal cortex [PFC] and right motor cortex) previously published from the same sample.²⁰ There was no significant relationship between KYN metabolites and cortical thickness in either football athlete groups (p 's > 0.05). The ratio of KYNA/3HK, however, was correlated with cortical thickness in the left ventromedial PFC in HC ($\rho_{21} = 0.48$, $r = 0.39$, $p = 0.026$; Fig. 3C). Further, total left and right hippocampal volume did not correlate with cortical thickness in either region for Ath or Ath-mTBI (p 's > 0.05), although left hippocampal volume was significantly correlated with thickness in both the right motor cortex and the left ventromedial PFC in HC ($\rho_{27} = 0.42$, $r = 0.42$, $p = 0.03$ and $\rho_{27} = 0.42$, $r = 0.43$, $p = 0.03$).

Discussion

The main finding of the current study is that collegiate-aged football Ath and Ath-mTBI have smaller DG and CA2-3 volumes in the left hemisphere compared with HC. To our knowledge, this is the first demonstration of volume differences in specific hippocampal subfields associated with football exposure in young adults. Football players with and without a history of concussion also had smaller volumes in the right hemisphere than HC; however, these differences did not reach statistical significance. These findings are

FIG. 1. Cornu Ammonis (CA)2-3 and dentate gyrus-CA4 volumes. Scatter plots show subfield volumes for each participant in each group. The group mean is demonstrated as a horizontal line in the scatter plot. Significant or trending toward significant planned comparisons are indicated by ^ at $p < 0.10$ and * at $p < 0.05$. HC, healthy control; Ath, athlete without concussion history; Ath-mTBI, athlete with concussion history.

consistent with our previous finding of smaller total hippocampal volumes in a slightly different cohort of collegiate-aged football Ath and Ath-mTBI.¹

These results are consistent with animal models of TBI that consistently demonstrate neuronal loss in CA3 and DG of the hippocampus.^{14–16,19} Smaller CA2-3 and dentate gyrus volumes have also been documented in several other human populations and disorders, including borderline personality disorder,²⁴ post-traumatic stress disorder,²⁶ and in adults with a history of childhood maltreatment.²⁵

We observed smaller hippocampal subfield volumes in athletes with and without a previous concussion diagnosis. It is likely that at least some of the athletes without a previously diagnosed concussion did actually have a concussion at some time during their football career, because athletes regularly underreport concussions.²⁷ It is also possible that repeated subconcussive head hits could also lead to hippocampal volume loss. For example, non-concussive head impact exposure has recently been correlated with

measures of white matter, including hippocampal white matter.²⁸ The volume differences observed here, however, were larger for athletes who reported a previously diagnosed concussion, suggesting that previous concussions are a potential risk factor for smaller hippocampal subfield volumes.

Although both left and right CA2-3 and DG-CA4 volumes were smaller in Ath and Ath-mTBI relative to HC, it is interesting that only the difference in the left hemisphere reached significance. We have previously reported reduced bilateral total hippocampal volumes in Ath and Ath-mTBI.¹ In the previous study, however, total hippocampal volumes were estimated using manually edited hippocampal masks. Here we used a fully automated method of estimating hippocampal subfield volumes.²² Moreover, this study included a slightly different cohort than our previous study. In addition, there was also a main effect of age between the groups in the present study. It is possible that this could also explain the differences between this study and our previous study.

FIG. 2. Quinolinic acid (QUIN) and kynurenine (KYN) levels. Scatter plots show QUIN and KYN levels for each participant in each group. The group mean is demonstrated as a horizontal line in the scatter plot. Significant or trending toward significant planned comparisons are indicated by ^ at $p < 0.10$ and * at $p < 0.05$. HC, healthy control; Ath, athlete without concussion history; Ath-mTBI, athlete with concussion history.

FIG. 3. Structural correlations with kynurenine metabolites. (A) *A priori* correlation analyses demonstrated that 3 hydroxykynurenine (HK) concentrations were inversely correlated with left and right hippocampal volume in athletes with a history of concussion (Ath-mTBI; squares). (B) The ratio of kynurenic acid (KYNA) over quinolinic acid (QUIN) was positively correlated with left hippocampal volume in athletes without a history of concussion (Ath; circles). (C) The ratio of KYNA over 3HK was positively correlated with cortical thickness in the left ventromedial pre-frontal cortex (PFC) in healthy controls (HC; triangles).

Lateralization of hippocampal volume differences, however, has been observed in other disorders. For example, volume differences in the left hippocampus have been observed in schizophrenia²⁹ and in adults with a history of childhood trauma.^{25,30} Intriguingly, our observation of left lateralized volume differences is also consistent with work in animals and tissue culture experiments on NMDA receptor subunits. NMDA receptors that contain NR2B subunits are more densely expressed in the left hippocampus than in the right,^{31,32} and NMDA-mediated excitotoxicity is dependent on NMDA receptors containing NR2B subunits.³³ Therefore, the left hippocampus may be more susceptible to excitotoxicity-induced injury.

Our results partially supported our hypothesis that KYN pathway metabolite concentration in blood would significantly differ in football athletes relative to HC. Interestingly, KYN levels were higher in HC relative to both athlete groups. KYN is the primary metabolite of tryptophan and could indicate the overall activity of the KYN pathway.⁵ A previous study found increased peripheral levels of KYN relative to tryptophan in patients with TBI compared with HC.¹² Tryptophan levels are not available for the current sample, and thus we cannot compare the relative overall activity of the KYN pathway. Several factors, however, could potentially explain the lower levels of KYN in athletes, including their carefully monitored diet, body composition, and consistent exposure to physical exercise. For example, there were differences in BMI across our groups, with both football groups having higher BMI than HC. Previous studies, however, have demonstrated that BMI is a misleading measure in football athletes,^{34,35} and more accurate measures of adiposity, such as percent body fat, are not available in this current sample. Future studies are needed to assess the relationship of visceral adiposity on KYN pathway metabolites in football athletes. In addition, a recent study demonstrated that exercise increases the metabolism of KYN to KYNA,³⁶ which could potentially explain the higher KYN levels observed in the control group observed here, because the majority of these participants were nonathletes.

Importantly, our observation of elevated levels of QUIN in Ath-mTBI relative to Ath is consistent with work in severe TBI. For example, patients with severe TBI have elevated levels of QUIN in cerebral spinal fluid.^{10,11} We have also recently reported increased plasma levels of QUIN and decreased ratios of KYNA/QUIN in football athletes at 1 day, 1 week, and 1 month post-concussion relative to healthy football athletes.³⁷ Our results raise the possibility that QUIN could potentially serve as a biomarker for a history of sports-related concussion, although future studies are needed to test this hypothesis.

Our third novel finding was that putatively neurotoxic and neuroprotective KYN pathway metabolites had group-specific relationships with hippocampal volume. In Ath-mTBI, there was a strong, inverse relationship between 3HK and bilateral hippocampus, with the strongest relationship between 3HK and DG-CA4 and CA2-3. 3HK is a putative neurotoxic metabolite found in the periphery and CNS that can cause oxidative damage and potentially cell death.⁶ Increased brain levels of 3HK have been observed postmortem and in animal models of other neurological and psychiatric disorders, including Huntington's disease and depression.³⁸

Although we were only able to measure peripheral levels of KYN pathway metabolites, 3HK can readily cross the blood-brain barrier along with tryptophan and KYN.³⁹ In the brain, 3HK is metabolized into QUIN in microglia and infiltrating peripheral macrophages, which can lead to excitotoxicity on release.^{7,40} In addition, the combination of QUIN and 3HK can have a synergistic effect on neurodegeneration.⁴¹ Interestingly, a previous study on adolescents with melancholic MDD found a positive correlation between 3-hydroxyanthranilic acid in plasma, a neurotoxic intermediate of the kynurenine pathway, and putamen choline levels, indicative of cell membrane turnover (e.g., from demyelination), which suggests that peripheral levels of kynurenine metabolites are associated with changes in the CNS.⁴²

It is important to note that we are not able to demonstrate neurodegeneration; however, the inverse correlation between hippocampal volume and 3HK levels is consistent with the “immunoexcitotoxic” hypothesis of TBI.⁴³ According to this hypothesis, an initial injury results in primed microglia that are hyperreactive to additional injuries. On addition insult, such as a head hit or even systemic infection, primed microglia release large amounts of oxidative stressors, proinflammatory cytokines, and excitotoxins including glutamate and QUIN than can potentially lead to NMDA-mediated neuronal excitotoxicity.^{5,43}

In contrast to what was observed in football athletes *with* a history of diagnosed concussion, we found that a putatively neuroprotective ratio of KYN pathway metabolites correlated positively with left hippocampal volume, specifically the DG-CA4, in Ath. Higher levels of KYNA relative to QUIN may theoretically be neuroprotective.^{2,3} KYNA is a glutamate receptor antagonist that can prevent excitotoxicity⁴⁴ and protect against oxidative stress.⁴⁵ KYNA has also shown to be neuroprotective in rodent models of TBI. For example, direct administration of KYNA to the hippocampus 30 min before experimental TBI reduces microglia and astrocyte response.⁴⁶ As with 3HK, it is not known whether the plasma levels measured here reflect brain levels of KYNA, particularly because KYNA cannot readily cross the blood–brain barrier.³⁹ Intravenous injection of KYNA after experimental TBI, however, was found to attenuate post-injury cognitive dysfunction, reduce edema in the hippocampus, and attenuate neuronal loss in CA3 observed at 2 weeks post-injury.^{47, 48} Thus, peripheral levels of KYNA can affect the CNS.

Finally, exploratory analyses demonstrated that KYN metabolites did not correlate with cortical thickness in regions in which football Ath-mTBI had thinner cortex, specifically the left ventromedial PFC and right motor cortex, for either football group.²⁰ A positive correlation between left ventromedial PFC thickness and KYNA/3HK was observed, however, in HC, and left hippocampal volume was significantly correlated with cortical thickness in both regions in HC but not in either football athlete group. The positive relationship between KYNA/3HK and left ventromedial PFC in HC is consistent with the hypothesis that KYNA/3HK is a neuroprotective measure given the respective actions of KYNA and 3HK in the CNS.^{2,3} Further, a post-mortem analysis of MDD and bipolar patients found an increased level of QUIN-positive cells in the anterior cingulate cortex, suggesting that KYN pathway abnormalities may also affect the ventromedial PFC.⁴⁹ Why the current study only found a relationship in HC is unclear.

Nevertheless, it is likely that individual differences in the KYN pathway affect other regions in Ath and Ath-mTBI athletes not hypothesized in this article. For example, we have observed relationships between KYN levels, as well as the ratio of KYN to tryptophan, and striatal volume in patients with MDD.⁵⁰ Additional studies are needed to determine whether individual differences in cortical thickness, as well as other neuroanatomical measures, are associated with differences in the KYN pathway in Ath and Ath-mTBI.

Limitations

First, there was a significant main effect of age between our groups. Although age was used as a covariate in all analyses in which comparisons across groups were performed, a nonlinear age effect could conceivably explain why we only observed a unilateral group difference in this sample. Second, we did not exclude for a history of attention deficit hyperactivity disorder in the current

study (ADHD), and three Ath-mTBI did self-report ADHD. Removal of these subjects, however, had no effect on the observed findings (see Supplementary information; see online supplementary material at ftp.liebertpub.com).

In addition, the large number of correlations between KYN metabolites and hippocampal volume could have resulted in false positives. Strict family-wise error correction, however, would likely be overly conservative because of the nonindependence of many of the investigated measures. Further, the concentration of KYN pathway metabolites was evaluated in the periphery, and we are not able to determine whether the peripheral levels reflect concentration levels in the brain. Nevertheless, data from the psychiatric literature suggest that peripheral measures of KYN metabolic pathway metabolites may be valid indicators of CNS KYN pathway metabolism. In two separate studies, higher levels of QUIN were found in the serum³ and cerebral spinal fluid⁵¹ of depressed patients relative to HC.

In the present study, there was a difference between groups in the timing of plasma freezing. Supplementary analyses, however, demonstrated no significant differences between samples stored immediately at -80°C and those stored at 4°C overnight and then frozen at -80°C .

Finally, although we discuss our results in the context of a mechanism of TBI-related neurodegeneration, we present only correlational evidence in a cross-sectional study design. Thus, the current observations could pre-date football exposure, or be explained by a variety of other factors not investigated, such as genetic predisposition, environment, neurodevelopmental history, and non-TBI injury history.

Summary

We observed that collegiate-age football athletes with and without a history of concussion have smaller CA2-3 and DG-CA4 hippocampal subfield volumes in the left hemisphere compared with HC. In addition, plasma levels of QUIN were higher in Ath-mTBI compared with Ath, converging with findings in cerebral spinal fluid of patients with severe TBI. We also observed group-specific correlations between KYN pathway metabolites and hippocampal subfield volumes. For football athletes with a history of concussion, the plasma levels of the putative neurotoxic metabolite 3HK inversely correlated with bilateral hippocampal volume, particularly the DG-CA4 and CA2-3. For football athletes without a history of concussion, left DG-CA4 volume positively correlated with KYNA/QUIN. These results suggest that the interaction between head injury exposure and individual differences in KYN pathway metabolism may provide a model for structural changes observed in subsets of otherwise healthy athletes.

Additional well-controlled, longitudinal studies in large samples across a variety of collision sports are needed to elucidate the effects of collision sport history on hippocampal structure, function, and behavior, and the potential mechanisms behind these changes. Further, given the similarity of our results to those previously described in other psychiatric disorders, the association between KYN pathway abnormalities and disorders that are often associated with TBI, such as MDD and PTSD, needs to be elucidated.

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Author Disclosure Statement

No competing financial interests exist.

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