Altered systemic inflammatory profiles in athletes with a history of concussion.

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ABSTRACT

Importance: The long-term health effects of concussion are unknown. Inflammation is pivotal to secondary injury processes and the etiology of neurodegenerative diseases. We characterized circulating inflammatory mediators in healthy male and female athletes with and without a history of concussion.

Methods: Blood samples from 53 healthy athletes (males, n = 29; females, n = 24) with (n = 28) and without (n = 25) previous concussion history were assessed for 29 inflammatory markers and 4 neuroinjury markers by immunoassay.

Results: Numerous chemokine concentrations were elevated in athletes with a history of concussion versus no history of concussion: monocyte chemoattractant protein (MCP)-4 ($p = 0.003$); thymus- and activation-regulated chemokine ($p = 0.001$); interferon gamma induced protein -10 ($p < 0.001$), interleukin-8 ($p = 0.019$), MCP-1 ($p = 0.006$), macrophage-derived chemokine ($p = 0.039$), and macrophage inflammatory protein (MIP)-1α ($p < 0.05$). Brain derived neurotrophic factor (BDNF) was also elevated ($p = 0.016$). A positive correlation was found between number of previous concussions and both TARC ($r = 0.45$, $p = 0.001$) and IP-10 ($R = 0.44$, $p = 0.001$). An inverse correlation was found between days from the last concussion and both IP-10 ($r = -0.45$, $p = 0.041$) and MIP-1β ($r = -0.49$, $p = 0.020$).

Interpretation: Athletes with concussion history displayed elevated systemic chemokine concentrations versus athletes without concussion history. Chemokines were associated with the number of concussions and time from last concussion. Further research is needed to investigate the pathophysiology of systemic inflammation chronically after concussion.

Introduction

With concern about the potential deficits produced by concussion, there has been an increased demand to delineate the pathophysiological mechanisms mediating long-term outcomes.[1] Presently, our conceptual understanding of the pathophysiology underlying concussion consists of an acute disturbance of neurobehavioral function together with damage to neuronal and glial cells.[2] Most often, symptoms are short-lived, resolving within days to weeks;[3-6] however, more recent objective neuroimaging and biomarker data have documented underlying functional and structural abnormalities which persist beyond symptom resolution.[7-9] How these changes are related to long-term health outcomes or risk for future concussion is poorly understood.

It is now recognized that secondary injury after neurotrauma is initiated by both local central nervous system (CNS) and systemic inflammation.[10,11] Until recently, inflammation has been overlooked in the pathogenesis of concussive injury (or mild traumatic brain injury; mTBI),[12] but more recent studies indicate inflammatory processes such as microglial activation and peripheral immune cell recruitment also occur in mTBI.[13,14] These processes play an important role in aiding the repair and regeneration of damaged brain cells.[15] However, when dysregulated, inflammation may also exacerbate tissue injury,[12,16] and is currently recognized as an integral component to numerous neurodegenerative diseases.[17] In view of this, experimental animal models of TBI have provided strong evidence supporting acute neuroinflammation post-injury that may persist for months or possibly years.[18,19] While the sequelae of inflammation following TBI in humans is less clear,[20] microglial activation has been identified up to 18 years after a single TBI.[21,22]

Inflammation post-concussion has been difficult to characterize due to practical limitations such as the inability to access tissue proximal to the site of injury, and the invasive nature of cerebral spinal fluid (CSF) acquisition. Nevertheless, peripheral blood samples have the potential to provide meaningful pathophysiological information between the CNS and periphery in response to brain injury, encompassing processes such as neuroinflammation and brain tissue injury in a relatively cost-effective, non-invasive manner.[23] In view of this, recent evidence has shown that increased circulating C-reactive protein levels post-injury may predict persistent post-concussive syndrome symptoms,[24] and coated platelet levels, which serve as an indirect inflammatory correlate, are elevated in mild TBI patients up to 9 years post-injury.[25] Yet, research examining the systemic inflammatory response in human concussion is limited, particularly concerning the role of key inflammatory mediators such as cytokines and chemokines.

Historically, there has been a lack of concussion research on females.[26] Despite this, available evidence suggests that females may be at a greater risk for concussion,[27,28] report more symptoms post-concussion,[27,29] and take longer to recover.[27,30] Such evidence is aligned with severe TBI research which has shown that women may sustain worse outcomes.[31] In addition, it is known that males and females show distinct immunological responses; women exhibit stronger cellular and humoral immune responses, are more prone to many autoimmune diseases, but are less susceptible to various of bacterial, viral, and fungal infections.[32,33] However, the relationship between concussion and the inflammatory response in relation to potential sex differences has not been investigated.

Thus, in this study we set out to examine a panel of systemic inflammatory cytokines and chemokines in a sample of male and female athletes, in order to characterize the relationship between previous concussions and peripheral inflammation.

2. METHODS

2.1 Participants

Participants were recruited from University of Toronto intercollegiate athletic "varsity" teams. A research team member provided an overview of the research project and requested consent to obtain blood samples and use the Sport Concussion Assessment Tool (SCAT) results for research purposes. Medical history was obtained by the team's therapist/trainer, followed by administration of the SCAT. Sixteen teams (8 male, 8 female) were contacted for research purposes, including the following sports: basketball, baseball, field hockey, football, ice hockey, lacrosse, rugby, soccer, wrestling and volleyball. Study procedures were approved by the university institutional review board (protocol reference # 27958), and all participants provided written informed consent prior to the study.

2.2 Measures

Sport Concussion Assessment Tool 3 (SCAT3): The SCAT3 combines aspects of several previously published concussion tools into eight components designed to assess concussion symptoms (number endorsed and severity), cognition (Sideline Assessment of Concussion or SAC and Maddocks questions), balance (firm conditions of the Balance Error Scoring System or BESS), Glasgow Coma Scale (GSC) and neurological signs (physical signs, coordination).[34] Each of the eight components are scored and recorded. The symptom score is comprised of a 22-item post-concussion symptom scale using a seven-point Likert scale rating. The symptom

severity is obtained by summing the rated symptom score for each symptom.[34] This symptom scale has been shown to be reliable and valid for the assessment of both symptom presence and severity.[29,34,35]

2.3 Blood Sample Collection

Venous blood samples were drawn from athletes after consent was obtained. Samples were drawn into a 10-mL K_2EDTA (with 4mM sodium metabisulfite [$Na_2S_2O_5$]) or 4-mL non-additive (Vacutainer, Becton Dickinson, NJ, USA) tube. After one hour, specimens were centrifuged at 1600 x *g* for 15 minutes at 4°C, the plasma supernatant was aliquoted and frozen at -70°C until analysis.

2.4 Biomarker Analysis

Thirty of thirty-three markers were analyzed using a Meso-Scale Discovery (MSD®) Sector imager™ 6000 with Discovery Workbench software (version 3.0.18), using MSD® 96-Well MULTI-ARRAY/-SPOT® V-plex Human Immunoassay Kits purchased from MSD (MD, USA) (see Table 1 for complete list of markers analyzed). Three additional neuroinjury markers were assessed using commercial ELISA technology; Glial fibrillary acidic protein (GFAP; EMD Millipore, HE, Germany), s100 calcium-binding protein B (s100B; EMD Millipore, HE, Germany), neuron specific enolase (NSE; R & D system, MPLS, USA). All assays were performed according to manufacturer's instructions, in duplicate, and without alterations to the recommended standard curve dilutions.

2.5 Statistical Analyses

A two-way analysis of variance (ANOVA) with between group factors - previous history of concussion (yes vs no) and sex (male vs female), was conducted on blood biomarker concentrations. Significant main effects and interactions were considered at $P < .05$ (2-tailed).

We reported the absolute adjusted differences with 95% confidence interval for both history of concussion and sex. In cases where the interaction between history and concussion and sex was significant, a Tukey's multiple comparisons test was employed to identify specific differences. To evaluate the relationship between inflammatory marker concentrations and number of previous concussions, a spearmans' rank correlation coefficient (rho) was calculated. To assess the relationship between inflammatory marker concentrations and the time from the last concussion, a Spearman's rho or Pearsons' product-moment correlation coefficient was calculated, where appropriate. All data were analyzed using GraphPad Prism Version 6.0f (GraphPad Inc., CA, USA) and Stata Version 13.1 (StataCorp, TX, USA).

[Insert Table 1 Here]

3. Results

3.1 Demographics and Clinical Characteristics

Physical characteristics and concussion histories of the two groups are listed in **Table 1**. The mean age for all athletes (n = 62) was 20.3 years (SD = 1.8; range 17-24) with no differences in age between groups (difference, -.86 [95% CI, −2.07 to .34]; $P = .15$). There was an equal representation of male and female athletes in both groups with no differences in reporting of history of migraines, learning disabilities, anxiety/depression/psychiatric disorders, or family history of psychiatric illness. Athletes reported a total score 3.1 (SD = 3.7) on the SCAT3, with no significant differences between groups of athletes with or without previous concussion history (difference, -.1.67 [95% CI, −4.09 to 0.39]; $P = .10$; table 1). In addition, symptom severity scores did not differ between groups (difference, -3.31 [95% CI, −7.52 to 0.90]; $P = .12$; **Table 1**).

3.2 Systemic inflammatory marker analysis

[Insert Table 2 Here]

3.2.1 Previous concussion history

Of the 62 athletes enrolled, 4 athletes were excluded due to the presence of seasonal allergies, and 5 athletes were excluded due to prescription drug use. Fifty-three patients remained for blood analysis (males, $n = 29$; females, $n = 24$). Mean concentrations of inflammatory markers stratified by concussion history are displayed in **Table 2**. Eight markers were significantly elevated in the peripheral blood of healthy athletes with a history of concussion vs. those without a history of concussion (**Table 2 & Figure 1**). BDNF was the only neurological injury marker elevated in athletes with a history of concussion (difference, 990.2 [95% CI, 191.6 – 1788.7]; $p = 0.016$, $\omega^2 = 0.10$) (**Table 2 & Figure 1, Panel A**). The remaining 7 markers elevated in healthy athletes with a history of concussion were chemokines. The greatest absolute difference in chemokine concentrations between healthy athletes with a history of concussion vs. those without was seen in MCP-4; levels were over 1.5 fold higher in athletes with a history of concussion (difference 14.3 [95% CI, 5.2 – 23.4]; $p = 0.003$, $\omega^2 = 0.12$) (**Table 2 & Figure 1, Panel E**). However the largest effect size was seen in TARC – healthy athletes with a history of concussion had levels approximately 1.5-fold higher than healthy athletes without a history of concussion (difference 17.1 [95% CI, 7.8 – 26.4]; $p = 0.001$, $\omega^2 = 0.22$) (**Table 2 and Figure 1, Panel H**). Additionally, healthy athletes without a history of concussion displayed elevations in IP-10 (difference, 72.9 [95% CI, 34.2 – 111.7]; $p < 0.001$, $\omega^2 = 0.20$), IL-8 (difference, 0.65 [95% CI, 0.11 – 1.2]; $p = 0.019$, $\omega^2 = 0.08$), MCP-1 (difference, 14.2 [95% CI, 4.2 – 24.1]; $p = 0.006$, $\omega^2 = 0.11$), MDC (difference, 117.3 [95% CI, 6.3 – 228.4]; $p = 0.039$, $\omega^2 = 0.06$), and

MIP-1α (difference, 1.8 [95% CI, 0.03 – 3.5]; $p < 0.05$, $\omega^2 = 0.09$) (**Table 2 and Figure 1)**.

[Insert Figure 1 Here]

[Insert Table 3 Here]

3.2.2 Sex

Peripheral blood concentrations of inflammatory and neuroinjury markers in healthy athletes dichotomized by sex are displayed in **Table 3**. Compared with males, regardless of concussion history, females had lower levels of a number of inflammatory mediators; Cytokines, IL-16 (difference, -117.3 [95% CI, -170.9 – -63.8]; $p < 0.001$, $\omega^2 = 0.27$) and TNF-α (difference, -0.3 [95% CI, -0.5 - -0.03]; $p = 0.03$, $\omega^2 = 0.08$) ; Chemokines, Eotaxin (difference, -21.5 [95% CI, -35.8 – -7.1]; $p = 0.004$, $\omega^2 = 0.13$), IP-10 (difference, -40.7 [95%CI, -79.4 – -2.0]; $p = 0.04$, $\omega^2 = 0.05$), IL-8 (difference, -0.7 [95% CI, -1.2 – -1.8]; $p = 0.010$, $\omega^2 = 0.1$), MCP-1 (difference, -11.4 [95% CI, -21.4 – -1.4]; $p = 0.026$, $\omega^2 = 0.07$) , and MCP-4 (difference, -19.0 [95% CI , -28.1 – -9.9]; $p < 0.001$, $\omega^2 = 0.22$) **(Table 3)**. Additionally, s100B levels were 1.5-fold higher in healthy female athletes vs. healthy male athletes (difference, 6.1 [95% CI, 2.8 – 9.3]; $p < 0.001$, $\omega^2 = 0.22$) **(Table 3)**.

An interaction was found between sex and history of concussion for IL-8 and MIP-1α (**Figure 2, Panels A & B, respectively**). Post hoc analysis revealed that healthy males with a history of concussion had significantly higher IL-8 levels compared to healthy males with no previous concussion history (difference, 1.2 [95% CI, 0.2 – 2.2]; $p = 0.012$); there were no significant differences in IL-8 concentrations between healthy female athletes with and without a history of concussion (**Figure 2, Panel A**). However, no specific differences between history of concussion and sex were identified for MIP-1α (**Figure 2, Panel B**)

[Insert Figure 2 Here]

3.2.3 Correlational Analysis

Associations were identified between systemic inflammatory chemokines and both number of previous concussions and days from last concussion (**Table 4).** A significant positive correlation was found between the number of previous concussions sustained by the athletes (range = 0-7) and concentrations of TARC ($r = 0.45$, $p = 0.001$) and IP-10 ($r = 0.44$, $p = 0.001$). (**Table 4).** In addition, an inverse correlation was found between the days from the last concussion (range = 279 – 2335) and IP-10 ($r = -0.45$, $p = 0.041$) and MIP-1β ($r = -0.49$, $p = 0.020$) concentrations (**Table 4**).

[Insert Table 4 Here]

4. Discussion

In the present study we identified differences in peripheral markers of inflammation between healthy athletes with and without a previous history of concussion. We found multiple circulating chemokines positively correlated with the number of previous concussion sustained, and inversely correlated with the time elapsed from the last concussion. Furthermore, we identified differences in systemic inflammatory markers between male and female athletes, with differential responses between sexes according to previous concussions.

The athletes in our study displayed symptom scores similar to previously reported values in healthy athletic populations.[36,37] However, our results indicate that individuals with a history of concussion manifest a systemic inflammatory profile that is characterized by elevations in markers with chemoattractant properties. The two most pertinent questions stemming from these findings are: (1) what are the possible biological mechanisms governing systemic chemokine

alterations in relation to concussion? (2) what are the clinical implications of these findings on patient health?

It is possible that chemokines may enter the systemic circulation from the site of injury in the CNS. Mechanical trauma and subsequent damage of brain tissue initiates local neuroinflammation.[15,38] Activated microglia release cytokines and chemokines, which may then travel into the systemic circulation across a damaged BBB, and subsequently recruit peripheral leukocytes to the injured brain tissue.[15,38] Furthermore, cell fragments and other markers of injury, termed DAMPs (damage associated molecular patterns) are also released from the CNS into the periphery where they may become immunogenic and cause subsequent systemic cytokine/chemokine release through their interactions with peripheral leukocytes.[39,40] While the presence of DAMPs such as s100B may be elevated for months after mTBI in humans,[41-43] that we did not find differences in s100B molecules between athletes with and without a history of concussion suggests that it may not be the primary mediator of chronic inflammation after concussion. However, GFAP, a DAMP by definition, but not previously examined with respect to its immunogenic role in the peripheral circulation, was detectable in 68% of samples from previously concussed athletes, as compared with 39.3% of samples from athletes with no prior concussions (Supplement 1). Hence, further research on the involvement on a multitude of DAMP molecules in chronic inflammation after concussion is warranted.

Alternatively, it is conceivable that individuals with a history of concussion may have altered sympathetic nervous system (SNS) function. SNS activation results in the release of norepinephrine and epinephrine into the circulation, a process previously shown to occur acutely following moderate and severe TBI.[44,45] This hyperadrenergic state can mediate inflammation through sympathetic terminals in innervated tissues, and also via receptor-mediated interactions

between catecholamines and peripheral leukocytes.[46,47] While the current study did not assess adrenergic function, our group and others have previously identified autonomic dysfunction post concussion with measures of heart rate variability.[48-50] Undoubtedly, this correlative evidence requires further investigation, though it is supportive of the general hypothesis that concussion may induce chronic alterations to sympathetic tone, and this may alter systemic inflammation.[51]

It is difficult to speculate on the clinical implications of the present findings, in part due to pleiotropic properties of chemokines. In pathological conditions, chemokines are primarily involved in coordinating leukocyte recruitment to injured tissues.[52,53] Animal models of TBI have implicated chemokines in the recruitment of macrophages and neutrophils to the CNS, and inhibition of this process has been associated with improved recovery and cognitive function.[54,55] Furthermore, it has been suggested that BBB breakdown after TBI is largely mediated by chemokines,[38] and the BBB may be damaged for months after concussion.[56] However, chemokines may also aid in neuronal repair, mediating regenerative processes such as axonal sprouting.[57,58] Indeed, we found an inverse correlation between systemic chemokine levels and days from last concussion, possibly reflective of ongoing recovery. This is further supported by our findings of elevated BDNF levels in athletes with a history of concussion; BDNF is the most abundant neurotrophin found in the brain, promoting neuronal health and survival. Our group previously showed elevated BDNF acutely after moderate and severe TBI,[42] and decreased BDNF levels were associated with patient mortality.[59] Our findings provide justification for further mechanistic experimentation to identify the underlying consequences of an altered systemic inflammatory profile chronically after concussion.

We found higher levels of inflammatory markers were positively associated with the number of reported previous concussions, supportive of previous studies suggesting multiple

concussions may have deleterious health effects.[60-62] In view of this, it has been speculated that resident microglial cells may be "primed" by an initial injury, resulting in an exaggerated inflammatory response to subsequent insults,[63] although it is still unclear how the systemic inflammatory response may react to repeated concussive injuries.

We observed significantly lower chemokine levels in females compared to males, regardless of concussion history, and sex-specific alterations in IL-8 and MIP-1α concentrations according to concussion history. While these novel findings are supportive of previous evidence regarding sex-specific responses following concussion,[3,5,27,30] the gap in sex-based inflammatory research in TBI prevents any speculation on their mechanistic interpretation. In addition, we found female athletes had higher s100B levels than males regardless of concussion history. While not related to concussion history directly, this finding is pertinent due to the prominence of s100B in concussion research. Previous studies addressing sex-specific differences in systemic s100B concentrations have been inconsistent,[64,65] possibly due to differences the biological fluids being measured (i.e., blood vs. CSF), and the analytical techniques employed. Yet, we did not find differences in s100B levels between athletes with and without a previous concussion history, and thus do not have evidence to implicate s100B in the chronic sequelae of concussion.

The current study was limited by a number of factors. A cross-sectional design limited the ability to evaluate pre-injury inflammatory marker levels in our athlete-cohort. Furthermore, a larger sample size would improve the strength of our findings, particularly for combined sex and concussion history stratification. Yet, despite these limitations, our results demonstrate systemic inflammatory alterations in athletes with previously diagnosed concussions, and provide evidence that a subset of these differences appear to be sex-specific.

5. Conclusion

Previous history of concussion is associated with alterations in systemic inflammatory biomarker concentrations in a sample of healthy athletes. Specifically, circulating chemokine levels are elevated in athletes with a history of concussion compared to athletes with no previous concussion history. Furthermore, peripheral chemokine concentrations are positively associated with the number of previous concussions sustained, and inversely associated with the time from the last concussion. In addition, systemic inflammatory profiles differ between male and female athletes, and sex-specific differences exist in the inflammatory profiles of athletes with a history of concussion vs. those with no previous concussion history. Future concussion studies should consider the role of systemic inflammatory mediators and sex when investigating the biological underpinnings of post-concussion recovery and long-term health outcomes.

Author Contributions

Dr. Hutchison and Mr. Di Battista had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Di Battista, Hutchison, Rhind, Baker, Richards.

Acquisition of data: Di Battista.

Analysis and interpretation of data: Di Battista, Hutchison, Rhind, Baker, Richards.

Drafting of the manuscript: Di Battista, Hutchison.

Critical revision of the manuscript for important intellectual content: Di Battista, Hutchison, Rhind, Baker, Richards.

Statistical analysis: Di Battista, Hutchison.

Administrative, technical or material support: Di Battista, Hutchison, Rhind, Baker, Richards.

Study supervision: Hutchison, Rhind, Baker, Richards.

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References

1. Blennow K, Hardy J, Zetterberg H. The neuropathology and neurobiology of traumatic brain injury. *Neuron*. 2012;76(5):886-899. doi:10.1016/j.neuron.2012.11.021.

2. Giza CC, Hovda DA. The new neurometabolic cascade of concussion. *Neurosurgery*. 2014;75 Suppl 4:S24-S33. doi:10.1227/NEU.0000000000000505.

3. Broglio SP, Cantu RC, Gioia GA, et al. National Athletic Trainers' Association position statement: management of sport concussion. *J Athl Train*. 2014;49(2):245-265. doi:10.4085/1062-6050-49.1.07.

4. McCrea M, Guskiewicz KM, Marshall SW, et al. Acute effects and recovery time following concussion in collegiate football players: the NCAA Concussion Study. *JAMA*. 2003;290(19):2556-2563. doi:10.1001/jama.290.19.2556.

5. McCrory P, Meeuwisse WH, Aubry M, et al. Consensus statement on concussion in sport: the 4th International Conference on Concussion in Sport held in Zurich, November 2012. In: Vol 47. 2013:250-258. doi:10.1136/bjsports-2013-092313.

6. Giza CC, Kutcher JS, Ashwal S, et al. Summary of evidence-based guideline update: evaluation and management of concussion in sports: report of the Guideline Development Subcommittee of the American Academy of Neurology. *Neurology*. 2013;80(24):2250-2257. doi:10.1212/WNL.0b013e31828d57dd.

7. Yuh EL, Hawryluk GWJ, Manley GT. Imaging concussion: a review. *Neurosurgery*. 2014;75 Suppl 4:S50-S63. doi:10.1227/NEU.0000000000000491.

8. Gosselin N, Saluja RS, Chen J-K, Bottari C, Johnston K, Ptito A. Brain functions after sports-related concussion: insights from event-related potentials and functional MRI. *Phys Sportsmed*. 2010;38(3):27-37. doi:10.3810/psm.2010.10.1805.

9. Murugavel M, Cubon V, Putukian M, et al. A longitudinal diffusion tensor imaging study assessing white matter fiber tracts after sports-related concussion. *J Neurotrauma*. 2014;31(22):1860-1871. doi:10.1089/neu.2014.3368.

10. Balu R. Inflammation and immune system activation after traumatic brain injury. *Curr Neurol Neurosci Rep*. 2014;14(10):484-484. doi:10.1007/s11910-014-0484-2.

11. Anthony DC, Couch Y. The systemic response to CNS injury. *Exp Neurol*. 2014;258:105-111. doi:10.1016/j.expneurol.2014.03.013.

12. Rathbone ATL, Tharmaradinam S, Jiang S, Rathbone MP, Kumbhare DA. A review of the neuro- and systemic inflammatory responses in post concussion symptoms: Introduction of the “post-inflammatory brain syndrome” PIBS. *Brain Behavior and Immunity*. 2015;46:1-16. doi:10.1016/j.bbi.2015.02.009.

13. Seifert T, Shipman V. The Pathophysiology of Sports Concussion. *Curr Pain Headache*

Rep. 2015;19(8):513. doi:10.1007/s11916-015-0513-0.

14. Karve IP, Taylor JM, Crack PJ. The contribution of astrocytes and microglia to traumatic brain injury. *British Journal of Pharmacology*. March 2015:n/a–n/a. doi:10.1111/bph.13125.

15. Finnie JW. Neuroinflammation: beneficial and detrimental effects after traumatic brain injury. *Inflammopharmacol*. January 2013. doi:10.1007/s10787-012-0164-2.

16. Gyoneva S, Ransohoff RM. Inflammatory reaction after traumatic brain injury: therapeutic potential of targeting cell–cell communication by chemokines. *Trends Pharmacol Sci*. 2015;36(7):471-480. doi:10.1016/j.tips.2015.04.003.

17. Faden AI, Wu J, Stoica BA, Loane DJ. Progressive inflammation-mediated neurodegeneration after traumatic brain or spinal cord injury. *British Journal of Pharmacology*. May 2015:n/a–n/a. doi:10.1111/bph.13179.

18. Nagamoto-Combs K, McNeal DW, Morecraft RJ, Combs CK. Prolonged microgliosis in the rhesus monkey central nervous system after traumatic brain injury. *J Neurotrauma*. 2007;24(11):1719-1742. doi:10.1089/neu.2007.0377.

19. Almeida-Suhett CP, Li Z, Marini AM, Braga MFM, Eiden LE. Temporal course of changes in gene expression suggests a cytokine-related mechanism for long-term hippocampal alteration after controlled cortical impact. *J Neurotrauma*. 2014;31(7):683-690. doi:10.1089/neu.2013.3029.

20. Sharp DJ, Jenkins PO. Concussion is confusing us all. *Pract Neurol*. 2015;15(3):172-186. doi:10.1136/practneurol-2015-001087.

21. Johnson VE, Stewart JE, Begbie FD, Trojanowski JQ, Smith DH, Stewart W. Inflammation and white matter degeneration persist for years after a single traumatic brain injury. *Brain*. 2013;136(Pt 1):28-42. doi:10.1093/brain/aws322.

22. Ramlackhansingh AF, Brooks DJ, Greenwood RJ, et al. Inflammation after trauma: microglial activation and traumatic brain injury. *Ann Neurol*. 2011;70(3):374-383. doi:10.1002/ana.22455.

23. Di Battista AP, Rhind SG, Baker AJ. Application of blood-based biomarkers in human mild traumatic brain injury. *Frontiers in Neurology*. 2013;4(44). doi:10.3389/fneur.2013.00044.

24. Su S-H, Xu W, Li M, et al. Elevated C-reactive protein levels may be a predictor of persistent unfavourable symptoms in patients with mild traumatic brain injury: A preliminary study. *Brain Behavior and Immunity*. 2014;38(C):111-117. doi:10.1016/j.bbi.2014.01.009.

25. Prodan CI, Vincent AS, Dale GL. Coated-platelet levels are persistently elevated in patients with mild traumatic brain injury. *J Head Trauma Rehabil*. 2014;29(6):522-526.

doi:10.1097/HTR.0000000000000010.

26. Comper P, Hutchison M, Magrys S, Mainwaring L, Richards D. Evaluating the methodological quality of sports neuropsychology concussion research: a systematic review. *Brain Inj*. 2010;24(11):1257-1271. doi:10.3109/02699052.2010.506854.

27. Dick RW. Is there a gender difference in concussion incidence and outcomes? *British Journal of Sports Medicine*. 2009;43 Suppl 1:i46-i50. doi:10.1136/bjsm.2009.058172.

28. Hootman JM, Dick R, Agel J. Epidemiology of collegiate injuries for 15 sports: summary and recommendations for injury prevention initiatives. *J Athl Train*. 2007;42(2):311-319.

29. Brown DA, Elsass JA, Miller AJ, Reed LE, Reneker JC. Differences in Symptom Reporting Between Males and Females at Baseline and After a Sports-Related Concussion: A Systematic Review and Meta-Analysis. *Sports Med*. 2015;45(7):1027-1040. doi:10.1007/s40279-015-0335-6.

30. Baker JG, Leddy JJ, Darling SR, Shucard J, Makdissi M, Willer BS. Gender Differences in Recovery From Sports-Related Concussion in Adolescents. *Clin Pediatr (Phila)*. September 2015. doi:10.1177/0009922815606417.

31. Kirkness CJ, Burr RL, Mitchell PH, Newell DW. Is there a sex difference in the course following traumatic brain injury? *Biological Research For Nursing*. 2004;5(4):299-310. doi:10.1177/1099800404263050.

32. Fish EN. The X-files in immunity: sex-based differences predispose immune responses. *Nat Rev Immunol*. 2008;8(9):737-744. doi:10.1038/nri2394.

33. Bouman A, Heineman MJ, Faas MM. Sex hormones and the immune response in humans. *Hum Reprod Update*. 2005;11(4):411-423. doi:10.1093/humupd/dmi008.

34. Guskiewicz KM, Register-Mihalik J, McCrory P, et al. Evidence-based approach to revising the SCAT2: introducing the SCAT3. *British Journal of Sports Medicine*. 2013;47(5):289-293. doi:10.1136/bjsports-2013-092225.

35. Galetta MS, Galetta KM, McCrossin J, et al. Saccades and memory: baseline associations of the King-Devick and SCAT2 SAC tests in professional ice hockey players. *J Neurol Sci*. 2013;328(1-2):28-31. doi:10.1016/j.jns.2013.02.008.

36. Snyder AR, Bauer RM, Health IMPACTS for Florida Network. A normative study of the sport concussion assessment tool (SCAT2) in children and adolescents. *Clin Neuropsychol*. 2014;28(7):1091-1103. doi:10.1080/13854046.2014.952667.

37. Jinguji TM, Bompadre V, Harmon KG, et al. Sport Concussion Assessment Tool-2: baseline values for high school athletes. *British Journal of Sports Medicine*. 2012;46(5):365-370. doi:10.1136/bjsports-2011-090526.

38. Chodobski A, Zink BJ, Szmydynger-Chodobska J. Blood–Brain Barrier Pathophysiology

in Traumatic Brain Injury. *Transl Stroke Res*. 2011;2(4):492-516. doi:10.1007/s12975-011-0125-x.

39. Akirav EM, Preston-Hurlburt P, Garyu J, et al. RAGE expression in human T cells: a link between environmental factors and adaptive immune responses. *PLoS ONE*. 2012;7(4):e34698. doi:10.1371/journal.pone.0034698.

40. Liesz A, Dalpke A, Mracsko E, et al. DAMP Signaling is a Key Pathway Inducing Immune Modulation after Brain Injury. *J Neurosci*. 2015;35(2):583-598. doi:10.1523/JNEUROSCI.2439-14.2015.

41. Topolovec-Vranic J, Pollmann-Mudryj M-A, Ouchterlony D, et al. The Value of Serum Biomarkers in Prediction Models of Outcome After Mild Traumatic Brain Injury. *J Trauma*. 2011;71:S478-S486. doi:10.1097/TA.0b013e318232fa70.

42. Buonora JE, Yarnell AM, Lazarus RC, et al. Multivariate analysis of traumatic brain injury: development of an assessment score. *Frontiers in Neurology*. 2015;6:68. doi:10.3389/fneur.2015.00068.

43. Nygren-de Boussard C, Fredman P, Lundin A, Andersson K, Edman G, Borg J. S100 in mild traumatic brain injury. *Brain Inj*. 2004;18(7):671-683. doi:10.1080/02699050310001646215.

44. Clifton GL, Ziegler MG, Grossman RG. Circulating catecholamines and sympathetic activity after head injury. *Neurosurgery*. 1981;8(1):10-14.

45. Hamill RW, Woolf PD, McDonald JV, Lee LA, Kelly M. Catecholamines predict outcome in traumatic brain injury. *Ann Neurol*. 1987;21(5):438-443. doi:10.1002/ana.410210504.

46. Elenkov IJ, Wilder RL, Chrousos GP, Vizi ES. The sympathetic nerve—an integrative interface between two supersystems: the brain and the immune system. *Pharmacol Rev*. 2000;52(4):595-638.

47. Catania A, Lonati C, Sordi A, Gatti S. Detrimental consequences of brain injury on peripheral cells. *Brain Behavior and Immunity*. 2009;23(7):877-884. doi:10.1016/j.bbi.2009.04.006.

48. La Fountaine MF, Heffernan KS, Gossett JD, Bauman WA, De Meersman RE. Transient suppression of heart rate complexity in concussed athletes. *Autonomic Neuroscience: Basic and Clinical*. 2009;148(1-2):101-103. doi:10.1016/j.autneu.2009.03.001.

49. Lagos L, Bottiglieri T, Vaschillo B, Vaschillo E. Heart Rate Variability Biofeedback for Postconcussion Syndrome: Implications for Treatment. *Biofeedback*. 2012;40(4):150-153. doi:10.5298/1081-5937-40.4.05.

50. Gall B, Parkhouse W, Goodman D. Heart rate variability of recently concussed athletes at rest and exercise. *Med Sci Sports Exerc*. 2004;36(8):1269-1274.

51. Hinson HE, Sheth KN. Manifestations of the hyperadrenergic state after acute brain injury. *Curr Opin Crit Care*. 2012;18(2):139-145. doi:10.1097/MCC.0b013e3283513290.

52. Sokol CL, Luster AD. The chemokine system in innate immunity. *Cold Spring Harb Perspect Biol*. 2015;7(5). doi:10.1101/cshperspect.a016303.

53. Luster AD. Chemokines--chemotactic cytokines that mediate inflammation. *N Engl J Med*. 1998;338(7):436-445. doi:10.1056/NEJM199802123380706.

54. Semple BD, Frugier T, Morganti-Kossmann MC. CCL2 modulates cytokine production in cultured mouse astrocytes. *J Neuroinflammation*. 2010;7(1):67. doi:10.1186/1742-2094-7-67.

55. Semple BD, Bye N, Ziebell JM, Morganti-Kossmann MC. Deficiency of the chemokine receptor CXCR2 attenuates neutrophil infiltration and cortical damage following closed head injury. *Neurobiology of Disease*. 2010;40(2):394-403. doi:10.1016/j.nbd.2010.06.015.

56. Korn A, Golan H, Melamed I, Pascual-Marqui R, Friedman A. Focal Cortical Dysfunction and Blood–Brain Barrier Disruption in Patients With Postconcussion Syndrome. *Journal of Clinical Neurophysiology*. 2005;22(1):1-9. doi:10.1097/01.wnp.0000150973.24324.a7.

57. Jaerve A, Müller HW. Chemokines in CNS injury and repair. *Cell Tissue Res*. 2012;349(1):229-248. doi:10.1007/s00441-012-1427-3.

58. Jaerve A, Schiwy N, Schmitz C, Mueller HW. Differential effect of aging on axon sprouting and regenerative growth in spinal cord injury. *Exp Neurol*. 2011;231(2):284-294. doi:10.1016/j.expneurol.2011.07.002.

59. Di Battista AP, Buonora JE, Rhind SG, et al. Blood Biomarkers in Moderate-To-Severe Traumatic Brain Injury: Potential Utility of a Multi-Marker Approach in Characterizing Outcome. *Frontiers in Neurology*. 2015;6:110. doi:10.3389/fneur.2015.00110.

60. Iverson GL, Echemendia RJ, LaMarre AK, Brooks BL, Gaetz MB. Possible lingering effects of multiple past concussions. *Rehabil Res Pract*. 2012;2012:316575. doi:10.1155/2012/316575.

61. Guskiewicz KM, Marshall SW, Bailes J, et al. Association between Recurrent Concussion and Late-Life Cognitive Impairment in Retired Professional Football Players. *Neurosurgery*. 2005;57(4):719-726. doi:10.1227/01.NEU.0000175725.75780.DD.

62. Bazarian JJ, Zhu T, Zhong J, et al. Persistent, long-term cerebral white matter changes after sports-related repetitive head impacts. *PLoS ONE*. 2014;9(4):e94734. doi:10.1371/journal.pone.0094734.

63. Witcher KG, Eiferman DS, Godbout JP. Priming the Inflammatory Pump of the CNS after Traumatic Brain Injury. *Trends in Neurosciences*. 2015;38(10):609-620. doi:10.1016/j.tins.2015.08.002.

64. Yang K, Xie G-R, Hu Y-Q, Mao F-Q, Su L-Y. The effects of gender and numbers of depressive episodes on serum S100B levels in patients with major depression. *J Neural Transm*. 2008;115(12):1687-1694. doi:10.1007/s00702-008-0130-8.

65. Nygaard O, Langbakk B, Romner B. Age- and sex-related changes of S-100 protein concentrations in cerebrospinal fluid and serum in patients with no previous history of neurological disorder. *Clin Chem*. 1997;43(3):541-543.

Tables

Table 1. Demographics and clinical characteristics
Unless otherwise stated, results are reported as the mean and percent - *n* (%).

Characteristics	No Concussion History (*n*=29)	Concussion History (*n*=24)
Demographics		
Age (years) – (mean ± SD)	19.8 ± 1.7	20.8 ± 1.9
Male Gender	15 (53.6)	13 (46.4)
Medical History		
Migraines	0 (0.0)	1 (4.2)
Learning disability	1 (3.4)	1 (3.3)
Anxiety/depression/psychiatric disorders	2 (6.9)	1 (4.2)
Family history of psychiatric illness	5 (17.2)	5 (20.1)
Sport		
Basketball	8 (27.5)	2(8.3)
Hockey	3 (10.3)	14 (58.3)
Lacrosse	1 (3.4)	0 (0.0)
Rugby	4 (13.8)	2 (8.3)
Volleyball	9 (31.0)	6 (25.0)
Wrestling	4 (13.8)	0 (0)
Current SCAT symptom scores – (mean ± SD)		
Total symptoms	2.1 ± 2.1	3.9 ± 4.2
Symptom severity	3.5 ± 4.4	6.5 ± 8.1

No significant differences were found between subjects with a history of concussion versus those without, by unpaired student's t-test ($p < 0.05$).

Table 2. Concentrations of inflammatory mediators in healthy Athletes stratified according to concussion history.

Markers	No Hx of Concussion (*n* = 28)	Hx of Concussion (*n* = 25)
	Cytokines	
IL-1α	3.4 ± 0.6	2.7 ± 0.6
IL-1β	--	--
IL-2	--	--
IL-4	--	--
IL-5	--	0.5 ± 0.1
IL-6	--	--
IL-7	2.2 ± 0.4	3.2 ± 0.4
IL-10	0.3 ± 0.0	0.3 ± 0.0
IL-12p40	112.7 ± 6.9	133.0 ± 7.7
IL-12p70	--	--
IL-13	--	--
IL-15	2.4 ± 0.1	2.5 ± 0.1
IL-16	250.4 ± 24.1	278.8 ± 19.6
IL-17A	2.2 ± 0.2	2.3 ± 0.2
TNF-α	1.6 ± 0.1	1.6 ± 0.1
TNF-β	0.2 ± 0.0	0.2 ± 0.0
GM-CSF	--	--
VEGF	42.1 ± 3.4	42.2 ± 3.4
IFN-γ	6.0 ± 0.5	5.5 ± 0.5
	Chemokines	
Eotaxin	84.3 ± 6.9	70.0 ± 2.8
Eotaxin-3	24.1 ± 2.5	27.9 ± 3.6
IP-10	158.9 ± 8.2	232.7 ± 18.5*
IL-8	1.9 ± 0.2	2.6 ± 0.2*
MCP-1	52.7 ± 2.2	67.2 ± 4.9*
MCP-4	26.6 ± 2.4	42.0 ± 4.7*
MDC	737.3 ± 31.0	853.1 ± 45.3*
MIP-1α	6.7 ± 0.6	7.6 ± 0.6*
MIP-1β	34.5 ± 2.7	40.0 ± 2.7
TARC	32.2 ± 2.5	49.4 ± 3.8*
	Neuroinjury Markers	
BDNF	1432.9 ± 213.5	2336.5 ± 332.0*
s100B	16.3 ± 1.2	16.3 ± 1.3
GFAP (ng/mL)	--	9.8 ± 1.6
NSE (ng/mL)	4.1 ± 0.2	4.2 ± 0.1

Interleukin (IL) -1α, -1β, -2, -4, -5, -6, -7, -10, -12p40, -12p70, -13, -15, -16, -17A, tumor necrosis factor (TNF) -α, -β, granulocyte macrophage colony-stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF), interferon-gamma (IFN-γ), eotaxin, eotaxin-3, interferon gamma-induced protein (IP) -10, IL-8. monocyte chemoattractant protein (MCP) -1, -4, macrophage derived chemokine, (CONT ON NEXT PAGE)

(MDC), macrophage inflammatory protein (MIP) -1α, -1β, thymocyte- and activation-regulated chemokine (TARC), brain derived neurotrophic factor (BDNF), s100 calcium binding protein beta (s100B), glial fibrillary acidic protein (GFAP), neuron specific enolase (NSE).

Data are presented as mean SEM in pg/mL unless otherwise stated.

* = adjusted $P < 0.05$ vs. athletes with no history of concussion.

"--" = below assay detection in $\geq$50% of samples analyzed.

Table 3. Concentrations of inflammatory mediators in healthy athletes stratified according to sex.

Markers	Males (*n* = 29)	Females (*n* = 24)
	Cytokines	
IL-1α	2.5 ± 0.5	3.7 ± 0.6
IL-1β	--	--
IL-2	--	--
IL-4	--	--
IL-5	0.6 ± 0.1	--
IL-6	0.7 ± 0.1	--
IL-7	2.9 ± 0.4	2.3 ± 0.3
IL-10	0.3 ± 0.0	0.3 ± 0.0
IL-12p40	120.4 ± 7.4	125.7 ± 7.7
IL-12p70	--	--
IL-13	--	--
IL-15	2.5 ± 0.1	2.5 ± 0.1
IL-16	318.6 ± 21.6	200.0 ± 13.9*
IL-17A	--	2.5 ± 0.2
TNF-α	1.7 ± 0.1	1.5 ± 0.1*
TNF-β	0.2 ± 0.0	0.2 ± 0.0
GM-CSF	--	--
VEGF	37.8 ± 2.5	47.2 ± 4.1*
IFN-γ	6.3 ± 0.4	5.3 ± 0.6
	Chemokines	
Eotaxin	88.0 ± 6.2	65.8 ± 3.5*
Eotaxin-3	28.2 ± 2.8	--
IP-10	213.0 ± 16.2	172.4 ± 13.7*
IL-8	2.5 ± 0.2	1.8 ± 0.2*
MCP-1	64.5 ± 4.4	53.6 ± 2.4*
MCP-4	42.4 ± 4.0	23.2 ± 2.1*
MDC	797.7 ± 35.8	784.8 ± 44.6
MIP-1α	6.9 ± 0.4	--
MIP-1β	39.0 ± 2.8	34.9 ± 2.7
TARC	43.2 ± 3.5	37.4 ± 3.8
	Neuroinjury Markers	
BDNF	1701.4 ± 268.7	2141.4 ± 320.1
s100B	13.7 ± 1.2	19.7 ± 1.0*
GFAP (ng/mL)	--	8.1 ± 1.2
NSE (ng/mL)	4.2 ± 0.1	4.0 ± 0.2

Interleukin (IL) -1α, -1β, -2, -4, -5, -6, -7, -10, -12p40, -12p70, -13, -15, -16, -17A, tumor necrosis factor (TNF) -α, -β, granulocyte macrophage colony-stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF), interferon-gamma (IFN-γ), eotaxin, eotaxin-3, interferon gamma-induced protein (IP) -10, IL-8. monocyte chemoattractant protein (MCP) -1, -4, macrophage derived chemokine, (MDC), macrophage inflammatory protein (MIP) -1α, -1β, (CONT ON NEXT PAGE)

thymocyte- and activation-regulated chemokine (TARC), s100 calcium binding protein beta (s100B), glial fibrillary acidic protein (GFAP), neuron specific enolase (NSE), brain derived neurotrophic factor (BDNF).

Data are presented as mean ± SEM in pg/mL, unless stated otherwise.

* = adjusted $P < 0.05$ vs. males.

"--" = below assay detection in $\geq$ 50% of samples analyzed.

Table 4. Correlations between inflammatory markers and concussion history indices

	Number of previous concussions		Days from last concussion	
Marker	**r**	**P value**	**r**	**P value**
BDNF	0.21	0.151	-0.06	0.795
IL-8	0.34	0.017*	-0.01	0.959
IP-10	0.44	0.001*	-0.45	0.041*
MCP-1	0.34	0.015*	-0.09	0.699
MCP-4	0.41	0.003*	0.17	0.439
MIP-1α	0.32	0.063	-0.49	0.054
MDC	0.22	0.108	-0.22	0.317
TARC	0.45	0.001*	-0.01	0.956
IL-7	0.36	0.009*	-0.09	0.692
MIP-1β	0.24	0.085	-0.49	0.020*

Abbreviations: BDNF, brain derived neurotrophic factor; IL, interleukin; IP-10, interferon-gamma induced protein - 10; MCP, monocyte chemoattractant protein; MDC, macrophage-derived chemokine; TARC, thymus and activation regulated chemokine; MIP, macrophage inflammatory protein.

* = $P < 0.05$ using spearman *r*.

Figure Legends

Figure 1. Changes in systemic inflammatory markers in athletes with a history of concussion. brain derived neurotrophic factor (BDNF), interleukin (IL) -8, interferon gamma-induced protein (IP) -10, monocyte chemoattractant protein (MCP) -1, -4, macrophage inflammatory protein (MIP) -1α, macrophage derived chemokine (MDC), and thymus and activation-regulated chemokine (TARC) concentrations in healthy athletes with (n = 28) versus without (n = 25) a previous history of concussion. Lines represent the mean and standard error of the mean (SEM). * = $p < 0.05$ versus athletes without a previous history of concussion by student's *t*-test with Welch's correction, where appropriate.

Figure 2. Alterations in systemic inflammatory markers according to concussion history and sex. interleukin (IL) -8 and macrophage inflammatory protein (MIP) -1α concentrations in healthy athletes with (male, *n* = 15; female, *n* = 13) versus without (male, *n* = 14; female *n* = 11) a previous history of concussion. Bars and lines represent the mean and standard error of the mean (SEM). * = $p < 0.05$ versus athletes without a previous history of concussion by Two-way ANOVA and Tukey's multiple comparisons test.