Inflammatory blood biomarker response after controlled subconcussive head impacts: a pilot randomized controlled trial

CURRENT STATUS: UNDER REVISION

Megan Elizabeth Huibregtse  
Indiana University School of Public Health - Bloomington

Keisuke Ejima  
Indiana University School of Public Health - Bloomington

Zhongxue Chen  
Indiana University School of Public Health - Bloomington

Zachary William Bevilacqua  
Indiana University School of Public Health - Bloomington

Alekhya Koppineni  
Indiana University School of Public Health - Bloomington

Rachel M. Kalbfell  
Indiana University School of Public Health - Bloomington

Keisuke Kawata  
kkawata@indiana.edu  
Indiana University Bloomington School of Public Health  
Corresponding Author  
ORCiD: 0000-0003-4135-9311

DOI: 10.21203/rs.2.15214/v1

SUBJECT AREAS  
Neurology

KEYWORDS  
subconcussive head impacts, soccer heading, acute inflammation, blood biomarkers
Abstract

Background: Chronic neuroinflammation has been implicated as a possible contributing mechanism in the development and progression of chronic traumatic encephalopathy. This neurodegenerative condition has been associated with longterm, repetitive exposure to subconcussive head impacts, defined as head impacts that do not induce clinical signs or symptoms of concussion. However, there is a gap in knowledge surrounding the acute inflammatory response to subconcussive head impacts. The present study aimed to test our hypothesis that plasma levels of two proinflammatory markers (CCL11, CCL2) and one anti-inflammatory marker (IL-10) would be significantly elevated after 10 repetitions of controlled subconcussive head impacts.

Methods: This randomized controlled trial included 39 healthy adult soccer players who were randomized into a heading (n=22) or kicking-control group (n=17). The heading group executed 10 headers with soccer balls projected at a speed of 25mph. The kicking-control group followed the same protocol with 10 kicks. Plasma samples were collected pre-, 0h post-, 2h post-, and 24h post-intervention. Samples were assayed for CCL11, CCL2, and IL-10, which are inflammatory markers that have shown to upregulate following traumatic brain injury. The longitudinal inflammatory marker data were analyzed using mixed-effect regression models.

Results: There were no significant group differences in the changes of plasma CCL11, CCL2, or IL-10 levels at post-intervention time points as compared with pre-intervention baseline. However, within the heading group, there was a statistically significant interaction between time and years of soccer heading experience in plasma CCL11 levels at 24h post-intervention (2.0 pg/mL per a single year of soccer heading experience, 95% CI: 0.8, 3.1, p = 0.001).

Conclusions: Ten soccer headings did not modulate the acute response in the three
inflammatory marker levels compared against a kicking-control group. However, the acute
CCL11 response may be influenced by the duration of prior exposure to subconcussive
head impacts. Our data provide a precedent for future field studies that prospectively
track head impact exposure and the time course of changes in circulating CCL11.

Background

Traumatic brain injury (TBI) has been shown to induce an acute inflammatory response,
producing both pro- and anti-inflammatory cytokines and chemokines to restore
homeostasis [1–6]. While this acute inflammatory response can be beneficial in the short-
term, chronic neuroinflammation, characterized by alterations in inflammatory mediators
and increased microglial activation, can exacerbate cellular damage and lead to neuronal
cell death [3, 7]. Persistent microglial activation has been detected up to 17 years after
moderate to severe TBI [8]. Even a history of multiple concussions has been shown to
 elevate circulating levels of inflammatory markers more than one year after the most
recent concussion in collegiate athletes [9].

Furthermore, chronic neuroinflammation has been implicated as a potential mechanism in
the development of chronic traumatic encephalopathy (CTE), which is currently defined as
a progressive tauopathy diagnosed post-mortem and characterized by an irregular pattern
of abnormal accumulation of phosphorylated tau in the brain parenchyma [10].

Retrospective association studies suggest that duration of contact sports career appears
to be a major factor to the development of CTE [11], whereby the severity of CTE and
duration of subconcussive head impact exposure have been associated with signs of
chronic neuroinflammation in the dorsolateral frontal cortex of former American football
players [12]. However, acute inflammation after these subconcussive head impacts in
young adults has yet to be investigated.

We identified three key inflammatory markers, eotaxin-1 (CCL11), monocyte

chemoattractant 1 (CCL2), and interleukin 10 (IL-10), that may have the potential to reflect the subtle inflammatory response induced by subconcussive head impacts. CCL11 is a pro-inflammatory chemokine that can be produced peripherally by sources such as epithelial and endothelial cells in gut and respiratory tissue [13] and within the CNS by epithelial cells in the choroid plexus [14] and by astrocytes in response to various insults [15] in order to promote microglial migration and activation at the site of injury. Cherry et al. [16] recently proposed a link between years of head impact exposure and cortical expression of CCL11 in deceased professional American football players diagnosed with CTE, supporting that levels of this chemokine may have the potential to reflect the cumulative subconcussive neural damage. CCL2, another pro-inflammatory chemokine produced by astrocytes in addition to endothelial cells and fibroblasts, has been shown to trigger migration of macrophages and monocytes from the periphery across the blood-brain barrier to the injury site within the CNS [17–19]. Severe TBI patients exhibited a sustained elevation in CCL2 in cerebrospinal fluid compared to healthy uninjured controls for ten days after injury [17]. Lastly, IL-10 has several anti-inflammatory pathways, including halting the production of pro-inflammatory cytokines, downregulating cytokine receptor expression, and inhibiting cytokine receptor activation [20]. This anti-inflammatory cytokine is expressed by microglia and astrocytes, in addition to a variety of immune cells in the periphery, such as Th2 cells, B cells, neutrophils, and macrophages [21]. IL-10 has been touted as a potential biomarker to gauge the severity of brain damage [22, 23], with various clinical studies reporting significant elevations in IL–10 after TBI [24].

Despite the previous TBI studies supporting the use of CCL11, CCL2, and IL–10 to gauge the severity of brain injury, an acute response profile of these markers to subclinical head insults remains unknown. Given their expression levels outside the brain, we carefully
designing the study to isolate subconcussive head impact effects by eliminating extraneous factors that are inherent to field studies, such as body and environmental temperature change, perspiration and hydration, and muscle damage. We conducted a randomized controlled trial using our controlled soccer heading paradigm [25] to evaluate the acute effect of subconcussive head impacts on plasma levels of CCL11, CCL2, and IL-10. The two aims of this study were to (a) examine the difference in the acute response profile of CCL11, CCL2, and IL-10 levels between soccer heading and soccer kicking-control groups and (b) evaluate the influence of participants’ years of heading experience on the changes in CCL11, CCL2, and IL-10 levels caused by subconcussive head impacts. Our primary hypothesis was that 10 soccer headings will induce acute and significant elevations in plasma levels of all three inflammatory markers relative to those of the kicking-control group. Our secondary hypothesis was that more soccer heading experience would exacerbate the plasma CCL11, CCL2, and IL-10 response to 10 soccer headings.

**Methods**

**Participants**

From August 2017 through March 2018, we recruited potential subjects who were enrolled at Indiana University-Bloomington, met the following inclusion criteria, and were free of exclusion criteria. For inclusion, subjects were required to have at least 3 years of soccer heading experience and be between the ages of 18 and 26. Subjects were excluded for a history of head injury during one year prior to the study; a history of vestibular, ocular, or vision dysfunction; or a history of neurological disorders. Our sample size calculation, based on previous subconcussion studies, [26–28] estimated that a minimum of 17 subjects per group would yield a statistical power of at least 0.80 with a significance level of = 0.05. Forty-two potential subjects were assessed for eligibility, and three were
excluded for not meeting inclusion criteria. As a result, 39 healthy adult soccer players were included in the study. Using a simple dice-based randomization method, participants were randomly assigned to either soccer heading (n = 22) or soccer kicking-control (n = 17: see Figure 1). After randomization, participants were unblinded as they needed to physically perform either heading or kicking, whereas biomarker experimenters remained blinded. Subject recruitment ended when at least 17 participants had been randomized to both groups.

Experimental design

This study employed a repeated measures design with four data collection time points (pre-intervention, and 0h, 2h, and 24h post-intervention). Between the pre-intervention and the 0h post-intervention time points, participants in the heading group performed 10 soccer headers while participants in the kicking-control group kicked the ball 10 times (see Soccer heading model subsection). Participants remained in the laboratory until the 2h post-intervention time point and did not engage in strenuous cognitive or physical activities. Participants were instructed to refrain from activities that would include head impacts until after the 24h post-intervention time point.

Soccer heading model

A well-established soccer heading model was used to induce subconcussive head impacts [25–27, 29]. See Bevilacqua et al. [25] for a video version of the soccer heading protocol. A triaxial accelerometer (SIM-G, Triax Technologies Inc, Norwalk, CT) was secured inside a custom headband and positioned directly below each participant’s external occipital protuberance to capture the linear and rotational acceleration (peak linear acceleration [PLA, g], peak rotational acceleration [PRA, krad/s²]) of each head impact. A JUGS soccer machine (JPS Sports, Tualatin, OR) was used to launch a size 5 soccer ball at 25 mph,
which is similar to a center pass from a player near the sideline to the center of the field. Participants in both groups stood approximately 40 ft away from the JUGS machine, which was set up to project the soccer ball at 40° to the horizontal. The participant’s distance away from the machine was calibrated by moving closer or farther away to ensure that the heading participants were in a position to head the soccer ball and the kicking-control participants would be properly situated to kick the ball. Heading participants were instructed to head the ball with their forehead and aim for research personnel standing approximately 16 ft in front and slightly to the side of the participant. Kicking-control participants received a similar set of instructions to kick the ball, rather than heading. Participants performed ten headers or kicks with a one-minute interval between each launch.

Blood sampling and immunoassays

At each of the four data collection time points, four milliliters of venous blood were collected into K₂ EDTA vacutainer tubes (BD Biosciences, Franklin Lakes, NJ). Plasma was separated by centrifugation (1500 g, 15 minutes, 4°C). CCL11 levels were tested using an enzyme-linked immunosorbent assay (ELISA) kit (Human CCL11/Eotaxin Quantikine ELISA kit, R&D Systems, Minneapolis, MN). The lowest detection limit of the assay is 5 pg/mL, and the assay covers a concentration range up to 1,000 pg/mL with an intra-assay precision of 3.4%—5.3% and an inter-assay precision of 8.4%—11.5%. CCL2 measurements were obtained using Human CCL2/MCP–1 Quantikine ELISA (R&D Systems, Minneapolis, MN). The lowest detection limit of the assay is 10 pg/mL, and the assay covers a concentration range up to 2,000 pg/mL with an intra-assay precision of 4.2%—5.9% and an inter-assay precision of 4.5%—5.9%. IL-10 measurements were obtained using Human IL-10 Quantikine ELISA kits (R&D Systems, Minneapolis, MN). The lowest detection limit of
the assay is 3.9 pg/mL, and the assay covers a concentration range of up to 500 pg/mL with an intra-assay precision of 2.5%—6.6% and an inter-assay precision of 5.6%—7.6%. Samples were loaded in duplicate into the ELISA plates according to manufacturer instructions. Fluorescent signals measured by a micro-plate reader (BioTek EL800, Winooski, VT) were converted into pg/mL as per standard curve concentrations and adjusted for the sample dilution factor, when appropriate. To eliminate the inter-assay effect on within-subject data, all samples from each subject for each marker were assayed on the same 96-well plate. The same experimenter performed all assays for each inflammatory marker. Assay data were unavailable for one kicking-control participant for CCL2 assays (outside assay detection range).

Statistical analysis

The primary outcome of this study was to assess the differences in change in acute plasma levels of CCL11, CCL2, and IL-10 between the kicking-control and heading groups. The secondary outcome was to examine the influence of prior soccer heading experience on changes in plasma inflammatory marker levels in response to 10 acute soccer headings.

Demographic differences (age, BMI, number of past concussions, years of soccer heading experience) were compared between the heading and kicking-control groups using Student’s independent t-tests. Sex was compared between the heading and kicking-control groups using Fisher’s exact test. In the primary aim, we tested the difference in plasma levels of CCL11, CCL2, and IL-10 between the kicking and heading groups over time using mixed-effects regression model (MRM), which enables us to account for repeated measurements of the inflammatory markers from the same individuals. We used plasma inflammatory marker levels as outcome variables, and treated group, time (pre, 0h post, 2h post, and 24h post), and group by time interactions as fixed effects. Individual baseline
difference was treated as a random effect. The models were adjusted for covariates such as age, sex, BMI, years of soccer heading experience, and number of previous concussions.

In the secondary aim, we evaluated the influence of years of soccer experience on the plasma inflammatory marker levels in response to 10 soccer headings in the heading group using another MRM. We used plasma inflammatory marker level as outcome variables, and time, years of soccer experience, and time by years of soccer heading experience as fixed effects. Individual baseline difference was treated as a random effect. The model was adjusted for age, sex, BMI, number of previous concussions, and the mean magnitude of head impact (PLA and PRA). As part of exploratory analysis, Pearson correlation coefficient was conducted to assess the potential correlation between years of soccer heading experience and one’s heading technique as reflected by mean impact magnitudes (PRA and PLA). In both MRMs, time was treated as a discrete variable, and 95% CI and p-values were assessed using the jackknife method [30]. All the analyses were performed for each inflammatory marker independently. All t-tests were two-tailed, and the level of significance was set a priori to p < 0.05. All analyses were conducted using statistical software R (version 3.4.1) with package “nlme.”

Ethics statement

This study protocol was performed in accordance with the Declaration of Helsinki and was approved by the Indiana University Institutional Review Board (Protocol No. 1610743422). Written informed consent to participate was obtained from all participants.

Results

Demographic differences between heading and kinking-control groups

Forty-two individuals were assessed for eligibility, and 39 individuals who met inclusion
criteria and were free of exclusion criteria proceeded to participate in the study. There was one voluntary withdrawal at 24h post-intervention (heading, n = 1; see Figure 1). There were no significant differences between the heading and kicking-control groups for sex, BMI, number of previous concussions, or years of soccer heading experience. There was a significant difference between the groups for age (p = 0.010), with the kicking-control group being slightly older than the heading group (see Table 1).

| Demographic and impact kinematic data for the heading and kicking groups. |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Demographics               | Heading                     | Kicking                     | P-value                    |
| n                           | 22                          | 17                          | -                           |
| Age, y                      | 20.05 ± 1.50                | 21.47 ± 1.77                | 0.010                       |
| Sex                         | 9M, 13F                     | 7M, 10F                     | 0.257                       |
| BMI                         | 23.24 ± 2.73                | 24.42 ± 3.13                | 0.216                       |
| No. of previous concussions | 0.64 ± 0.95                 | 0.59 ± 1.70                 | 0.911                       |
| Soccer heading experience, y| 9.14 ± 3.81                 | 10.63 ± 5.02                | 0.305                       |
| Impact kinematics            |                             |                             |                             |
| PLA, g                      | 33.45 ± 4.36                | . a                        | -                           |
| PRA, krad/s²                | 3.63 ± 0.78                 | . a                        | -                           |

Note: All data are expressed as mean ± standard deviation, except for n (number of participants per group) and sex. BMI, body mass index; No., number; PLA, peak linear acceleration; PRA, peak rotational acceleration; g, gravitational force equivalent; krad, kiloradian per squared second.

Primary outcome: Differences in changes in plasma inflammatory marker levels between heading and kicking-control groups

There were no significant differences in post-intervention changes in plasma CCL11, CCL2, and IL-10 levels from baseline between the heading and kicking-control groups. Table 2 summarizes the effects of heading on the changes in CCL11, CCL2, and IL-10 expression.
Table 2. Differences in change from pre-intervention in plasma CCL11, CCL2, and IL-10 between heading and kicking-control group as a reference

|               | CCL11 (pg/mL) | CCL2 (pg/mL) | IL-10 (pg/mL) |
|---------------|---------------|--------------|---------------|
|               | Estimate (95% CI) | P-value | Estimate (95% CI) | P-value | Estimate (95% CI) |
| 0h Post – Pre | 2.2 (-4.3, 8.8) | 0.506 | 8.6 (-18.3, 35.5) | 0.530 | 0.6 (-0.8, 1.9) |
| 2h Post – Pre | 2.2 (-6.2, 10.5) | 0.612 | 1.2 (-20.2, 22.7) | 0.909 | 0.7 (-1.1, 2.5) |
| 24h Post – Pre | 6.4 (-3.2, 16.1) | 0.190 | 15.0 (-29.2, 59.3) | 0.506 | 0.8 (-1.0, 2.6) |

Secondary outcome: influence of prior soccer heading experience on change in plasma inflammatory marker levels in response to the heading intervention

We investigated the effects of soccer heading experience on the changes in plasma inflammatory marker levels in response to acute 10 soccer headings. Our model indicates that when individuals without any soccer heading experience sustain 10 acute soccer headings, plasma CCL11 levels are estimated to significantly decrease by −12.9 [95% CI: −22.2, −3.5, p = 0.007] at 24h post-heading compared with the baseline. The model further demonstrates that years of soccer heading experience significantly modulates the magnitude of changes in plasma CCL11 after 10 headings. Specifically, each unit increase (one year) in individuals’ heading experience is estimated to elevate their change in CCL11 levels from baseline by 2.0 pg/mL (95% CI: 0.8, 3.1) at 24h post-heading, which was supported by a statistically significant time by soccer-heading-experience interaction at 24h post-heading (p = 0.001). For example, if a participant had 10 years of soccer heading experience prior to the study, the effect is magnified by 10 (i.e., the change in CCL11 at 24 post is 19.5 pg/mL higher than ones with zero years of soccer heading experience). Using the estimated parameters, the time course changes of the plasma CCL11 from the pre-heading time point are illustrated for hypothetical participants with 0,
5, 10, and 15 years of soccer experience (Figure 2).

Plasma CCL2 or IL–10 levels and their changes after the heading intervention were not significantly modulated by soccer heading experience. We found a significant time effect for the change in plasma IL–10 at 2h post-heading (–1.4 pg/mL [95%CI: –2.6, –0.1, p = 0.037]) for individuals without soccer heading experience. However, no other significant effects were observed at any other time point for IL–10 with or without heading experience or at any time points for changes in plasma CCL2 with or without heading experience. Table 3 summarizes the time by soccer heading experience interaction effects for the changes in the three plasma inflammatory markers.

|                   | CCL11 (pg/mL) | CCL2 (pg/mL) | IL-10 (pg/mL) |
|-------------------|--------------|--------------|---------------|
|                   | Estimate (95% CI) | P-value | Estimate (95% CI) | P-value | Estimate (95% CI) |
| 0h Post – Pre     | -0.1 (-1.5, 1.2) | 0.855       | -0.7 (-4.6, 3.3) | 0.745   | 0.1 (-0.1, 0.2)   |
| 2h Post – Pre     | 0.6 (-0.6, 1.8)  | 0.352       | 1.4 (-2.6, 5.4) | 0.497   | 0.1 (0.0, 0.2)    |
| 24h Post – Pre    | 2.0 (0.8, 3.1)   | 0.001*      | 5.8 (-1.8, 13.5)| 0.136   | 0.0 (-0.2, 0.2)   |

The relationship between years of soccer heading experience and head impact magnitude

We identified noteworthy findings from our exploratory analysis, which tested the relationship between one’s years of heading experience and head impact magnitude.

There was a significant negative correlation between years of heading experience and mean peak rotational head acceleration (r = –0.57, p = 0.005, Figure 3A); the correlation between years of heading experience and mean peak linear head acceleration was nonsignificant (r = –0.34, p = 0.121, Figure 3B).

Discussion

The current study used controlled soccer heading model to tease out the effects of
subconcussive head impacts on acute inflammatory response, as surrogated by circulating CCL11, CCL2, and IL-10 levels. The three chief findings from this study were: (1) Plasma CCL11, CCL2, and IL-10 response to the heading or kicking intervention were not significantly different between the heading and the kicking-control groups at any time points after intervention; (2) There was a heterogeneous inflammatory response in CCL11 levels over time to subconcussive head impacts; and (3) The heterogeneity in the CCL11 changes was partly modulated by one’s years of soccer heading experience. These data add to the growing body of literature regarding the effects of repetitive head impacts on young adult brain, as the scope of brain injury research expands to include these subclinical head impacts.

Our data indicate that 10 soccer headers were insufficient or negligible mechanical force to induce significant elevations in acute plasma levels of CCL11, CCL2, and IL-10, whereas more severe forms of TBI have shown clear evidence that traumatic forces to the brain triggers an acute inflammatory response. For instance, moderate and severe TBI patients exhibited significant elevations in both CCL2 and IL-10 at hospital admission as compared to those of health controls, with the elevation in IL-10 levels persisted beyond 24h post-admission [31]. Additionally, CCL11 was useful in predicting fatality in moderate and severe TBI such that non-survivors had significantly higher plasma concentrations of CCL11 with odds ratio of 1.90 at admission and 1.90 at 6h after admission compared to survivors [31]. However, sports-related concussion, which is considered as mild TBI, did not alter plasma levels of CCL11, CCL2, and IL-10 [6], which supports that acute concussive and subconcussive head impacts alone may not necessarily trigger a robust neuroinflammatory response.

Our data shed new light on a potential factor that modulates one’s neuroinflammatory response to subconcussive head impacts, whereby greater years of soccer heading
experience was associated with greater increases in plasma CCL11 at 24h after 10 headers. In fact, the MRM results suggest that an individual without any soccer heading experience would exhibit a decrease in plasma CCL11 by 24h post-heading. There are two studies reporting the similar observation. Di Battista et al. [31] assessed plasma CCL11 levels in patients with severe traumatic brain injury (severe TBI) at admission, 6h, 12h, and 24h post-admissions. The researchers observed a significant decline in plasma CCL11 levels at the 12h and 24h post-admission time points compared to healthy control’s referential levels. These trends were notable in ischemic stroke patients, where a significantly lower plasma CCL11 was identified at 24h after stroke events compared to healthy control levels. Furthermore, the lower post-stroke CCL11 levels were predictive of stroke severity and poorer functional outcomes [32]. One potential reason for these observations is that CCL11, known as a pro-inflammatory factor, increases its expression concurrently with inflammatory cytokines such as TNF-alpha and interleukins–1 beta (IL–1) [33] to induce neural inflammation [34]. Yet, neural system injury triggers a surge of immunosuppressive factors like IL–10 [31], whose function is to suppress the release of pro-inflammatory markers, including CCL11 [35]. As a result, the acute increases in plasma IL–10 and decreases in plasma CCL11 levels were identified in patients with moderate-severe TBI [32] and stroke [31]. However, in our study cohort, we did not observe significant changes in IL–10 levels. The specific mechanism of the CCL11’s response to neural injury remains an open-ended question and warrants further investigation.

There are clinical implications of our CCL11 data. Parajuli et al demonstrated that CCL11 does not directly damage neurons but does correspond with increased microglial recruitment and production of reactive oxygen species, suggesting a potential mechanism by which upregulated expression of CCL11 over time in response to head impacts may
contribute to neurodegenerative processes such as CTE [15]. Broglio et al. postulated that exposure to concussions and subconcussive head impacts may accelerate the aging-related decline in cognitive function [36]. This hypothesis was supported by the recent work of Ritzel et al., who showed that mice show signs of accelerated immune aging post-TBI [37]. Long-term exposure to subconcussive head impacts may mimic the effects of aging in the brain over time, which has shown to be characterized by increases in CCL11 in plasma and CBF, which has been linked to cognitive impairments [38–40]. This study provides preliminary evidence for an exposure-dependent acute inflammatory response in healthy young adults, specifically a positive association between years of soccer heading experience and relative change in plasma CCL11 in response to a short bout of subconcussive head impacts. Future research should examine the effects of prior exposure and a larger dose of subconcussive head impacts on plasma CCL11, such as an entire season of soccer or American football.

Using a soccer heading model allowed us to examine the acute inflammatory response to subconcussive head impacts in young adults while eliminating the effects of exercise, impact type, and environmental fluctuations. Including a baseline, pre-intervention measurement and multiple post-intervention time points allowed us to observe the acute profile of plasma CCL11, CCL2, and IL-10 levels. Nonetheless, the results of this investigation should be interpreted in consideration of the several limitations. First, we rely on participants’ self-reported estimates of soccer heading exposure, which in reality varies widely by gender, primary position played, level of play, and playing style [41, 42]. However, the significant, negative correlation between mean peak rotational acceleration and self-reported years of heading experience suggests that the more experienced participants performed the 10 headers with better technique, supporting our reliance on self-reported estimate of heading experience. Second, we did not control for sleep quality,
diet, menstrual cycle phase, or hormonal contraceptive use. Last, we did not collect data after the 24h post-intervention time point, preventing us from determining when or if the experience-dependent increase in CCL11 returned to baseline levels.

Conclusion
Performing ten soccer headers did not elicit significant differences in plasma CCL11, CCL2, or IL-10 levels between the heading and kicking-control groups. However, the relationship between years of prior soccer heading experience and relative change in CCL11 at 24h after heading suggests that circulating CCL11 may have the potential utility to be used as an indirect inflammatory indicator of cumulative head impact exposure in athletes. This study provides valuable reference data for future field studies that prospectively track head impact exposure and the time course of changes in circulating CCL11.

Declarations

Ethics approval and consent to participate
Subjects gave written informed consent before eligibility screening and participation. All procedures in this study followed NIH guidelines and were approved by the Indiana University Institutional Review Board.

Consent for publication
Not applicable.

Availability of data and material
The dataset generated and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests
The authors declare that they have no competing interests.

Funding
This work was partly supported from the Indiana Spinal Cord & Brain Injury Research Fund from the Indiana State Department of Health (to K. Kawata: ISCBIRF 0019939) and IU School of Public Health faculty research grant program (to K. Kawata: FRGP 2246237).

Authors’ contributions

KK designed the study. MEH, ZWB, AK, and RMK collected the data and carried out the experiments. MEH, KK, ZC, and KE analyzed and interpreted the data. MEH drafted the manuscript and created the figures. ZC, KE, and KK reviewed the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors would like to thank Ms. Angela Wirsching for her assistance with subject recruitment, scheduling, and data collection.

References

1. Yang SH, Gangidine M, Pritts TA, Goodman MD, Lentsch AB: Interleukin 6 mediates neuroinflammation and motor coordination deficits after mild traumatic brain injury and brief hypoxia in mice. Shock 2013, 40:471-475.

2. Mouzon BC, Bachmeier C, Ferro A, Ojo JO, Crynen G, Acker CM, Davies P, Mullan M, Stewart W, Crawford F: Chronic neuropathological and neurobehavioral changes in a repetitive mild traumatic brain injury model. Ann Neurol 2014, 75:241-254.

3. Morganti-Kossman MC, Lenzlinger PM, Hans V, Stahel P, Csuka E, Ammann E, Stocker R, Trentz O, Kossmann T: Production of cytokines following brain injury: beneficial and deleterious for the damaged tissue. Mol Psychiatry 1997, 2:133-136.

4. Ghirnikar RS, Lee YL, Eng LF: Inflammation in traumatic brain injury: role of cytokines and chemokines. Neurochem Res 1998, 23:329-340.

5. Nizamutdinov D, Shapiro LA: Overview of Traumatic Brain Injury: An Immunological
Context. *Brain Sci* 2017, 7.

6. Di Battista AP, Churchill N, Rhind SG, Richards D, Hutchison MG: *Evidence of a distinct peripheral inflammatory profile in sport-related concussion. J Neuroinflammation* 2019, 16:17.

7. Morganti-Kossmann MC, Rancan M, Stahel PF, Kossmann T: *Inflammatory response in acute traumatic brain injury: a double-edged sword. Curr Opin Crit Care* 2002, 8:101-105.

8. Ramlackhansingh AF, Brooks DJ, Greenwood RJ, Bose SK, Turkheimer FE, Kinnunen KM, Gentleman S, Heckemann RA, Gunanayagam K, Gelosa G, Sharp DJ: *Inflammation after trauma: microglial activation and traumatic brain injury. Ann Neurol* 2011, 70:374-383.

9. Di Battista AP, Rhind SG, Richards D, Churchill N, Baker AJ, Hutchison MG: *Altered Blood Biomarker Profiles in Athletes with a History of Repetitive Head Impacts. PLoS One* 2016, 11:e0159929.

10. McKee AC, Cairns NJ, Dickson DW, Folkerth RD, Keene CD, Litvan I, Perl DP, Stein TD, Vonsattel JP, Stewart W, et al: *The first NINDS/NIBIB consensus meeting to define neuropathological criteria for the diagnosis of chronic traumatic encephalopathy. Acta Neuropathol* 2016, 131:75–86.

11. Mez J, Daneshvar DH, Kiernan PT, Abdolmohammadi B, Alvarez VE, Huber BR, Alosco ML, Solomon TM, Nowinski CJ, McHale L, et al: *Clinicopathological Evaluation of Chronic Traumatic Encephalopathy in Players of American Football. JAMA* 2017, 318:360-370.

12. Cherry JD, Tripodis Y, Alvarez VE, Huber B, Kiernan PT, Daneshvar DH, Mez J, Montenigro PH, Solomon TM, Alosco ML, et al: *Microglial neuroinflammation contributes to tau accumulation in chronic traumatic encephalopathy. Acta Neuropathol Commun* 2016, 4:112.

13. Amerio P, Frezzolini A, Feliciani C, Verdolini R, Teofoli P, De Pita O, Puddu P: *Eotaxins and CCR3 receptor in inflammatory and allergic skin diseases: therapeutical implications.*
Curr Drug Targets Inflamm Allergy 2003, 2:81–94.

14. Baruch K, Ron-Harel N, Gal H, Deczkowska A, Shifrut E, Ndifon W, Mirlas-Neisberg N, Cardon M, Vaknin I, Cahalon L, et al: CNS-specific immunity at the choroid plexus shifts toward destructive Th2 inflammation in brain aging. Proc Natl Acad Sci U S A 2013, 110:2264–2269.

15. Parajuli B, Horiuchi H, Mizuno T, Takeuchi H, Suzumura A: CCL11 enhances excitotoxic neuronal death by producing reactive oxygen species in microglia. Glia 2015, 63:2274–2284.

16. Cherry JD, Stein TD, Tripodis Y, Alvarez VE, Huber BR, Au R, Kiernan PT, Daneshvar DH, Mez J, Solomon TM, et al: CCL11 is increased in the CNS in chronic traumatic encephalopathy but not in Alzheimer’s disease. PLoS One 2017, 12:e0185541.

17. Semple BD, Bye N, Rancan M, Ziebell JM, Morganti-Kossmann MC: Role of CCL2 (MCP-1) in traumatic brain injury (TBI): evidence from severe TBI patients and CCL2/-/- mice. J Cereb Blood Flow Metab 2010, 30:769–782.

18. Semple BD, Kossman T, Morganti-Kossmann MC: Role of chemokines in CNS health and pathology: a focus on the CCL2/CCR2 and CXCL8/CXCR2 networks. J Cereb Blood Flow Metab 2010, 30:459-473.

19. Deshmane SL, Kremlev S, Amini S, Sawaya BE: Monocyte chemoattractant protein–1 (MCP-1): an overview. J Interferon Cytokine Res 2009, 29:313–326.

20. Strle K, Zhou JH, Shen WH, Broussard SR, Johnson RW, Freund GG, Dantzer R, Kelley KW: Interleukin–10 in the brain. Crit Rev Immunol 2001, 21:427–449.

21. Lobo-Silva D, Carriche GM, Castro AG, Roque S, Saraiva M: Balancing the immune response in the brain: IL–10 and its regulation. J Neuroinflammation 2016, 13:297.

22. Kumar RG, Boles JA, Wagner AK: Chronic Inflammation After Severe Traumatic Brain Injury: Characterization and Associations With Outcome at 6 and 12 Months Postinjury. J
Head Trauma Rehabil 2015, 30:369-381.

23. Schneider Soares FM, Menezes de Souza N, Liborio Schwarzbold M, Paim Diaz A, Costa Nunes J, Hohl A, Nunes Abreu da Silva P, Vieira J, Lisboa de Souza R, More Bertotti M, et al: Interleukin-10 is an independent biomarker of severe traumatic brain injury prognosis. Neuroimmunomodulation 2012, 19:377–385.

24. Garcia JM, Stillings SA, Leclerc JL, Phillips H, Edwards NJ, Robicsek SA, Hoh BL, Blackburn S, Dore S: Role of Interleukin-10 in Acute Brain Injuries. Front Neurol 2017, 8:244.

25. Bevilacqua ZW, Huibregtse ME, Kawata K: In Vivo Protocol of Controlled Subconcussive Head Impacts for the Validation of Field Study Data. J Vis Exp 2019.

26. Hwang S, Ma L, Kawata K, Tierney R, Jeka JJ: Vestibular Dysfunction after Subconcussive Head Impact. J Neurotrauma 2017, 34:8-15.

27. Kawata K, Tierney R, Phillips J, Jeka JJ: Effect of Repetitive Sub-concussive Head Impacts on Ocular Near Point of Convergence. Int J Sports Med 2016, 37:405-410.

28. Oliver JM, Jones MT, Kirk KM, Gable DA, Repshas JT, Johnson TA, Andreasson U, Norgren N, Blennow K, Zetterberg H: Serum Neurofilament Light in American Football Athletes over the Course of a Season. J Neurotrauma 2016, 33:1784-1789.

29. Wirsching A, Chen Z, Bevilacqua ZW, Huibregtse ME, Kawata K: Association of Acute Increase in Plasma Neurofilament Light with Repetitive Subconcussive Head Impacts: A Pilot Randomized Control Trial. J Neurotrauma 2019, 36:548-553.

30. Efron B, Stein C: The Jackknife Estimate of Variance. Ann Statist 1981, 9:586-596.

31. Di Battista AP, Rhind SG, Hutchison MG, Hassan S, Shiu MY, Inaba K, Topolovec-Vranic J, Neto AC, Rizoli SB, Baker AJ: Inflammatory cytokine and chemokine profiles are associated with patient outcome and the hyperadrenergic state following acute brain injury. J Neuroinflammation 2016, 13:40.
32. Roy-O’Reilly M, Ritzel RM, Conway SE, Staff I, Fortunato G, McCullough LD: CCL11 (Eotaxin-1) Levels Predict Long-Term Functional Outcomes in Patients Following Ischemic Stroke. *Transl Stroke Res* 2017, 8:578–584.

33. Chung KF, Patel HJ, Fadlon EJ, Rousell J, Haddad EB, Jose PJ, Mitchell J, Belvisi M: Induction of eotaxin expression and release from human airway smooth muscle cells by IL-1beta and TNFalpha: effects of IL-10 and corticosteroids. *Br J Pharmacol* 1999, 127:1145-1150.

34. Kitaura M, Nakajima T, Imai T, Harada S, Combadiere C, Tiffany HL, Murphy PM, Yoshie O: Molecular cloning of human eotaxin, an eosinophil-selective CC chemokine, and identification of a specific eosinophil eotaxin receptor, CC chemokine receptor 3. *J Biol Chem* 1996, 271:7725–7730.

35. Garcia-Zepeda EA, Rothenberg ME, Ownbey RT, Celestin J, Leder P, Luster AD: Human eotaxin is a specific chemoattractant for eosinophil cells and provides a new mechanism to explain tissue eosinophilia. *Nat Med* 1996, 2:449-456.

36. Broglio SP, Eckner JT, Paulson HL, Kutcher JS: Cognitive decline and aging: the role of concussive and subconcussive impacts. *Exerc Sport Sci Rev* 2012, 40:138–144.

37. Ritzel RM, Doran SJ, Barrett JP, Henry RJ, Ma EL, Faden AI, Loane DJ: Chronic Alterations in Systemic Immune Function after Traumatic Brain Injury. *J Neurotrauma* 2018, 35:1419–1436.

38. Villeda SA, Luo J, Mosher KL, Zou B, Britschgi M, Bieri G, Stan TM, Fainberg N, Ding Z, Eggel A, et al: The ageing systemic milieu negatively regulates neurogenesis and cognitive function. *Nature* 2011, 477:90–94.

39. Hoefer J, Luger M, Dal-Pont C, Culig Z, Schennach H, Jochberger S: The “Aging Factor” Eotaxin-1 (CCL11) Is Detectable in Transfusion Blood Products and Increases with the Donor’s Age. *Front Aging Neurosci* 2017, 9:402.
40. Bettcher BM, Fitch R, Wynn MJ, Lalli MA, Elofson J, Jastrzab L, Mitic L, Miller ZA, Rabinovici GD, Miller BL, et al: MCP-1 and eotaxin-1 selectively and negatively associate with memory in MCI and Alzheimer's disease dementia phenotypes. Alzheimers Dement (Amst) 2016, 3:91-97.

41. Reynolds BB, Patrie J, Henry EJ, Goodkin HP, Broshek DK, Wintermark M, Druzgal TJ: Effects of Sex and Event Type on Head Impact in Collegiate Soccer. Orthop J Sports Med 2017, 5:2325967117701708.

42. Harriss A, Johnson AM, Walton DM, Dickey JP: Head impact magnitudes that occur from purposeful soccer heading depend on the game scenario and head impact location. Musculoskelet Sci Pract 2019, 40:53-57.

Figures
Study design flow chart Subjects were randomized to the heading or kicking-control interventions. Blood samples were collected at baseline (pre-intervention) and at three post-intervention time points (0h, 2h, and 24h post-intervention).
Figure 2

Hypothetical time course of plasma CCL11 by years of experience. The time course changes of the plasma CCL11 from the pre-heading time point are illustrated for hypothetical participants with 0, 5, 10, and 15 years of soccer experience based on the MRM results for the secondary outcome. Error bars represent SEM. Cubic smoothing splines are fit to the mean estimations.
Correlations between years of soccer heading experience and head impact kinematics (A) Mean peak rotational acceleration (PRA, krad/s²) was significantly and negatively correlated with participants’ self-reported years of soccer heading experience ($r = -0.57, p = 0.005$). (B) Mean peak linear acceleration (PLA, g) was not significantly correlated with years of soccer heading experience ($r = -0.34, p = 0.121$).

Supplementary Files

This is a list of supplementary files associated with the primary manuscript. Click to download.

CONSORT-2010-Checklist-MS-Word.docx