Acute changes in plasma neurofilament light chain are moderated by subconcussive head impacts in college football players

Leah H. Rubin[1], Leroy Wesley[3], Jong Hyun Lee[3], Keisuke Kawata[4], Ryan Tierney[4], Henrik Zetterberg[4,5,6,7], Kaj Blennow[4,5], Dianne Langford[3] *

[1] *Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD*

[2] *Department of Neuroscience, Lewis Katz School of Medicine at Temple University, Philadelphia, PA*

[3] *Department of Kinesiology, School of Public Health, Indiana University, Bloomington, IN*

[4] *Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, The Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden*

[5]*Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden*

[6]*Department of Molecular Neuroscience, UCL Institute of Neurology, Queen Square, London, United Kingdom*

[7]*UK Dementia Research Institute at UCL, London, United Kingdom*

*Corresponding Author: Dianne Langford, PhD

Address: Lewis Katz School of Medicine at Temple University, MERB 750, Department of Neuroscience, 3500 North Broad Street, Philadelphia, PA 19140, USA

E-mail: tdl@temple.edu

Phone: (215) 707-5487

Fax: (215) 707-4888

Key Words: Subconcussive, traumatic brain injury, neurofilament light chain, Biomarker, Head Impact Kinematics

Abstract

Repetitive subconcussive head impacts in contact/collision sports such as American football may contribute to long-term brain changes. However, the lack of data to measure the effects of repeated subconcussive blows limits our understanding of their potential contributions to neuropathological alterations including neuronal damage. Here we examined the impact of subconcussive head impacts as measured by an accelerometer embedded mouth guard on changes in blood levels of the neuronal protein neurofilament light (NFL) chain in 24 Division I college football players. Plasma levels of NFL as well as other biomarkers (e.g., plasma tau, S100β, near point of convergence) and clinical symptoms were assessed in this cohort at baseline and pre-post practices. The impact of frequency and linear and rotational head accelerations recorded via the mouth guard were examined in relation to NFL changes. Increases in NFL levels from baseline to post-practice were moderated by frequency and magnitude of head impacts ($p=0.04$). Players sustaining higher force and more frequent head impacts ($n=17$) showed significant increases in NFL levels from baseline to post-practice ($p<0.001$). However, there were no changes in NFL blood levels among players ($n=7$) sustaining lower force/less frequent head impacts ($p=0.61$). The greater number of hits and linear and rotational head accelerations were also associated with greater pre- to post-practice NFL level increases ($p's<0.05$). Greater pre- to post-practice increases in NFL were associated with greater pre- to post-practice increases in S100β ($p<0.001$), but not with total tau. Acute changes in NFL, similar to S100β, may be a clinically useful peripheral marker in tracking acute brain damage in collegiate football players.

Introduction

Athletes engaged in collision or contact sports are at high risk for sustaining repeated head injuries with the majority of injuries being mild in nature. Milder forms of traumatic brain injury (mTBI) that do not elicit signs or symptoms associated with concussion are termed subconcussion. Notably, repeated subconcussive impacts can lead to significant neurological alterations (Bailes et al., 2013) and these alterations may differ from those observed in athletes sustaining a concussion (Di Battista et al., 2016). To date, there is a paucity of studies addressing the effects of subconcussive head impacts on changes in the brain. Rather, the majority of mTBI studies focus on changes in clinical parameters and biomarkers following the diagnosis of a single concussion. Given the difficulties associated with detecting concussions and the dearth of knowledge regarding the adverse neuropathological and neurobiological sequelae of subconcussive head impacts, our research represents a high priority area.

Minimally invasive approaches to detect brain changes following repeated subconcussive impacts are needed. To track longitudinal changes in biomarkers indicative of repetitive brain injury from subconcussive blows, brain-derived blood-borne biomarkers are more practical than lumbar puncture for cerebrospinal fluid analyses. In a recent series of studies where blood was collected before and after repeated subconcussive head impacts sustained during college football summer training camp practices, we demonstrated that greater frequency and magnitude of subconcussive impacts were associated with greater increases in S100β plasma

levels from pre- to post-impact time points (Kawata et al., 2017a). However, subconcussive head impact exposure was not associated with changes in plasma total Tau protein (Kawata et al., 2017b). Taken together, these results illustrate the need to assess various biomarkers that can indicate disruptions in different cell populations and brain regions, and in different biological processes and systems.

Along with some of the more extensively studied blood biomarkers such as S100β and Tau, neurofilament light chain (NFL) has recently gained more attention as a viable biomarker for neuronal damage associated with TBI (Al Nimer et al., 2015; Shahim et al., 2016), as well as for several neurodegenerative diseases including frontotemporal dementia (Rohrer et al., 2016), Huntington's disease (Byrne et al., 2017), multiple sclerosis (Disanto et al., 2017), Parkinson's disease (Hansson et al., 2017), and amyotrophic lateral sclerosis (Lu et al., 2015). Neurofilament light chain is an important neuronal cytoskeletal element involved in normal axonal and dendritic structure, growth and function (Lepinoux-Chambaud and Eyer, 2013). Unlike Tau that is largely associated with thin unmyelinated axons of interneurons, NFL is a major component of large myelinated axons projecting into the white matter in subcortical brain regions (Shahim et al., 2016; Zetterberg et al., 2013). These large myelinated axons are believed to be more susceptible to TBI, and therefore may be a better marker for TBI than Tau (Shahim et al., 2016). Moreover, studies assessing changes in levels of NFL in other neurodegenerative diseases report robust correlations between CSF and plasma levels of NFL (Gisslen et al., 2016; Lu et al., 2015) and propose that NFL may therefore provide a valuable biomarker for on-going CNS injury associated with its release from degenerating neurons (Gisslen et al., 2016; Jonsson et al., 2010).

In a subset of collegiate football players from our previously published cohort studies (Kawata et al., 2017a; Kawata et al., 2017b; Kawata et al., 2016b), we assessed the effects of repeated subconcussive hits on changes in plasma NFL. We expected that the repetitive football subconcussive impacts would alter NFL levels particularly among players experiencing more frequent and higher magnitude impacts compared with players sustaining less frequent and lower magnitude impacts. We also expected that individual differences in the frequency and magnitude of head impacts at any given practice would be associated with changes in NFL blood levels. Given that repeated subconcussive hits to head are likely to result in complex biological changes, we examined the inter-relationships between plasma NFL levels and our previous data on plasma total Tau, plasma S100β, near point of convergence, and self-reported clinical symptoms pre- and post-practice, and whether these relationships differ among players sustaining higher versus lower numbers and magnitudes of head impacts.

Methods

Participants

Thirty-three Division I college football players volunteered to participate in the study, and from this cohort, 24 participants' data were included in the current study. Participants gave written informed consent and the Temple University Institutional Review Board approved the study. The study was conducted pre-season during a series of summer training camp practices. Baseline, and pre-post pairs of measures were collected from participants. Participants sustained no concussions during the course of the study. Inclusion criteria were being an active member of a college football team. Exclusion criteria were a history of head, neck, ocular or face injury 6

months prior to study or any neurological disorders. Participants refrained from the use of substances influencing the central nervous system (e.g., stimulants), and alcohol use was prohibited. Demographic information (e.g., age, body mass index) was collected. History of concussion and years of football experience were self-reported.

Head Impact Measurements

The Vector™ mouth guard (i1 Biometrics™, Inc., Kirkland, WA, USA) was used to measure the frequency (number of hits) and magnitude of head accelerations (peak linear and rotational accelerations) as previously described (Kawata et al., 2017a; Kawata et al., 2017b; Kawata et al., 2016b). After placing briefly in boiling water to soften the mold, mouth guards were molded to each participant's bite for a secure custom fit. A triaxial accelerometer (ADXL377, Analog Devices, Norwood, MA, USA) with 200*g* maximum per axis to measure linear acceleration is employed by the mouth guards. A triaxial rotational rate gyroscope (L3GD20H, ST Microelectrics, Geneva, Switzerland) is used for rotational kinematics (Camarillo et al., 2013; Kawata et al., 2017a; Kawata et al., 2016b). Data from the accelerometer and gyroscope were low-pass filtered at 180 and 40 Hz cutoff, respectively. When a preset threshold for a peak linear acceleration (PLA) magnitude exceeded 10.0 *g*, 16 pre-trigger and 80 post-trigger samples with a standard hit duration of 93.75 milliseconds of all impact data were wirelessly transmitted through the antenna transmitter to the sideline antenna and computer, then stored on a secure Internet database as previously described (Kawata et al., 2017a; Kawata et al., 2016b). From raw impact data, the number of hits, PLA, and peak rotational acceleration (PRA) were used for further analyses. Consistent with our previous work, players were categorized into lower and higher impact groups based on the sum from 3-practice impact kinematic data collection (Kawata et al., 2017a; Kawata et al., 2016b) (**Table 1**).

Blood Collection

Venous blood samples were collected at baseline prior to any practices and from pre- and post-practices into vacutainer sterile tubes with the EDTA anticoagulant (BD Bioscience). Plasma was separated by centrifugation (1,500 x *g*, 15 minutes) and stored at -80ºC until analyses as previously described (Kawata et al., 2017a; Kawata et al., 2016b).

NFL measurements

NFL concentration was measured by digital array technology (Simoa; Quanterix Corporation) using an in house kit, as previously described (ref: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4739412/).

S100β measurements

S100β levels were determined using sandwich-based ELISA kits according to manufacturer's instructions (EMD Millipore, Billerica, MA), and as described in our recent publication (Kawata et al., 2017a).

Total Tau measurements

Tau concentrations in plasma samples were measured by digital array technology (Simoa; Quanterix Corporation) using a commercial kit (Quanterix Corporation) as previously described (Kawata et al., 2017b).

Near Point of Convergence

The near point of convergence (NPC) was calculated for each participant at baseline and pre-, post-practices as previously described (Kawata et al., 2016b). The assessment was repeated twice, and mean NPC was used for analysis.

Symptom Checklist

Participants were instructed to rate the presence of any symptoms at each time point using the symptom checklist, which is a subset of the Sports Concussion Assessment Tool 3, as previously described (Kawata et al., 2017a; Kawata et al., 2016b).

Statistical Analyses

A multilevel (hierarchical) model was conducted in SAS PROC MIXED (version 9.4, Cary, NC) to examine whether lower- and higher- impact groups showed different patterns of NFL levels across baseline, and pre- and post-practice assessments. Multilevel models were selected to handle the nested design with repeated measures (24 players over three time points: baseline, pre- and post-practices). Variables included in the initial model were group (lower and higher), time (linear trend), and the group by trend interaction. A significant group by trend interaction indicates that the pattern of NFL levels across time points depends on head impact. Based on this model, we conducted additional analyses (spearman correlations) to examine if kinematics influenced the change from pre- to post-practice assessment on NFL levels. To determine the specificity of these findings, we also used multilevel models to determine whether years of football experience (<10 years vs. $\geq$10 years) or concussion history (yes vs. no) moderated the change in NFL from baseline to post-practice assessment. Models were also conducted examining impact by time interactions on changes in S100β, Tau, NPC and symptoms, as we wanted to know in this subset of data whether these markers changed as a function of impact in the same or different manner as NFL. Exploratory spearman correlation analyses were conducted to determine the pattern of associations between changes in NFL, S100β, Tau, NPC, and symptoms (post - pre). Significance was set at $p<0.05$.

Results

Demographic and kinematic data for the overall sample of 24 players and by impact group are shown in **Table 1**. The higher (n=17) and lower (n=7) impact groups were similar in age, body mass index, prior concussions, and years of football experience. Importantly, the higher and lower impact groups differed in head impact kinematics (number of hits, 35 vs. 6; PLA, 940 vs.99*g*; 57,482 vs. 7,589 rad/s^2; HIC, 766 vs. 355, respectively).

The mean NFL levels by group did not differ at baseline, F (1, 113)=0.11, p=0.74 (**Figure 1**). Regarding group differences, changes in NFL levels from baseline to post-practice assessment differed for those in the higher compared to lower impact group (F for group x linear trend$_{1, 113}$, =3.97, p=0.04). In the higher impact group, there was a significant increase in NFL levels from baseline to post-practice assessment (B, unstandardized coefficient for linear trend [SE], 0.25 [0.06], p<0.0001). However, in the lower impact group, there was no change in NFL levels from baseline to the post-practice assessment (B for the linear trend [SE], 0.04 [0.09], p=0.61). This pattern was specific to head impact as there were no significant interactions between years of football experience, concussion history or time on NFL levels (p's>0.22). Notably, the number of hits, sum of PRA, PLA, and HIC were associated with changes in NFL levels (**Figure 2**). Specifically, the greater number of hits, PRA, PLA, and HIC were associated with a greater increase in NFL levels from pre- to post-practice (p's<0.05).

Consistent with our previous publication (Kawata et al., 2017a) and NFL data, changes in S100β levels from baseline to post-practice assessment also differed for those in the higher compared to lower impact group (*F* for group x linear trend$_{1, 99}$ =13.58, p=0.0004; **Figure 3**). Although both the higher (B for the linear trend [SE], 0.07 [0.008], p<0.0001) and lower impact (B for the linear trend [SE],

0.02 [0.01], p=0.02) groups showed increases in S100β levels from baseline to post-practice assessment, the magnitude of the increase was greater among players in the higher impact group. Tau, NPC, and symptoms did not show the same pattern across the time points as a function of head impact. For Tau, levels from baseline to post-practice assessment differed for those in the higher compared to lower impact group (*F* for group x linear trend$_{1, 113}$ =4.72, p=0.03). However, the pattern was such that only the lower impact group showed declines in Tau levels (B for the linear trend [SE], 2.16 [0.29], p<0.0001). For NPC and symptoms, higher impact players demonstrated higher levels of both compared to lower impact players (p's<0.05).

Exploratory correlations were conducted to examine associations between changes in NFL, S100β, Tau, NPC, and symptoms. Greater increases in NFL were associated with greater increases in S100β from the pre- to post-practice assessment (p<0.0001; **Figure 4**). Greater increases in S100β were also associated with greater increases in NPC (r_s=0.38, p=0.03); trend with Tau (r_s=0.31, p=0.09). There were no other significant associations between the changes in these markers from the pre- to post-practice assessment.

Discussion

Reliable, specific, and minimally invasive biomarkers are needed to gauge acute and cumulative CNS injury in the absence of documented concussion. For the first time in football players, we document that the frequencies and magnitudes of repeated subconcussive heads impacts is associated with changes in plasma NFL levels. This pattern emerged when comparing two extreme groups, one sustaining significantly more impacts at greater magnitudes compared to the second, lower impact group. We also noted that individual differences in the frequencies and magnitudes of repeated subconcussive heads impacts also was associated with

changes in plasma NFL levels from pre- to post-practice. This pattern was specific to head impacts and not to other measured factors such as years of football experience or concussion history. NFL may distinguish individuals that sustain greater numbers of hits and greater magnitudes of hits from those that have fewer and less forceful impacts, especially if more in depth studies are conducted that can construct impact dosage thresholds for an individual.

NEED COMMENT ON LINK BETWEEN NFL CHANGES AND S100B CHANGES BUT NOT WITH TAU, NPC, OR SYMPTOMS. WHAT DOES THIS MEAN?

Results from our studies highlight the need for assessing multiple biomarkers within an individual or within groups of individuals sustaining head impacts. Within the present cohort, changes in NFL, Tau, S100β, NPC, and symptoms have been measured. Peripheral NFL and S100β appear more important for tracking acute CNS changes due to repetitive subconcussive head impacts; whereas, NPC appeared more sensitive to tracking CNS changes over a longer duration (Kawata et al., 2017a; Kawata et al., 2017b; Kawata et al., 2016b). As pointed out by a recent review, a number of considerations should be taken into account when interpreting change sin a blood-borne brain-derived protein marker including the protein's origin, the implications for its presence in the blood and the biological significance of changes in blood levels (Kawata et al., 2016a). Consideration of the biological role that proposed biomarker proteins play when assessing changes in levels post-injury may guide researchers to better understand potential pathological mechanisms and specific cellular injuries. This train of thought may inform as to whether cells have been 1) injured and are simply releasing normal proteins due to mechanical cellular damage, 2) have increased levels of the protein and are releasing the excess, 3) are releasing aberrant proteins such as hyperphosphorylated Tau, or a combination.

The present study has a number of limitations. In addition to the small sample size, one limitation is the absence of CSF samples from participants to confirm whether changes in CSF and plasma NFL levels correlate. This may be important since increased plasma NFL without increased CSF NFL could indicate injuries outside of the CNS such as peripheral nervous system neurons (Trojanowski et al., 1986) given that injuries to extremities were not tracked in our cohort. However, studies in neurodegenerative disease do report robust associations between CSF and peripheral NFL levels (Gisslen et al., 2016; Lu et al., 2015) suggesting that peripheral NFL levels may measures on-going CNS injury.

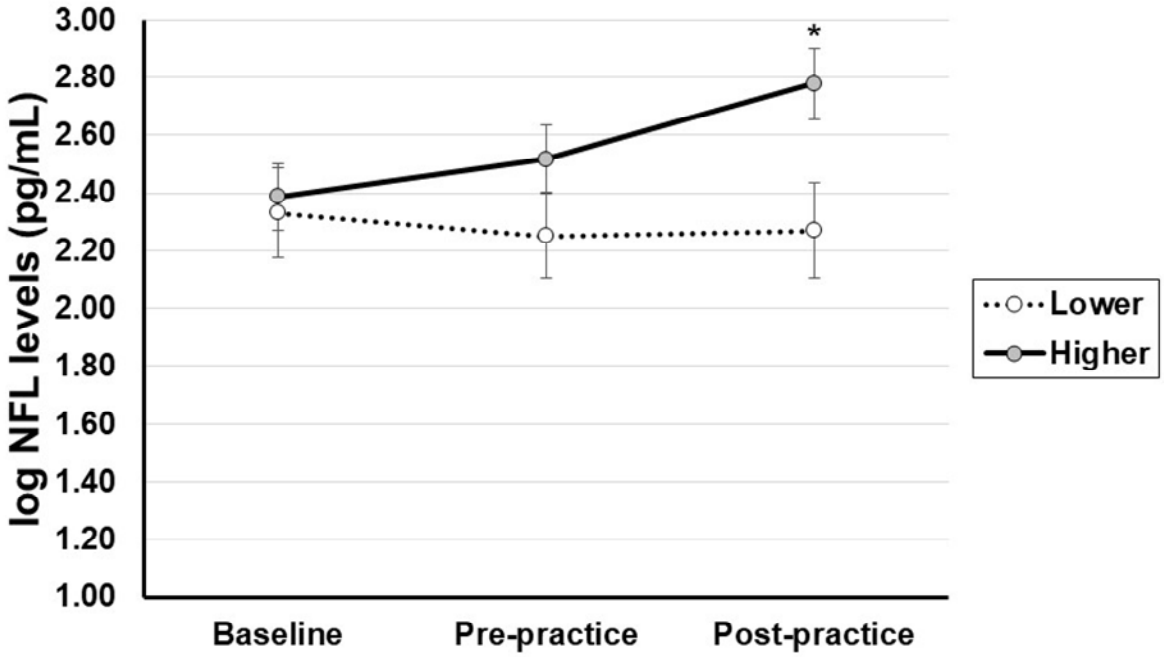
Table 1. Demographics, position, and head impact kinematics.

		Group		
Variables	Players (n=24)	Low Impact (n=7)	High Impact (n=17)	p-value
Demographics				
Age, median (IQR)	20.5 (20 – 21.5)	21 (20 - 22)	20 (19 - 21)	0.43
Body mass index, median (IQR)	28.75 (26.5 – 31.6)	26.5 (25.8 – 28)	30.3 (28.2 – 32)	0.06
Years football experience, median (IQR)	10 (5 – 15)	10 (4 - 15)	10 (6 – 15)	0.70
# of previous concussions, n (%)				0.72
None	13 (54)	4 (57)	9 (53)	
1	9 (38)	2 (29)	7 (41)	
3	2 (8)	1 (14)	1 (6)	
Position, n (%)				0.03
Linemen (OL, DL)	7 (29)	1 (14)	6 (35)	
Linebacker, Tight End	6 (25)	0 (0)	6 (35)	
Skill Players (WR, DB, RB, QB)	9 (38)	4 (57)	5 (30)	
Special team	2 (8)	2 (29)	0 (0)	
Impact Kinematics, median (IQR)[†]				
# of hits	23 (11.5-38.5)	6 (1 – 9)	35 (23 – 41)	<0.001
PLA (*g*)	665 (391 – 1083)	99 (41 – 377)	940 (661 – 1148)	<0.001
PRA (rad/sec^2)	47026 (22306 – 65016)	7589 (1671 – 22082)	57482 (44612 – 75473)	<0.001

Head injury criterion	572 (355 – 1101)	355 (72 – 911)	766 (534 – 1375)	<0.001

Note. OL, offensive lineman. DL, defensive lineman. WR, wide receiver. DB, defensive back. RB: running back. QB, quarterback. PLA, peak linear acceleration. PRA, peak rotational acceleration. IQR=interquartile range. †Based on the sum from 3-practice impact kinematic data collections (see methods section). Variables reported as n (%) were analyzed with Chi-square tests. Variables reported as median/IQR were analyzed with Wilcoxon-Mann-Whitney test.

Figure 1. Estimated log NFL levels from baseline to post-practice assessment by higher and lower impact players.



*p<0.0001 for the linear change from baseline to post-practice for the higher impact group.

Figure 2. Association between kinematic factors and changes in log NFL levels (post minus pre). Associations are depicted between change in NFL levels to total number of hits (A), sum of PLA (B), sum of PRA, and (C) head injury criterion. PLA, peak linear acceleration; PRA, peak rotational acceleration; HIC=head injury criterion; r_s=Spearman's Rho.

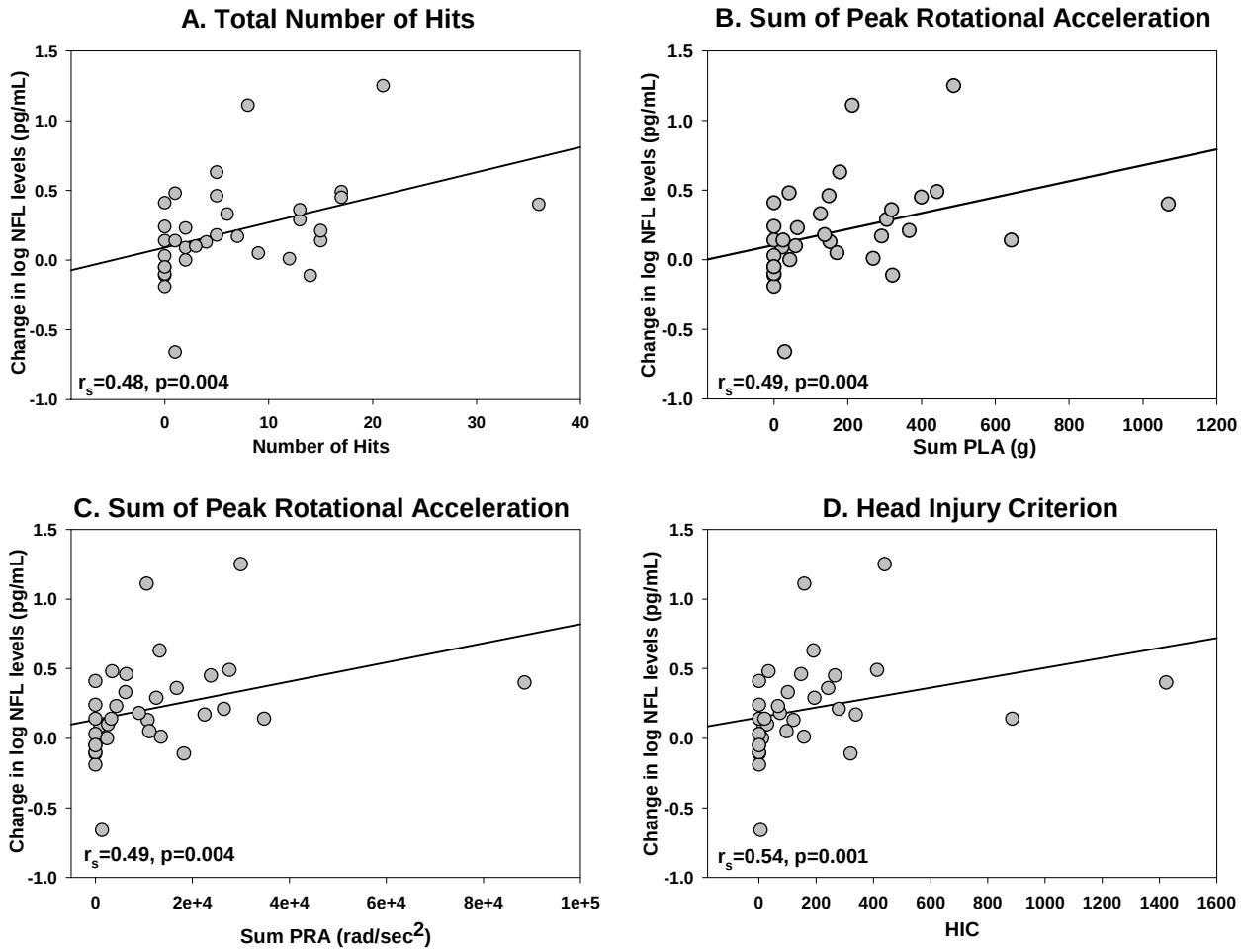
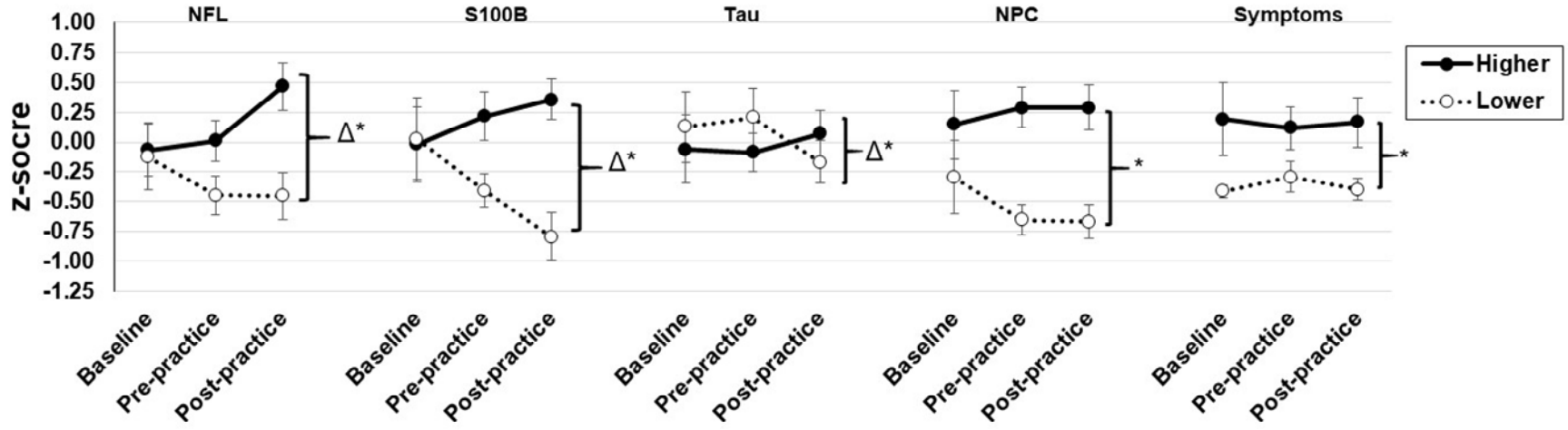
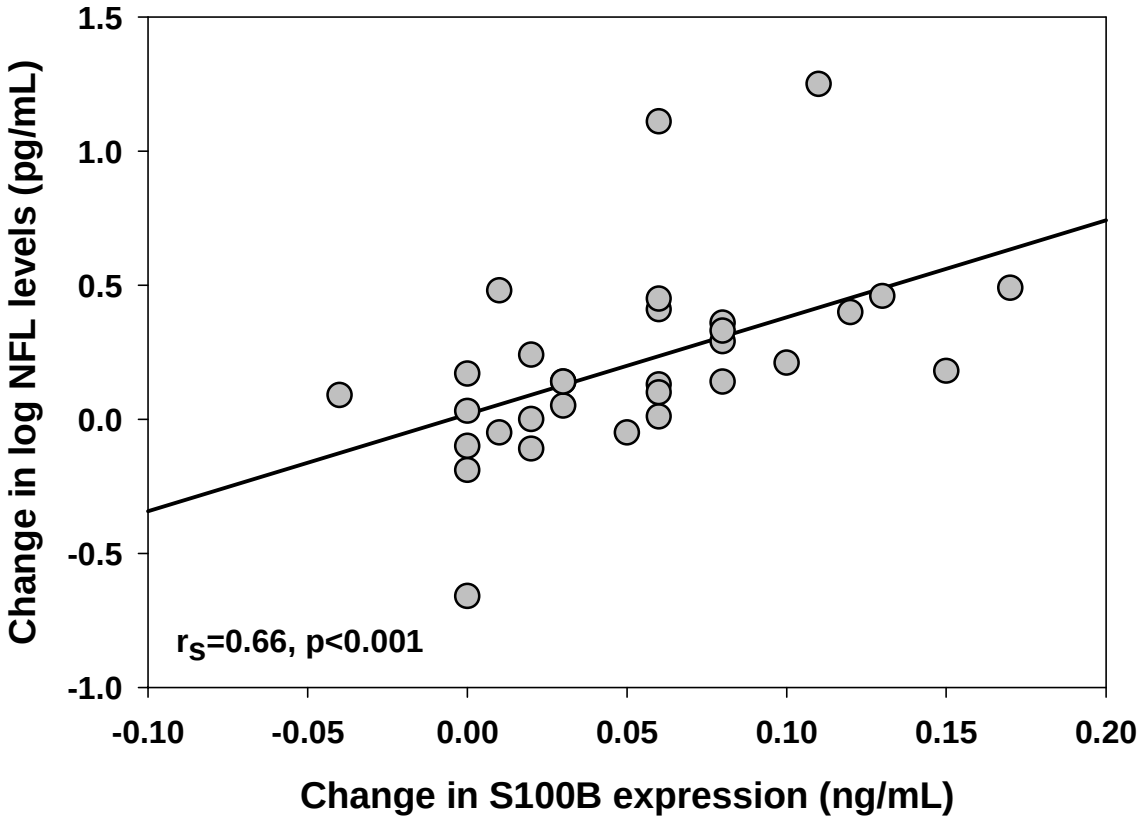


Figure 3. Changes in NFL, S100β, Tau, Near Point Convergence (NPC), and symptoms as a function of head impact.



Note. Δ*=significant interaction between time and impact group at $p<0.05$. *$p<0.05$ for overall difference in marker by impact group.

Figure 4. Changes in S100β in relation to changes in NFL levels (post – pre).



References

Al Nimer, F., E. Thelin, H. Nystrom, A.M. Dring, A. Svenningsson, F. Piehl, D.W. Nelson, and B.M. Bellander. 2015. Comparative Assessment of the Prognostic Value of Biomarkers in Traumatic Brain Injury Reveals an Independent Role for Serum Levels of Neurofilament Light. *PloS one*. 10:e0132177.

Bailes, J.E., A.L. Petraglia, B.I. Omalu, E. Nauman, and T. Talavage. 2013. Role of subconcussion in repetitive mild traumatic brain injury. *Journal of neurosurgery*. 119:1235-1245.

Byrne, L.M., F.B. Rodrigues, K. Blennow, A. Durr, B.R. Leavitt, R.A.C. Roos, R.I. Scahill, S.J. Tabrizi, H. Zetterberg, D. Langbehn, and E.J. Wild. 2017. Neurofilament light protein in blood as a potential biomarker of neurodegeneration in Huntington's disease: a retrospective cohort analysis. *Lancet neurology*. 16:601-609.

Camarillo, D.B., P.B. Shull, J. Mattson, R. Shultz, and D. Garza. 2013. An instrumented mouthguard for measuring linear and angular head impact kinematics in American football. *Annals of biomedical engineering*. 41:1939-1949.

Di Battista, A.P., S.G. Rhind, D. Richards, N. Churchill, A.J. Baker, and M.G. Hutchison. 2016. Altered Blood Biomarker Profiles in Athletes with a History of Repetitive Head Impacts. *PloS one*. 11:e0159929.

Disanto, G., C. Barro, P. Benkert, Y. Naegelin, S. Schadelin, A. Giardiello, C. Zecca, K. Blennow, H. Zetterberg, D. Leppert, L. Kappos, C. Gobbi, J. Kuhle, and G. Swiss Multiple Sclerosis Cohort Study. 2017. Serum Neurofilament light: A biomarker of neuronal damage in multiple sclerosis. *Annals of neurology*. 81:857-870.

Gisslen, M., R.W. Price, U. Andreasson, N. Norgren, S. Nilsson, L. Hagberg, D. Fuchs, S. Spudich, K. Blennow, and H. Zetterberg. 2016. Plasma Concentration of the Neurofilament Light Protein (NFL) is a Biomarker of CNS Injury in HIV Infection: A Cross-Sectional Study. *EBioMedicine*. 3:135-140.

Hansson, O., S. Janelidze, S. Hall, N. Magdalinou, A.J. Lees, U. Andreasson, N. Norgren, J. Linder, L. Forsgren, R. Constantinescu, H. Zetterberg, K. Blennow, and F.s. Swedish Bio. 2017. Blood-based NfL: A biomarker for differential diagnosis of parkinsonian disorder. *Neurology*. 88:930-937.

Jonsson, M., H. Zetterberg, E. van Straaten, K. Lind, S. Syversen, A. Edman, K. Blennow, L. Rosengren, L. Pantoni, D. Inzitari, and A. Wallin. 2010. Cerebrospinal fluid biomarkers of white matter lesions - cross-sectional results from the LADIS study. *European journal of neurology : the official journal of the European Federation of Neurological Societies.* 17:377-382.

Kawata, K., C.Y. Liu, S.F. Merkel, S.H. Ramirez, R.T. Tierney, and D. Langford. 2016a. Blood Biomarkers for Brain Injury: What Are We Measuring? *Neuroscience and biobehavioral reviews*.

Kawata, K., L.H. Rubin, M. Takahagi, J.H. Lee, T. Sim, V. Szwanki, A. Bellamy, R. Tierney, and D. Langford. 2017a. Subconcussive Impact-Dependent Increase in Plasma S100beta Levels in Collegiate Football Players. *Journal of neurotrauma*. 34:2254-2260.

Kawata, K., L.H. Rubin, L. Wesley, J. Lee, T. Sim, M. Takahagi, A. Bellamy, R. Tierney, and D. Langford. 2017b. Acute changes in plasma total Tau levels are independent of subconcussive head impacts in college football players. *Journal of neurotrauma*.

Kawata, K.M.L.H.R., PhD, MPH; Jong Hyun Lee; Thomas Sim; Masahiro Takahagi, MEd;, M.A.B. Victor Szwanki, MS; Kurosh Darvish, PhD; Soroush Assari, BS, MS; Jeffrey D.

Henderer, MD;, and P.D.L. Ryan Tierney, PhD. 2016b. Association of Football Subconcussive Head Impacts

With Ocular Near Point of Convergence. *JAMA Ophthalmology*.

Lepinoux-Chambaud, C., and J. Eyer. 2013. Review on intermediate filaments of the nervous system and their pathological alterations. *Histochemistry and cell biology*. 140:13-22.

Lu, C.H., C. Macdonald-Wallis, E. Gray, N. Pearce, A. Petzold, N. Norgren, G. Giovannoni, P. Fratta, K. Sidle, M. Fish, R. Orrell, R. Howard, K. Talbot, L. Greensmith, J. Kuhle, M.R. Turner, and A. Malaspina. 2015. Neurofilament light chain: A prognostic biomarker in amyotrophic lateral sclerosis. *Neurology*. 84:2247-2257.

Rohrer, J.D., I.O. Woollacott, K.M. Dick, E. Brotherhood, E. Gordon, A. Fellows, J. Toombs, R. Druyeh, M.J. Cardoso, S. Ourselin, J.M. Nicholas, N. Norgren, S. Mead, U. Andreasson, K. Blennow, J.M. Schott, N.C. Fox, J.D. Warren, and H. Zetterberg. 2016. Serum neurofilament light chain protein is a measure of disease intensity in frontotemporal dementia. *Neurology*. 87:1329-1336.

Shahim, P., M. Gren, V. Liman, U. Andreasson, N. Norgren, Y. Tegner, N. Mattsson, N. Andreasen, M. Ost, H. Zetterberg, B. Nellgard, and K. Blennow. 2016. Serum neurofilament light protein predicts clinical outcome in traumatic brain injury. *Scientific reports*. 6:36791.

Trojanowski, J.Q., N. Walkenstein, and V.M. Lee. 1986. Expression of neurofilament subunits in neurons of the central and peripheral nervous system: an immunohistochemical study with monoclonal antibodies. *J Neurosci*. 6:650-660.

Zetterberg, H., D.H. Smith, and K. Blennow. 2013. Biomarkers of mild traumatic brain injury in cerebrospinal fluid and blood. *Nature reviews. Neurology*. 9:201-210.