Journal of Visualized Experiments

In-vivo protocol of controlled subconcussive head impacts for validation of field study data

--Manuscript Draft--

Article Type:	Invited Methods Article - JoVE Produced Video
Manuscript Number:	JoVE59381R1
Full Title:	In-vivo protocol of controlled subconcussive head impacts for validation of field study data
Keywords:	Subconcussion, Soccer Heading, subconcussive head impact, Chronic Traumatic Encephalopathy, Repeated Measures, Methodology, Concussion
Corresponding Author:	Keisuke Kawata UNITED STATES
Corresponding Author's Institution:	
Corresponding Author E-Mail:	kkawata@indiana.edu
Order of Authors:	Zachary W. Bevilacqua
	Megan E. Huibregtse
	Keisuke Kawata
Additional Information:	
Question	**Response**
Please indicate whether this article will be Standard Access or Open Access.	Standard Access (US$2,400)
Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations.	Bloomington, Indiana, United States of America

SCHOOL OF PUBLIC HEALTH
INDIANA UNIVERSITY
Department of Kinesiology
Bloomington

Aaron Berard, PhD
Science Editor
Journal of Visualized Experiments

11-10-18

Dear Dr. Berard,

Please accept our submission entitled, "*In-vivo* protocol of controlled subconcussive head impacts: validation of field study data" for publication in the Journal of Visualized Experiments. The manuscript has not been submitted for publication elsewhere. This manuscript outlines a subconcussive soccer heading model (SSHM) which is emerging as a reliable and leading methodology for examining the neural impact of subconcussive hits to the head. Our manuscript details a stepwise explanation of the SSHM, as well as the background and necessity for researching subconcussion. Furthermore, we supplement the efficacy of the SSHM by presenting results from a previous study of ours, in which the SSHM was utilized. Lastly, we show how the SSHM exhibits vast clinical relevance and implications, which will undoubtedly intrigue the scientific community.

Thank you for the invitation to submit.

Sincerely,

Keisuke Kawata, PhD, ATC

TITLE:

In Vivo Protocol of Controlled Subconcussive Head Impacts for the Validation of Field Study Data

AUTHORS & AFFILIATIONS:

Zachary W. Bevilacqua[1], Megan E. Huibregtse[1], Keisuke Kawata[1,2]

[1]Department of Kinesiology, College of Arts and Sciences, Indiana University, Bloomington, IN, USA

[2]Neuroscience Program, College of Arts and Sciences, Indiana University, Bloomington, IN, USA

Corresponding author:
Keisuke Kawata (kkawata@indiana.edu)
Department of Kinesiology, School of Public Health,
1025 E. 7th Street, C215, Bloomington, IN 47405
Tel: (812)-855-5244

Email addresses of co-authors:
Megan E. Huibregtse (mhuibreg@indiana.edu)
Zachary W. Bevilacqua (zwbevila@iu.edu)

KEYWORDS:

Subconcussion, soccer heading, chronic traumatic encephalopathy, repeated measures, methodology, concussion

SUMMARY:

The subconcussive soccer heading model is a safe and concise methodological approach to isolate and measure the effects of subconcussive head impacts.

ABSTRACT:

Subconcussive hits pose a threat to neuronal health as they have shown to induce neuronal structural damage and functional impairment without causing outward symptomology and appear to be a key contributor to an irreversible neurodegenerative disease, chronic traumatic encephalopathy (CTE). In addition, athletes can incur more than 1,000 of these hits per season. The subconcussive soccer heading model (SSHM) is a relevant, reproducible, and leading method of isolating and examining the effects of these subconcussive head impacts. By controlling variables such as ball traveling speed, the frequency of impacts, interval, ball placement to the head, as well as by measuring head impact magnitude, the SSHM provides the scientific community with a superior avenue of investigating the acute subconcussive effects on neuronal health. In this paper, we demonstrate the utility of SSHM in studying a time-course expression of neurofilament-light polypeptide (NF-L) in plasma in a repeated measures fashion. NF-L is an axonal injury marker that has previously been shown to be elevated in boxers and football players following subconcussive head trauma. Thirty-four adult aged soccer players were recruited and randomly assigned to either a soccer heading (n = 18) or kicking (n = 16) group. The heading group executed 10 headers with soccer balls projected at a velocity of 25 mph over 10 min. The kicking

group followed the same protocol with 10 kicks. Plasma samples were obtained before and at 0 h, 2 h, and 24 h after heading/kicking and assessed for NF-L expressions. The heading group showed a gradual increase in plasma NF-L expression and peaked at 24 h after the heading protocol, whereas the kicking group remained consistent across the time points. These results confirmed the NF-L data from clinical field studies, encouraging the use of SSHM to validate clinical subconcussion data.

INTRODUCTION:

Long-term, repetitive exposure to subconcussive head impacts has been proposed as one of the key contributors for developing the neurodegenerative disease CTE[1–5]. Each year, approximately 2.5 million high school and college athletes engage in contact sports that frequently induce these subconcussive insults through rapid acceleration-deceleration of the body and head[6,7]. Specifically, contact sport athletes may experience several 100 up to a 1,000 of such impacts per season[6,8,9]. Additionally, other populations, such as military men and women, have registered more than 300,000 head injuries since 2001, which has manifested into the recent diagnosis of CTE within a retired military veteran[10]. This diagnosis parallels with 110 postmortem CTE brains of American football players and four postmortem soccer players to present a burgeoning public health issue[11,12]. In light of the staggering prevalence, head impact research must shift its gaze to incorporate sound, precise methods of analyzing the acute debt subconcussive hits are inducing in a variety of arenas.

The SSHM presented here is one that satisfies the current methodological need to safely induce common mechanical stresses placed upon neural tissue during contact sport activities. The implementation of this model allows investigators to meticulously manage ball traveling speed, the frequency of impacts, interval, ball placement to the head, as well as measurements of head impact magnitude[13,14]. While these factors are virtually impossible to control in the field setting, the SSHM provides an outlet for researchers to isolate the effects of subconcussive head impacts. Furthermore, through the elimination of confounding variables seen during play (e.g., effects from vigorous exercise, body damage, body temperature change, and hydration/perspiration), the SSHM provides a superior method of validating clinical observations.

The SSHM has direct parallels to head impacts seen specifically within the realm of sport. As such, the literature has already begun to show its utility and corroborate the findings of cumulative head impact burden from other investigators. For example, we have demonstrated that the burden of subconcussive head impacts significantly drive neuro-ophthalmologic dysfunction amongst soccer athletes[13,15]. In addition, as few as 10 subconcussive impacts have been shown to immediately perturb vestibular function which can be normalized after 24 h of resting[16]. In this methodological report, we describe the application of SSHM to safely study the effects of subconcussive head impacts and introduce one of our findings that repetitive subconcussive head impacts gradually increase the concentration of a neuron-derived blood biomarker, namely NF-L[14]. This finding not only substantiates previous results of NF-L presence due to repetitive subconcussive blows to the head[17,18] but also validates that the SSHM can reproduce such findings in a controlled clinical manner.

PROTOCOL:

The authors verify that the Indiana University Institutional Review Board approved the study (protocol # 1610743422) and written informed consent was obtained.

NOTE: The SSHM was integrated into a repeated measures design that is intended to investigate changes among dependent variables within subjects at 0 h, 2 h, and 24 h post-intervention compared to individual pre-intervention values. This study design allows researchers to track changes for a 24 h period, which is the typical timeframe between athletic practices. In the present study, soccer players were randomly assigned to either a soccer heading (*n* = 18) or a soccer kicking group (*n* = 16).

1. Setup

1.1. Following baseline measurement collection (pre-intervention), begin the SSHM with the positioning of a soccer ball launcher approximately 40 ft away from the subject, as well as ensuring the soccer ball is inflated to 9 psi.

1.2. The face of the machine displays two identical dials, which regulate the speed of the left and right wheels, and an on/off switch in between them. Set both these dials to a standardized speed of choice.

NOTE: For this demonstration purpose, the ball traveling speed has been set to 30 mph. This speed was chosen as it simulates a soccer throw-in from the sideline to midfield. Soccer players frequently perform this maneuver during practice and games.

1.3. Place 3 inch blocks underneath the wheels of the ball launcher to allow for the desired trajectory (no blocks needed for kicking subjects). Once this is complete, the ball launcher is then angled to 40°, which is measured as the angle between the ground and the midline of the rotating wheels.

NOTE: The angle measurement is taken with a goniometer, and the machine can be adjusted after loosening a knob located along the blue rails where the soccer ball is loaded.

1.4. Once the soccer machine is properly set, fit the subjects with a triaxial accelerometer embedded within a head-band pocket and positioned directly below the external occipital protuberance (inion) to monitor linear and rotational head accelerations.

1.5. Start up the corresponding software for the accelerometer, and enter the subject's information accordingly. At this point, the subject is ready to begin the familiarization trials of the intervention (heading, kicking).

2. Familiarization trials

2.1. Position the subject approximately 40 ft in front of the ball launcher.

2.2. Be sure to explain to the subject that the ball launcher will volley the soccer ball to them and that they simply need to simulate intervention contact with the ball (i.e., heading subjects will catch the ball with their hands in front of their forehead before head-to-ball contact is made, kicking subjects will "trap" the ball on the ground with their foot instead of volleying the ball back, and standing subjects will remain static and not make contact with the ball).

2.3. When the subject understands and feels ready, have the researcher turn the ball launcher on, load the soccer ball onto the blue rails, and finally push the ball into the rotating wheels after a 3-2-1 countdown.

2.4. After stopping the ball as described previously (step 2.2), have the subject roll the ball back.

2.5. Repeat steps 2.3 and 2.4 two to four additional times (no rest time required between) to ensure that the subject positioning is correct and interaction with the ball will be safe and controlled. This concludes the familiarization trials.

3. Intervention

3.1. Verbally confirm that the subject is ready. Once confirmed, give instructions to the heading subjects to only make forehead contact with the ball; tell the subjects to avoid potential impacts to the crown, parietal, and temporal lobes. Instruct the kicking subjects to kick the ball only while it is in flight as ball contact with the ground will attenuate the subsequent impact to the foot.

3.2. Instruct both heading and kicking subjects to volley the ball to a target (additional researcher) approximately half the distance between them and the machine. As best they can, ask subjects to do this in a manner that will mimic the arched trajectory the ball took during its flight toward the subject.

3.3. Activate the triaxial accelerometer and begin the recording.

3.4. Load the soccer ball onto the blue rails, push the ball into the rotating wheels after a 3-2-1 countdown, and make sure appropriate contact is made.

3.5. Repeat step 3.4 nine more times with a 60 s rest in between bouts. If the subject forgoes interaction with the ball (due to inopportune placement or suspected contact with the body in an area that must be avoided), then volley the ball to the subject again promptly, without any rest period.

3.6. In between each head contact with the ball, verify that the triaxial accelerometer registered an impact (using triaxial software; kicking subjects should not register G-force).

3.7. Once the intervention is concluded, turn off the ball launcher and stop the triaxial recording (important, as the movement required to remove the headband could record another "impact"). Once the recording has stopped, remove the headband.

REPRESENTATIVE RESULTS:

The results represented here were interpreted from a previous article[14], in which the SSHM was utilized as previously described. In this particular study, we aimed to show how the SSHM could induce changes in plasma levels of NF-L, which is an axonal injury marker that is hypothesized to filter out of the cranium and into the peripheral blood following head impacts.

SSHM and head kinematics

The present data were derived from 34 subjects who were eligible for analysis (heading group: *n* = 18, and kicking [control] group: *n* = 16). There were no significant differences in any demographic characteristic between the groups. Demographics and head impact kinematics are detailed in **Table 1**. Head impact kinematic data showed that the heading group experienced a median linear head acceleration of 31.8 *g* per head impact (IQR: 31.1–34.5 *g*) and a median rotational head acceleration of 3.56 krad/s^2 per head impact (IQR: 2.93–4.04 krad/s^2). In contrast, the kicking (control) group did not result in detectable levels of head acceleration (as expected) (**Table 1**).

SSHM effects on NF-L biomarker levels

The SSHM was able to produce the following outcomes. See **Figure 1** for the visual representation of the results[14]. (i) There was a gradual increase in plasma NF-L expression, as illustrated by a statistically significant time effect for the head impact group, $F(1,31) = 9.17$, $p = 0.0049$. For example, 0.03 pg/mL of NF-L is estimated to increase every hour after 10 headers (SE = 0.001). (ii) There was no significant time effect for the kicking (control) group, $F(1,31) = 1.20$, $p = 0.28$. (iii) Follow-up paired *t*-tests with Bonferroni correction within the heading group revealed that a significant difference appeared at 24 h post-heading (3.68 ± 0.30 pg/mL) compared to pre-heading (3.12 ± 0.29 pg/mL, $p = 0.0013$; Cohen's d = 1.898). (iv) Linear regression, adjusting for the baseline NF-L level, was used to assess the between-group difference at the 24 h post-intervention time point and distinguished that the NF-L level in the heading group was significantly higher than the kicking (control) group with an estimated mean difference of 0.66 pg/mL (SE = 0.22, $p = 0.0025$).

FIGURE AND TABLE LEGENDS:

Table 1: Demographics and impact kinematics by group.

Figure 1: Changes in plasma NF-L levels before and after subconcussive impacts. In the heading group, NF-L was elevated at 24 h post-heading compared with pre-heading and 0 h post-heading time points, but the kicking (control) group remained static across all time points. The heading group's NF-L level at 24 h post-heading was higher than that of the kicking (control) group. Data are presented as the mean ± SEM. NF-L = neurofilament light; SEM = standard error of the mean.

The figure is reproduced from Wirsching et al.[14] with permission from Mary Ann Liebert, Inc., New Rochelle, NY.

DISCUSSION:

While contact sports like American football might appear to be driving the need for a concise research model to study subconcussive impacts, other sports like soccer may account for the dominant share of subconcussive exposure around the globe as approximately 265 million people participate in what is perhaps the world's most popular sport[19]. However, while the majority of the suspected long-term neurodegenerative effects of subconcussion have been autopsied in American football players, the resemblance of football head impacts to soccer headers is surprisingly large. For instance, 10 bouts of soccer heading in the current study induced approximately 300 *g* and 35 krad/s^2, which were near identical to previous reports using similar subconcussion models[20,21]. These impact kinematics were comparable to the hits observed in American football, where the average college football player incurs 7.0–9.4 hits during practice and 25 hits during games, with a mean peak linear acceleration per hit of 28.8–32.0 *g*[22–25]. Furthermore, other contact sports, such as ice hockey, have displayed similar or greater peak linear head accelerations than these results. For example, a recent study examining both male and female collegiate ice hockey athletes revealed that, over the course of a season, these athletes would register peak linear accelerations of 41.6 *g* (36.6–49.5 *g*) and 40.8 *g* (36.5–49.9 *g*) for males and females, respectively (median [IQR])[26]. Additionally, the head impact telemetry system that was utilized in this ice hockey study revealed that nearly one-third of males (28.0% [21.2%–33.5%]) and females (29.3% [24.8%–32.1%]) (median [IQR]) were inflicted on the front of the head[26]. These results directly parallel to the head impacts induced by using the SSHM, which indicates the vast clinical utility of the current methodology.

There is, however, one limitation that should be addressed when attempting to extrapolate soccer and American football head impacts to other contact sports like ice hockey. Head impacts in ice hockey are not routine drills and are penalized, and they usually are the result of deliberate contact with another player. In fact, subconcussive head impact incidence for collegiate ice hockey athletes is known to be substantially low: 1.3 (1.0–1.7) per practice and 6.3 (3.5–9.0) per game for males and 0.9 (0.6–1.0) per practice and 3.7 (2.5–4.9) per game for females[26].

The present study is not the first to explore the expression of NF-L following head trauma; however, it is the first to isolate head trauma as the cause for NF-L expression outside the central nervous system. Oliver et al. assessed for serum NF-L expression within American football athletes at eight different time points over the course of a season (~6 months)[17]. Players were categorized into two groups, starters and nonstarters, with an assumption that starter athletes will be exposed to larger quantities of head hits, whereas nonstarters will incur lower quantities of head hits than the starters. The authors identified significant increases in serum NF-L expressions in the starter group over time across the season[17]. Conversely, the nonstarter group remained consistent throughout the season. Similarly, Shahim et al. analyzed serum NF-L levels in boxers following a subacute phase of subconcussive head trauma (7–10 days prior)[18]. In their analysis, boxers were categorized into two groups (mild vs. severe head impact groups) based on an anecdotal threshold of <16 vs. ≥16 head hits during a boxing match, respectively. The serum

expression of NF-L was significantly elevated in the severe head impact group when compared to the mild impact group, suggesting a dose-dependent response to head impact and biomarker expression[18]. While the presented literature elicits strong reasoning for NF-L as a marker of axonal injury, neither study exhibits robust control of confounding variables seen throughout sport (thermogenesis, hydration status, body contacts, head kinematics, etc.). This gap in methodology underpins the need for the SSHM. By using the SSHM, Wirsching et al.[14] and Wallace et al.[27] were able to control aforementioned confounding variables, coupled with the monitoring location, magnitude, and quantity of head impacts, and study the head impact dose-response profile of NF-L levels in the blood. Therefore, the SSHM provides safe and standardized means to study the effect of subconcussive head impacts and validate clinical findings.

The SSHM shows promise as being a relevant, reproducible, and leading method of isolating and examining the effect of subconcussive head impacts irrespective of sport; however, there are a few limitations to consider when adopting this method. First, the SSHM has a strong ability to control for environmental covariates (previously described); however, this means that a temperature-controlled indoor facility is required. Second, in an effort to ensure safe and controlled head contacts, we set participant exclusion criteria to a minimum of 5 years of soccer heading experience. This cutoff was used to eliminate potential injurious head contacts due to novice heading technique. Finally, this protocol requires specific equipment and multiple researchers to conduct, which may not be accessible unanimously.

The SSHM provides an invaluable modality for researchers to confidently validate results found in field studies. This confidence stems from the ability of the SSHM to control for both internal and external confounding factors such as impact location and quantity, body damage, exercise effect, and perspiration, to mention a few. Furthermore, because the SSHM has shown similar head impact magnitudes as other sports like ice hockey and American football, the claim can be made that this methodology bears vast clinical relevance not only to soccer athletes but also to American football, ice hockey, rugby, and boxing athletes. Lastly, because the SSHM does not segregate populations, as do some unisex sports like American football, all genders and ethnicities can be studied.

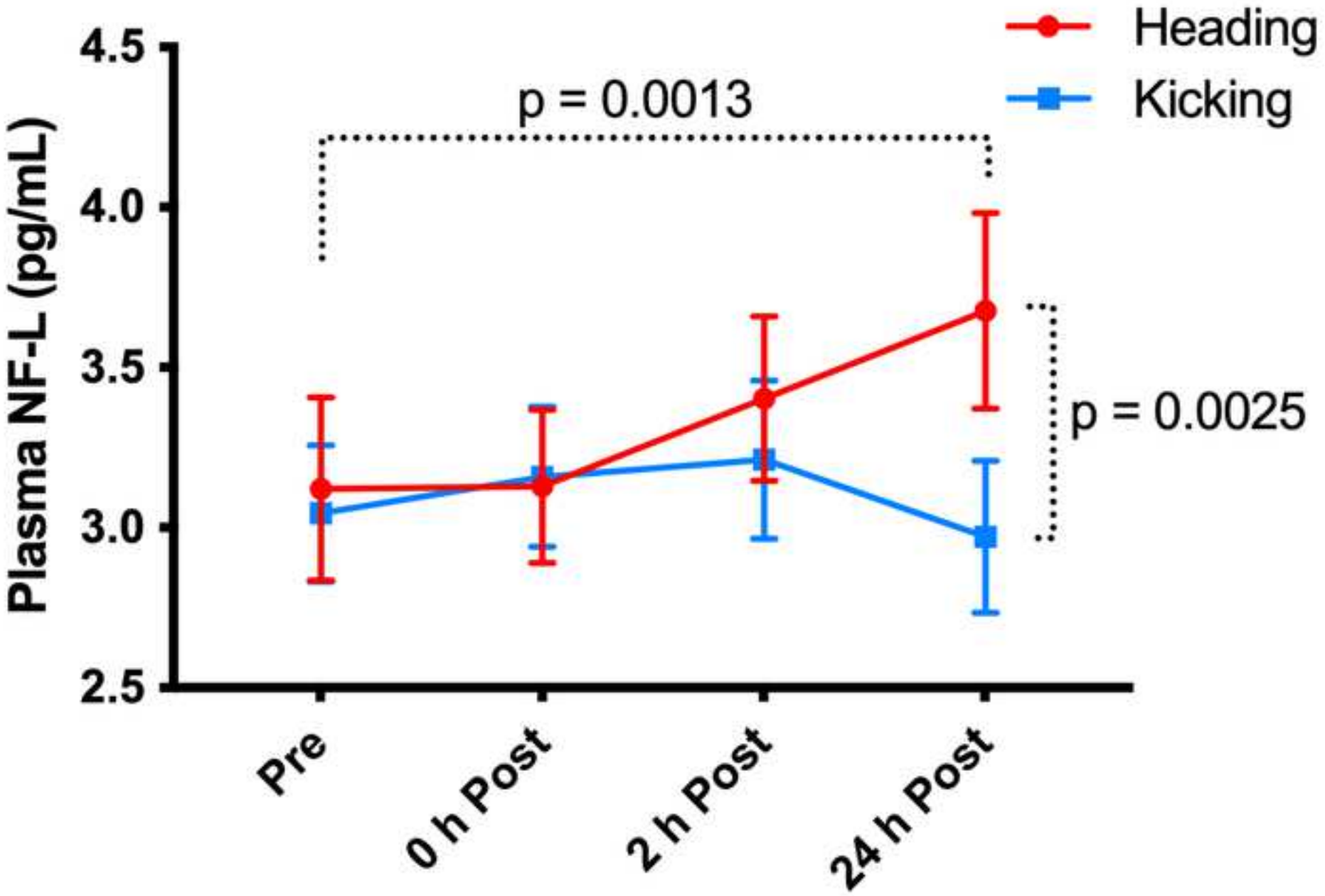
ACKNOWLEDGMENTS:
The authors would like to acknowledge Ms. Angela Wirsching, who was a key contributor to the research we cite in the representative results section.

DISCLOSURES:
The authors have nothing to disclose.

REFERENCES:
1. Goldstein, L.E. et al. Chronic traumatic encephalopathy in blast-exposed military veterans and a blast neurotrauma mouse model. *Science Translational Medicine.* **4** (134), 134ra60 (2012).
2. Kraus, M.F. et al. White matter integrity and cognition in chronic traumatic brain injury: a diffusion tensor imaging study. *Brain: A Journal of Neurology.* **130** (Pt 10), 2508-2519 (2007).

3. McKee, A.C. et al. The spectrum of disease in chronic traumatic encephalopathy. *Brain: A Journal of Neurology.* **136** (Pt 1), 43-64 (2013).
4. Mez, J. et al. Clinicopathological Evaluation of Chronic Traumatic Encephalopathy in Players of American Football. *JAMA.* **318** (4), 360-370 (2017).
5. Tagge, C.A. et al. Concussion, microvascular injury, and early tauopathy in young athletes after impact head injury and an impact concussion mouse model. *Brain: A Journal of Neurology.* **141** (2), 422-458 (2018).
6. Bailes, J.E., Petraglia, A.L., Omalu, B.I., Nauman, E., Talavage, T. Role of subconcussion in repetitive mild traumatic brain injury. *Journal of Neurosurgery.* **119** (5), 1235-1245 (2013).
7. Estimated probability of competing in college athletics | NCAA.org – The Official Site of the NCAA. http://www.ncaa.org/about/resources/research/estimated-probability-competing-college-athletics. Accessed September 20, 2018 (2018).
8. Schnebel, B., Gwin, J.T., Anderson, S., Gatlin, R. In vivo study of head impacts in football: a comparison of National Collegiate Athletic Association Division I versus high school impacts. *Neurosurgery.* **60** (3), 490-496 (2007).
9. Guskiewicz, K.M. et al. Measurement of head impacts in collegiate football players: relationship between head impact biomechanics and acute clinical outcome after concussion. *Neurosurgery.* **61** (6), 1244-1253 (2007).
10. Alfonsi, S. Combat veterans coming home with CTE - CBS News. https://www.cbsnews.com/news/60-minutes-combat-veterans-coming-home-with-cte-brain-injury/ (2018).
11. Ward, J., Williams, J., Manchester, S. 111 N.F.L. Brains. All But One Had C.T.E. - The New York Times. https://www.nytimes.com/interactive/2017/07/25/sports/football/nfl-cte.html. Accessed September 20, 2018 (2017).
12. Ling, H. et al. Mixed pathologies including chronic traumatic encephalopathy account for dementia in retired association football (soccer) players. *Acta Neuropathologica.* **133** (3), 337-352 (2017).
13. Kawata, K., Tierney, R., Phillips, J., Jeka, J.J. Effect of Repetitive Sub-concussive Head Impacts on Ocular Near Point of Convergence. *International Journal of Sports Medicine.* **37** (5), 405-410 (2016).
14. Wirsching, A., Chen, Z., Bevilacqua, Z.W., Huibregtse, M.E., Kawata, K. Association of Acute Increase in Plasma Neurofilament Light with Repetitive Subconcussive Head Impacts: A Pilot Randomized Control Trial. *Journal of Neurotrauma.* doi: 10.1089/neu.2018.5836 (2018).
15. Coon, S. *Acute Effects of Sleep Deprivation on Ocular-Motor Function as Assessed by King-Devick Test Performance*. Master's thesis. Bloomington, Indiana Indiana University (2018).
16. Hwang, S., Ma, L., Kawata, K., Tierney, R., Jeka, J.J. Vestibular Dysfunction after Subconcussive Head Impact. *Journal of Neurotrauma.* **34** (1), 8-15 (2017).
17. Oliver, J.M. et al. Serum Neurofilament Light in American Football Athletes over the Course of a Season. *Journal of Neurotrauma.* **33** (19), 1784-1789 (2016).
18. Shahim, P., Zetterberg, H., Tegner, Y., Blennow, K. Serum neurofilament light as a biomarker for mild traumatic brain injury in contact sports. *Neurology.* **88** (19), 1788-1794 (2017).
19. FIFA Communications Division, Information Services. FIFA Big Count 2006: 270 million people active in football.

https://www.fifa.com/mm/document/fifafacts/bcoffsurv/bigcount.statspackage_7024.pdf. Accessed April 9, 2018 (2007).
20. Dorminy, M. et al. Effect of soccer heading ball speed on S100B, sideline concussion assessments and head impact kinematics. *Brain Injury.* 1-7 (2015).
21. Bretzin, A.C., Mansell, J.L., Tierney, R.T., McDevitt, J.K. Sex Differences in Anthropometrics and Heading Kinematics Among Division I Soccer Athletes. *Sports Health.* **9** (2), 168-173 (2017).
22. Crisco, J.J. et al. Frequency and location of head impact exposures in individual collegiate football players. *Journal of Athletic Training.* **45** (6), 549-559 (2010).
23. Duma, S.M. et al. Analysis of real-time head accelerations in collegiate football players. *Clinical Journal of Sport Medicine.* **15** (1), 3-8 (2005).
24. Reynolds, B.B. et al. Practice type effects on head impact in collegiate football. *Journal of Neurosurgery.* 1-10 (2015).
25. Kawata, K. et al. Association of Football Subconcussive Head Impacts With Ocular Near Point of Convergence. *JAMA Ophthalmology.* **134** (7), 763-769 (2016).
26. Wilcox, B.J. et al. Head impact exposure in male and female collegiate ice hockey players. *Journal of Biomechanics.* **47** (1), 109-114 (2014).
27. Wallace, C. et al. Heading in soccer increases serum neurofilament light protein and SCAT3 symptom metrics. *BMJ Open Sport & Exercise Medicine.* **4** (1), e000433 (2018).



Table

Variables	Heading	Kicking Control	P-value
n	18	16	-
Sex	7M 11F	6M 10F	-
Age, y	20.3 ± 1.5	21.2 ± 1.4	0.089
BMI, kg/m^2	23.2 ± 2.4	24.4 ± 3.2	0.236
No. of previous concussion	0.78 ± 1.0	0.63 ± 1.7	0.753
Soccer heading experience, y	9.5 ± 3.6	10.0 ± 4.5	0.725
Head impact kinematics, median (IQR), a			
PLA, g	31.8 (31.1 – 34.5)	- b	-
PRA, $krad/s^2$	3.56 (2.93 – 4.04)	- b	-

Note: BMI, body mass index. IQR, interquartile range. PLA, peak linear acceleration. PRA, peak rotational acceleration. krad, kiloradian. a, Based on the sum of 10 soccer headers. b, Soccer kicking did not cause a detectable level of head acceleration.

Name of Material/ Equipment	Company
JUGS Soccer Machine	JUGS Sports
SIM-G Triaxial Accelerometer	Triax Technologies

Catalog Number

http://jugssports.com/products/soccer-machine.html

https://www.triaxtec.com/workersafety/wp-content/uploads/2017/08/SIM-G-User-Manual_V4-2-01.pdf

Comments/Description

jove JOURNAL OF VISUALIZED EXPERIMENTS
1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT

Title of Article: *In-vivo* protocol of controlled subconcussive head impacts: validation of field study data

Author(s): Zachary W. Bevilacqua MS, ATC, Megan E. Huibregtse, and Keisuke Kawata PhD, ATC

Item 1 (check one box): The Author elects to have the Materials be made available (as described at http://www.jove.com/author) via: ☐ Standard Access ☐ Open Access

Item 2 (check one box):

☐ The Author is NOT a United States government employee.

☐ The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.

☐ The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

ARTICLE AND VIDEO LICENSE AGREEMENT

1. Defined Terms. As used in this Article and Video License Agreement, the following terms shall have the following meanings: "**Agreement**" means this Article and Video License Agreement; "**Article**" means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; "**Author**" means the author who is a signatory to this Agreement; "**Collective Work**" means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; "**CRC License**" means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode; "**Derivative Work**" means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; "**Institution**" means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; "**JoVE**" means MyJove Corporation, a Massachusetts corporation and the publisher of *The Journal of Visualized Experiments;* "**Materials**" means the Article and / or the Video; "**Parties**" means the Author and JoVE; "**Video**" means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion of the Article, and in which the Author may or may not appear.

2. Background. The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3. Grant of Rights in Article. In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to **Sections 4** and **7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in **Item 1** above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

4. Retention of Rights in Article. Notwithstanding the exclusive license granted to JoVE in **Section 3** above, the Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. Grant of Rights in Video – Standard Access. This **Section 5** applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. Grant of Rights in Video – Open Access. This **Section 6** applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this Section 6 is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

7. Government Employees. If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. Likeness, Privacy, Personality. The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

9. Author Warranties. The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

10. JoVE Discretion. If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have

full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including, without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

11. Indemnification. The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in *JoVE* or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

12. Fees. To cover the cost incurred for publication, JoVE must receive payment before production and publication the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

13. Transfer, Governing Law. This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to me one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement required per submission.

CORRESPONDING AUTHOR:

Name:	Keisuke Kawata
Department:	Kinesiology
Institution:	Indiana University Bloomington
Article Title:	*In-vivo* protocol of controlled subconcussive head impacts: validation of field study data
Signature:	Keisuke Kawata
Date:	11/11/18

Please submit a signed and dated copy of this license by one of the following three methods:

1) Upload a scanned copy of the document as a pfd on the JoVE submission site;
2) Fax the document to +1.866.381.2236;
3) Mail the document to JoVE / Attn: JoVE Editorial / 1 Alewife Center #200 / Cambridge, MA 02139

For questions, please email submissions@jove.com or call +1.617.945.9051

Author Response:

We addressed and modified the following editorial comments in the revised manuscript. Thank you for the direct instruction.

Editorial comments:
Changes to be made by the author(s) regarding the manuscript:
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.
2. Please check either the Standard Access or Open Access checkbox in the Author License Agreement (ALA). Please then scan and upload the signed ALA to your Editorial Manager account.
3. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. "This figure has been modified from [citation]."
4. Figure 1: Please include a space between all numerical values and their corresponding time units (0 h, 2 h, 24 h).
5. Please upload each Table individually to your Editorial Manager account as an .xls or .xlsx file. Each table must be accompanied by a title and a description after the Representative Results of the manuscript text.
6. Please revise the title to avoid the use of the colon.
7. Please include a space between all numerical values and their corresponding units: 24 h, 37 °C, 60 s, 40 ft; etc.
8. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. You may use the generic term followed by "(see table of materials)" to draw the readers' attention to specific commercial names. Examples of commercial sounding language in your manuscript are: JUGS machine, JPS Sports, SIM-G; Triax Technologies, Inc., etc.
9. Please include an ethics statement before the numbered protocol steps, indicating that the protocol follows the guidelines of your institution's human research ethics committee.
10. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).
11. Please revise the protocol to contain only action items that direct the reader to do something (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as "could be," "should be,"

and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible. Please move the discussion about the protocol to the Discussion.
12. Discussion: Please also discuss the critical steps within the protocol.
13. References: Please do not abbreviate journal titles.
14. Table of Materials: Please remove trademark (™) and registered (®) symbols.

Reviewers' comments:

Please note that the reviewers raised some significant concerns regarding your method and your manuscript. Please thoroughly address each concern by revising the manuscript or addressing the comment in your rebuttal letter.

Reviewer #1:

The Authors of this manuscript use an automated machinery to induce control head impacts in players. A cohort of "kickers" is used as a control. The manuscript is concisely written and reports results that were already published. The main and perhaps fatal flaw of the design is to use a marker (NF-L) which has been rarely studied in clinical context, while ignoring S`100B and GFAP which are better known. The reason for this comment is not this Reviewer's preference of one marker over another, but rather a concern in the space of general diagnostics. An elevation is meaningless unless a value for controls vs. injured is available. In a previous study with S100B in football players, subconcussive head hits were often associated with an increase of S100B above the published and validated control values. In other words, an elevation of a marker (e.g., blood glucose) within a normal clinical range is meaningless, and does not suggest a diabetic pathology.

Author Response:
We thank the reviewer for his/her comments on the use of NF-L as a marker for brain injury. We would like to clarify the rationale of using the marker in this manuscript. As mentioned, extensive research has been conducted to better understand S100B and GFAP (along with UCH-L1 and Tau) in clinical studies, and indeed, NF-L has lesser volumes of evidence to date. This is exactly why our SSHM can be of paramount use in validating the clinical results. Shahim et al.[1] and Oliver et al.[2,3] have reported that blood levels of NF-L raise in concert with subconcussive head impacts in boxers and football players. However, as elaborated in our discussion section, these studies do not control any extraneous factors, and thus the effects of subconcussive head impact on NF-L is suggestive at best. In this manuscript, we demonstrated that using the laboratory subconcussion model we were able to validate their clinical findings by controlling for extraneous factors that are inherent to field studies (temperature change, exercise effect, body hit, and orthopedic stress). It is also important to emphasize that this manuscript is a methodological paper submitted to the JoVE (which introduces innovative methodologies).

While it is best to evaluate other markers (S100B, GFAP, Tau, UCH-L1, NF-H, NSE, SNTF: see our review papers[4,5] on their strength and clinical significance), that is not the intent of this manuscript. Also, the sentence on "an elevation is meaningless unless a value for controls vs. injured is available" is confusing to us. We demonstrate the elevation in NF-L which was significantly elevated within-group (heading) and between group (heading vs. kicking control). The clinical significance of such increase is yet to be delineated by follow-up studies, as with any other blood biomarkers. The reviewer's logic is not clear to us. Could you please elaborate?

An additional factor relates to use of human subjects as experimental tools. If NF-L increases are in your opinion clinically important, were the volunteers aware of the risk of CTE, or brain/axonal damage? It is a hard for me to understand how your IRB committee approved this study. Was the risk of long-term consequences deemed acceptable?

Author Response:
Our apologies for not including an IRB statement. The study has been approved by Indiana University IRB and Temple University IRB (where Dr. Kawata currently and previous affiliate). Subconcussion poses a public health concern and it is important to understand the mechanism of head impact and physiological/biological response. Therefore, our protocol of 10 soccer headers (which is regularly performed in soccer players, as well as players in American football, ice hockey, rugby, and boxing incurring similar levels of head impacts per practice/game), is considered safe and advantageous means to study the subconcussive effect. Subjects were also told regarding risks of head impacts and signed informed consent. The ethical statement was added to the revised manuscript. Thank you for pointing this out.

Methods: "frontal lobe" is not a synonym of forehead and a forehead hit will affect other regions of the brain as well.

Author Response:
It is corrected in the revised manuscript.

REFERENCES

1. Shahim P, Zetterberg H, Tegner Y, Blennow K. Serum neurofilament light as a biomarker for mild traumatic brain injury in contact sports. *Neurology.* 2017;88(19):1788-1794.
2. Oliver JM, Jones MT, Kirk KM, et al. Serum Neurofilament Light in American Football Athletes over the Course of a Season. *Journal of neurotrauma.* 2016;33(19):1784-1789.
3. Oliver JM, Jones MT, Kirk KM, et al. Effect of Docosahexaenoic Acid on a Biomarker of Head Trauma in American Football. *Med Sci Sports Exerc.* 2016;48(6):974-982.
4. Kawata K, Tierney R, Langford D. Blood and cerebrospinal fluid biomarkers. *Handbook of clinical neurology.* 2018;158:217-233.

5. Kawata K, Liu CY, Merkel SF, Ramirez SH, Tierney RT, Langford D. Blood biomarkers for brain injury: What are we measuring? *Neuroscience and biobehavioral reviews.* 2016;68:460-473.

Reviewer #2:

Manuscript Summary:
In this manuscript, the authors describe a soccer heading protocol that provides a means to generate subconcussive head impacts under controlled circumstances. In particular, in a pre/post repeated measures design, participants headed a soccer ball 10 times in a 10 minute period. For the proof of concept purposes of this methods paper, the authors measured a blood biomarker (neurofilament light) which is associated with axonal injury. This was done immediately prior to and after the bout of soccer heading and then at 2 hours and 24 hours after the protocol. For comparison, a control group of participants did all the same procedures except instead of heading the ball they kicked it. Ball speed and trajectory were controlled by a JUGS soccer machine. The results showed a significant increase in NF-L in the heading but not the kicking group.

The manuscript is clearly written and addresses a new experimental manipulation that has been published on only a small number of times. All of the relevant methodological details are present in the manuscript that would allow another scientist to replicate the procedures. I only have a couple of suggestions for the authors.

Major Concerns:

1. Some discussion of the safety and ethics associated with the protocol appears warranted. Researchers wanting to implement this protocol will need to convince their local ethics panel that the injuries that are induced by the head impacts are temporary in nature. One approach is to have the participants come back for additional testing (e.g., by serving as their own controls) to demonstrate that the relevant dependent variable has returned to baseline.

Author Response:
Thank you for the pointer and our apologies for not including the ethical statement. We have included an ”ethics statement” outlining our approval and compliance with IRB.

2. An additional paper by our group (Wallace et al., 2018. BMJ Open Sport and Exercise Science) examining NF-L changes following a more intense bout of heading has recently been published and may be worth including in the discussion regarding dose-response effects.

Author Response:
We included Wallace et al. paper in the discussion section.

3. The NF-L results are only different at the 24 hour time point between the heading and kicking groups. Although this is not the primary purpose of the manuscript, it might be worth discussing.

Author Response:
Although the reviewer's recommendation is important, we feel that elaborating upon NF-L specifically would detract from the primary goal of the manuscript, which is to showcase the SSHM, and not the biomarkers it can be used with. We believe that the NF-L finding has been discussed sufficiently in the discussion section; therefore, we respectfully choose not to further elaborate on the NF-L result. Readers should refer to Wirsching et al.

Minor Concerns:

1. The Alfonsi (2018) reference appears to be incomplete.

Author Response:
This was corrected.

From: "Ballen, Karen" <KBallen@liebertpub.com>
Date: Wednesday, December 19, 2018 at 7:50 PM
To: "Kawata, Keisuke" <kkawata@indiana.edu>
Subject: FW: Permission to reproduce Figure - URGENT

Dear Dr. Kawata:

Copyright permission is granted for this request. Please give proper credit to the journal and to the publisher: Mary Ann Liebert, Inc., New Rochelle, NY.

Kind regards,

Karen Ballen
Manager, Reprints, Permissions, and Open Access

From: Kawata, Keisuke <kkawata@indiana.edu>
Sent: Wednesday, December 19, 2018 10:12 AM
To: Ballen, Karen <KBallen@liebertpub.com>
Subject: Re: Permission to reproduce Figure

Hello Ms. Allen,

Sorry to bug you but I haven't heard from you on the previous request. I'm not sure if you received my note below.

Could you please get back to me at your convenience?

Thank you,

Keisuke Kawata, Ph.D., ATC.
Assistant Professor
Department of Kinesiology
School of Public Health-Bloomington
Indiana University
1025 E. 7th Street
Bloomington, IN 47405
Office: C215
Phone: (812)855-5244
Fax: (812)855-3193
Email: kkawata@indiana.edu

On Dec 13, 2018, at 3:22 PM, Kawata, Keisuke <kkawata@indiana.edu> wrote:

Thanks for responding to my call.

I'm emailing to inquire permission to reproduce the Figure 2 of our recent paper. We are preparing a methodological manuscript to JoVE (journal of visualized experiments), highlighting our soccer heading model that we used for J Neurotrauma paper. We'd like to reproduce the Figure 2 as an example finding derived from the soccer heading model. The title of the manuscript for the JoVE is: *In-vivo protocol of controlled subconcussive head impacts: validation of field study data*.

Please let me know if there is any other information needed and whether or not the publisher grants permission to reproduce the figure with citation.

Thank you so much for your time,

Keisuke Kawata, Ph.D., ATC.,
Assistant Professor
Department of Kinesiology
School of Public Health-Bloomington
Indiana University
1025 E. 7th Street
Bloomington IN 47405
Office: C215
Phone: (812) 855-5244
Fax: (812) 855-3193
Email: kkawata@indiana.edu