**Serum neurofilament light concentration does not increase following exposure to low velocity football heading**

Kieran Austin1, Ben J Lee1, Tessa R Flood1, Jamie Toombs2, Mina Borisova3,4, Mike Lauder1 Amanda Hesslegrave3,4, Henrik Zetterberg3,4,5,6, Neal A Smith1

**Contact Information for Corresponding Author:**

Mr Kieran Austin

Institute of Sport

University of Chichester

College Lane

Chichester

PO19 6PE

[K.Austin@chi.ac.uk](mailto:N.Smith@chi.ac.uk)

+44 (0) 1243 816361

ORCiD: 0000-0002-5438-8587

**Author Affiliations:**

1Institute of Sport, University of Chichester, Chichester, UK

2Centre for Discovery Brain Sciences, UK Dementia Research Institute, The University of Edinburgh, UK

3Department of Neurodegenerative Diseases, University College London, London, UK

4UK Dementia Research Institute at UCL, London, UK

5Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, the Sahlgrenska Academy at the University of Gothenburg, Mölndal, Sweden

6Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden.

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**ABSTRACT**

**Objectives**

To investigate if heading frequency and impact biomechanics in a single session influence the concentration of serum neurofilament light (NF-L), a sensitive biomarker for axonal damage, up to 7 days after heading incident at ball velocities reflecting basic training drills.

**Methods**

Forty-four males were randomized into either control (n=8), 10 header (n=12), 20 header (n=12) or 40 header (n=12) groups. Linear and angular head accelerations were quantified during heading. Venous blood samples were taken at baseline, 6 hours, 24 hours and 7 days after heading. Serum NF-L was quantified using Quanterix NF-L assay kit on the Simoa HD-1 Platform.

**Results**

Serum NF-L did not alter over time (p=0.44) and was not influenced by number of headers [p=0.47; mean(95% CI) concentrations at baseline 6.00 pg/ml (5.00 – 7.00 pg/ml); 6 hours post 6.50 pg/ml (5.70 – 7.29 pg/ml); 24 hours post 6.07 pg/ml (5.14 – 7.01 pg/ml); and 7 days post 6.46 pg/ml (5.45 – 7.46 pg/ml)]. There was no relationship between percentage change in NF-L and summed session linear and angular head accelerations.

**Conclusion**

In adult men, heading frequency or impact biomechanics did not affect NF-L response during a single session of headers at ball velocities reflective of basic training tasks.

**Keywords**: Soccer, Heading, Brain Injury, Axonal Damage, Biomechanics

**INTRODUCTION**

Football (or soccer in North America) is unique in that players purposefully use their head to interact with the ball, known as heading. There is concern over the potential negative effects of long-term exposure to repetitive concussive and sub-concussive head impacts [1]. With over 270 million players worldwide, and several reports of neurodegenerative diseases in the brains of retired athletes [2,3], heading may present a public health concern. Recently, retired professional football players were found to have a higher risk of mortality due to neurodegenerative diseases compared to the general population, and whilst findings have been used to infer potential links between heading and brain injury, no information pertaining to heading was collected [4]. Experimental research assessing the effects of heading on brain health remains contested [5].

Sensitive, objective and minimally invasive measures are needed to track longitudinal changes in brain status. Neurofilament light protein (NF-L), abundant in large-calibre myelinated axons that project into deep brain layers and help to form the scaffolding of the neuronal cytoskeleton, has been proposed as a marker specific to axonal damage [6]. Serum NF-L has also shown utility in identifying head trauma in American Football athletes [7] and boxers [8]. Whilst usually identified in cerebrospinal fluid, where no effects of controlled headings in football have been identified [9], recent technological and methodological advances allow NF-L to be quantified in the blood with up to a 1000-fold more sensitivity [10]. A single football training session, with variable heading exposures (between 7-33 headers), did not lead to an increase in serum NF-L [11]. Conversely, serum NF-L was elevated one hour after a bout of 10 headers at a ball launch velocity of 11.2 m/s [12] and 24 hours following a bout of 40 headers at a ball launch velocity of 21.5 m/s [13]. In the latter, the absence of an omnibus statistical test and large variability within and between groups should be noted. Furthermore, while the ball velocities used by Wallace et al may be emulative of in game scenarios such as corners, goal kicks and clearances, they are much higher than those seen in training drills. While the 11.2 m/s velocity used by Wirsching et al is lower, it too is still higher than those seen in common training drills. Preliminary data in women’s football shows that training can account for over 50% of headers experienced within a season [14]. While the literature has not confirmed this finding within the men’s game, it highlights the possibility that a large proportion of career headers are completed outside of match-play. Although no peer reviewed data is currently available on training scenario ball velocities, pilot data has indicated that basic heading drills rarely exceed 8 m/s (high self-feed; where a ball is thrown high in the air to be headed), with the majority of basic drills falling below this (short throw and return headers 3.5 – 4.5 m/s; long thrown and return headers 5.5 – 7 m/s).

To enhance the prospective understanding of head injury, knowledge of the specific biomechanics of impacts and their relationship to objective and sensitive measures of injury needs to be established. Impact biomechanics typically involves the quantification of linear and angular accelerations of the skull, a proxy for brain motion identified by skin or mouthguard mounted accelerometers [15]. These accelerations are often reported within sub-concussive research, but their relationship with sensitive measures of injury rarely examined, and the validity and reliability of skin mounted accelerometers is highly questionable [16]. If numerous head impacts occur within a sport, the overall linear and angular skull accelerations can be summed to identify an overall session or overall cumulative impact load [14]. Recently, Rubin et al. (2019) showed that greater pre-to-post training changes in plasma NF-L were associated with greater number of hits sustained within American football players (32 vs 4 hits for high vs low impact group respectively) and greater magnitude (summed linear acceleration 899 vs 70g and summed angular acceleration 55,457 vs 5514 rads/s-2 for high vs low impact group respectively). Such an approach could prove useful in understanding the relationship between football heading impact biomechanics and changes in measures of brain injury. Quantification of summed linear and summed angular accelerations could also control, or at least mediate, the effect inter-individual technique differences have on impact biomechanics.

The aims of the current study were to assess how the number of headers, fed at a ball velocity emulative of training scenarios, completed in a single session affected serum NF-L levels over time. Secondly, we assessed the relationship between summed linear or angular accelerations experienced through heading and change in serum NF-L. It was hypothesized that heading would increase NF-L over time and would be increased to a greater degree with more headers. It was also hypothesized that those with greater summed session linear and summed session angular head accelerations would see greater increases in NF-L.

**METHODS**

**Population and Procedure**

The study followed a randomized control trial design, with 44 male participants (age = 23.7 ± 4.8 years; height = 179.9 ± 6.5 cm; mass = 82.4 ± 13.1 kg) with no history of head injury within the last year and recreational experience of heading randomly assigned to either a control group (Control; *n* = 8), a 10 header group (10H; *n* = 12), a 20 header group (20H; *n* = 12) or 40 header group (40H; *n* = 12). Based on an *a priori* sample size analysis, 36 or higher participants were required to achieve a medium effect size, assuming correlation among repeated measures of 0.5, at an alpha of 0.05 and power of 80%. A greater number of participants were included to offset potential attrition. Participants supplied written informed consent. All participants were required to not partake in activities involving head impacts over the course of the study, and to not have completed activities involving head impacts for the two weeks prior to baseline testing, as biomarker levels have previously been shown to not be elevated 10 days after a heading session [9].Participants attended a preliminary hour-long session, followed by three follow up sessions. In the primary session, baseline venous blood samples were taken, followed by the heading protocol. Blood samples were then taken six hours after heading, 24 hours’ after heading and seven days’ after heading. The control group did not partake in the heading protocol, and instead returned six hours following their baseline blood sample.

The heading protocol consisted of participants completing 10, 20 or 40 headers fed from a researcher standing on a balcony 4 meters above and 4.7 meters in front of the participant. Heading doses were chosen in line with previous research [9,13,12]. A size 5 football inflated to 12 psi was dropped from a standardized height and position towards the participant, who was instructed simply to direct the ball to a 1m x 1m square taped to a crash mat 4.7 meters in front of the participant. The height of drop elicited a ball velocity of 8 ± 0 .1 m.s-1. No instructions were given regarding how forcefully to head the ball. There was at least a 30 second break between headers.

**Blood Sampling Procedures and Analysis**

Blood samples were collected form an antecubital vein via venepuncture into 10 mL Serum tube (Sarstedt, Nümbrecht, Germany) following 20 minutes of supine rest. Samples were left at room temperature for 30 minutes to coagulate, before being centrifuged at 1500g for 15 minutes. Once separated, serum was aliquoted and stored at -80 **°**C. Serum NF-L was measured by Single molecule array (Simoa) on an HD-1 analyser (Quanterix, Billerica, USA) as previously described [19]. Lower limit of quantification for the NF-L assay was 0.696 pg/mL when compensated for a four-fold sample dilution. Intra-assay coefficient of variation for low- and high concentration quality control samples was less than 11%. The mean coefficient of variation for all samples was < 5%. The minimum clinically important difference (MCID) for serum NF-L (i.e. the smallest change in NFL that would identify as important) was predefined as a change of >10 pg.mL-1 [20].

**Impact Kinematics**

Impact Kinematics were collected using a 10-camera three-dimensional motion capture system (3D MoCap; Vicon T40S, Oxford, UK) sampling at 1000Hz. Participants wore a neoprene swim cap with a chin strap, with six spherical reflective markers attached at the occipital protuberance, above the left and right ears, and three tracking markers attached in a triangle formation at the posterior surface of the head above the occipital protuberance (Figure 1). Six hemispherical markers were attached to the ball. Data was collected and gap filled using Vicon Nexus V2.9.2 (Vicon, Oxford, UK).

\*\*\*\* Please insert Fig. 1 near here \*\*\*\*

**Kinematic Data Processing**

Kinematic dependant variables included peak linear, peak angular, summed linear and summed angular acceleration of the head. Pre-header, post-header and change in ball velocity were analysed as an estimation of impact quality and to determine impact contact time. Motion data were imported into Visual 3D (C-Motion, Rockville, USA), where head and ball segments were modelled as spherical segments. The head segment was orientated so that movement in the *x* axis reflected flexion/extension; the *y* axis lateral flexion; and *z* axis axial rotation of the head/neck junction. Joint angles were defined using the *xyz* cardan sequence. Raw ball marker trajectories were low pass filtered with a cut off frequency of 50Hz, determined via visual inspection.

The first and second derivative of ball segment centre of mass (COM) displacement data were calculated to determine ball velocity (m/s-1) and acceleration (m/s-2) respectively, and the second derivative of the head segment COM displacement calculated to determine head linear acceleration (gravitational units; *g*). A root mean square was applied to all three variables to determine resultant ball velocity, resultant ball acceleration and resultant linear head acceleration. The resultant angular acceleration (rads/sec-2) of the head was determined using the root mean square of the second derivative of the angle between the head segment and global laboratory origin.

Ball contact time was defined between the first instance of ball acceleration exceeding 700 m.s-2 until the frame ball acceleration decreased below the same threshold. This threshold

was chosen via visual inspection of ball kinematic data that best fit ball contact. Incoming and outgoing ball velocity was defined as the average of the 15 frames 10 frames before and 10 frames after these contact points respectively, to account for roll on and off caused by ball data filtering. Peak linear and angular resultant head accelerations were defined as the respective peak values during ball contact time.

**Automatic Time-Frequency Filtering Procedure for Head Kinematics**

Conventional biomechanical filters utilise a singular cut off frequency, which are inappropriate for impact events due to the amplification of the frequency content of the impacting body’s motion [21]. An alternative strategy is to use a filter with a time-varying cut-off frequency [22–24]. In summary, when an impact produces an expansion of the signal frequency content of a motion capture marker, the filtering algorithm increases the cut-off value to optimise the signal to noise ratio [22]. From Visual 3D, raw head marker trajectories were exported to Matlab (MathWorks, Massachusetts, USA) to be process through custom code [22], before being imported back into Visual 3D. The process has been shown to best match reference accelerometery in pendulum impact data [23] and lower limb kinematics within football kicking [22,25].

**Statistical Analysis**

Preliminary statistical analysis was conducted using JASP (JASP Team, 2016; jasp-stats.org). Following satisfied normal distribution checks, five one-way ANOVA’s assessed differences between heading groups for Incoming Ball Velocity, Outgoing Ball Velocity, Change in Ball Velocity, Mean Peak Linear Head Acceleration and Mean Peak Angular Head Acceleration, to ensure groups did not differ on average in terms of impact magnitude or quality.

Summed peak linear and summed peak angular accelerations were calculated as the sum of all respective impact accelerations for each individual. The relationship between summed head accelerations and NF-L response was assessed using a Pearson’s Product Moment correlation, assessing the relationship between summed linear/angular head acceleration and percentage change in NF-L from baseline at 6 hours, 24 hours and 7 days, for a total of six correlations. Significance was set at *p*<0.05 for all statistical tests.

A two-way mixed effects ANCOVA was used to assess interaction, group, and time effects on NF-L after controlling for baseline NF-L levels. Five participants (11% of total) were missing one NF-L timepoint point each (3% of total data points). After Little’s test confirmed that data was missing completely at random (MCAR; χ2 = 6.8, DF = 9, p = 0.65 [26]), multiple imputation was used to fill missing data points [27]. Five imputations were completed using predictive mean matching, using all continuous repeated measures variables as predictors. ANCOVA analysis was completed in R Studio (R Core Team, 2019) using the “mice” [28] and “rstatix” packages [29].

**RESULTS**

There was no difference between heading groups for incoming ball velocity, outgoing ball velocity, mean peak linear head acceleration or mean peak angular head acceleration (Table 1).

After controlling for baseline NF-L concentrations, there were no group x time interaction effect (F(6,150.3) = 0.933, p = 0.47) and no main effect for time (F(2,7775) = 0.825, p = 0.44). There was a main effect for heading condition after controlling for baseline NF-L concentrations (F(3,1019.33) = 3.03, p = 0.02). Scheffe adjusted post hoc tests only showed the control group to have significantly greater concentrations of serum NF-L than the twenty header group (p = 0.04). Group and time effects for serum NF-L concentration are presented in figure 2.

\*\*\*\* Please insert Table 1 near here \*\*\*\*

\*\*\*\* Please insert Fig. 2 near here \*\*\*\*

There was no relationship between summed session linear head acceleration and NF-L percent change at six-hour post, 24 hours post or seven days post heading, and no relationship between summed angular head acceleration and NF-L percent change at six hours post, 24 hours post or seven days post heading (Table 2). Summed linear head accelerations ranged from 63.8 – 818.8 *g*, and summed angular head accelerations ranged from 11,909 – 122,473 rads.s-2.

\*\*\*\* Please insert Table 2 near here \*\*\*\*

**DISCUSSION**

The aims of the study were to assess how number of common training ball velocity headers completed in a single session affected serum NF-L levels over time, and to assess the relationship between summed head accelerations experienced through heading and change in serum NF-L over time. Contrary to the experimental hypotheses, NF-L did not increase over time regardless of heading dose and change in NF-L was not related to summed session linear or angular head accelerations.

**Serum NF-L**

Current findings for heading groups conflict with those of previous literature investigating how football heading effects serum NF-L concentrations. Previous research saw increases at and one-hour [12] and 24 hours [13] post heading respectively, no such pattern was observed in the current study. Reasons for this conflict of results could be due to differences in ball velocity, where a ball launch velocity of 11.2 m/s [12] and 21.4 m/s [13] were observed compared to the 8.03 m/s used in the current study. It could be possible that the higher ball velocity prompting higher head impact magnitudes seen by Wirsching *et al.,* (2019) could have induced a more pronounced NF-L reaction, but it is curious that even in the high heading incidence groups in the current study, no uniform change was present. Although the change in serum NF-L observed by Wirsching et al. was statistically different (+0.66 pg.mL-1), it is questionable as to whether such a small change has any clinical relevance is also considerably lower than the suggested clinically important change of 10 pg.mL-1 [20].While post hoc tests in the current study did show the control group to have significantly higher concentrations of serum NF-L than the twenty header group (Mean[95% CI]; 11.297 [9.814 - 12.781] vs 9.183 [7.819 – 10.547] respectively), this difference fell well below the aforementioned proposed value for clinically important change, and were not interpreted further in lieu of significantly time or interaction effects. Hence, the current data shows that, even up to an extremely high heading frequency, a single session of headers at ball velocities reflective of those used in common training drills does not produce a measurable change in markers for axonal damage. While this could indicate that practice sessions with similar drills are relatively safe, chronic loading effects are yet to be established.

**Heading Kinematics**

The current study utilised 3D MoCap, combined with a time varying filtering algorithm to maintain signal integrity, to quantify impact kinematics. There were no differences in mean peak linear or angular accelerations between groups. For all groups, linear head accelerations showed a mean of 15.3 ± 5.6 *g* and angular head accelerations a mean of 2143 ± 609 rads/s2. The values reported are less than those seen by previous research showing an increase in NF-L following exposure to heading (31.1 – 34.5 *g* & 2930 – 4040 rads/s2 [12]). While this is unsurprising given the greater ball velocity used within the previous research, it should be noted that the validity of sensors such as those used by Wirsching *et al.* has been questioned [16]. While not representative of in game football heading, the protocol in the current study used is similar to simple ‘self-serve’ or ‘throw-and-return’ heading drills often used with beginners. Therefore, it could be possible that such drills used to introduce technique to beginners do not produce a stimulus capable of measurable axonal damage, although it should be stressed that cumulative practices and maturation effects of beginner players could heavily influence this and will require further study.

**Session Summed Head Accelerations**

To the author’s knowledge, this is the first study to investigate a relationship between whole session summed head accelerations and expression of markers of brain injury during football heading. No such relationship was found. Previous work demonstrated a relationship between summed linear and angular head accelerations and changes in log serum NF-L levels within American Football players [17], with a similar range of summed linear and angular accelerations reported as the current study. Difference here could be due to the mechanism of injury, whereby American Football tackles produced a greater peak angular acceleration of the head. It has been hypothesized that as NF-L is abundant in long, large calibre myelinated axons, it may be more susceptible to rotational impacts, which were not present in the current study either due to chosen technique of the individual or the nature of the heading protocol used [30].

An alternative explanation for lack of differences could be surrounding the nature of a ‘session summed head accelerations’, whereby it is hard to delineate the effects of the impact magnitude or impact incidence. For instance, for a given overall sum, would fewer impacts at a higher magnitude produce different outcomes to more impacts at a lower magnitude? There is also a possibility that time between impacts could factor as a confounding element during the quantification of head loading, as has been noted in previous literature [31]. It is clear that further, longitudinal work surrounding overall cumulative head loading is needed.

**Limitations and Future Considerations**

The current study only utilises a single session of headers at a single ball velocity, and hence is not representative of longitudinal exposure to varying heading situations experienced by football players. The study only utilises adult male participants, whereas it has been shown that females and adolescents could experience different levels of microtrauma in relation to head impacts [32,33]. Finally, although the 3D MoCap system and time varying filter algorithm have been validated for use in lower limb accelerometery, they are yet to be experimentally validated in quantifying head impact biomechanics. Whilst values produced are in agreement with previous literature, comparison with a gold standard is still warranted.

**Conclusions**

The current study provides evidence that low ball velocity football heading does not result in a measurable increase of a proposed marker for axonal damage within in a single session in adult males, regardless of incidence rate or cumulative load applied. While this could suggest high doses of low impact magnitude head impacts are safe in acute doses, further study is needed with regards to the suitability of biomarkers used and longer-term cumulative effects of sub-concussive impacts.

**WHAT ARE THE NEW FINDINGS?**

* A single session of low ball velocity football headers, emulative of common training scenarios, does not increase serum NF-L.
* The number of headers completed in a single session does not affect NF-L response.
* There is no relationship between summed session impact biomechanics and NF-L response.

**DATA SHARING STATEMENT**

All data shall be made available upon request.

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**TABLES**

**Table 1.** Descriptive (mean ± SD) and inferential (One-way ANOVA) statistics of kinematic data.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Heading Group | | | *F* Value | Sig. |
|  | 10H | 20H | 40H |
| Mean Peak Linear Head Acceleration (g) | 17.3 ± 8.1 | 13.8 ± 4.1 | 14.8 ± 3.8 | 1.261 | 0.297 |
| Mean Peak Angular Head Acceleration (rads/s/s) | 2350 ± 784 | 1986 ± 506 | 2092 ± 484 | 1.143 | 0.331 |
| Incoming Ball Velocity (m/s) | 8.00 ± 0.09 | 8.02 ± 0.16 | 8.05 ± 0.11 | 0.475 | 0.626 |
| Outgoing Ball Velocity (m/s) | 8.63 ± 1.23 | 8.70 ± 1.17 | 8.42 ± 1.12 | 0.177 | 0.838 |

**Table 2.** Pearson product moment correlations assessing relationships between summed session head accelerations and percentage change in NF-L from baseline at each timepoint

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Summed Session Linear Acceleration (g) | | Summed Session Angular Acceleration (rads.s2) | |
|  | *R* | Sig. | *r* | Sig. |
| Change in NF-L at 6 Hours (%) | -0.018 | 0.92 | -0.062 | 0.72 |
| Change in NF-L at 24 Hours (%) | 0.143 | 0.42 | 0.113 | 0.53 |
| Change in NF-L at 7 Days (%) | 0.165 | 0.34 | 0.215 | 0.215 |

**FIGURES**

**Figure 1**

**A picture containing ball, looking, sitting, holding

Description automatically generated**

**Figure 2**

**A picture containing object, antenna

Description automatically generated**

**FIGURE LEGENDS**

**Figure 1.** Head mounted marker placements. 1 = Occipital Protuberance; 2 = Left Mastoid; 3 = Right Mastoid; 4 = Tracking marker superior to left ear; 5 = Tracking marker superior to right ear; 6 = Tracking marker superior to Occipital Protuberance.

**Figure 2.** Mean group serum NF-L (± 95% confidence interval) across time points for Control (n = 8), 10 headers (10 H, n = 12), 20 headers (20 H, n = 12) and 40 headers (40 H, n = 12).