KC IRB
Protocol Summary

Protocol Number: 1610743422R001

Title: Effects of soccer heading on ocular-motor function, brain-derived blood biomarker, and neuronal activity

Status: Submitted to IRB

Expiration Date: 01/16/2018

Last Approval Date:

Investigator: Kawata, Keisuke

Protocol Details

Type: Full Board

Application Date: 12/18/2017

IU or Investigator held IND/IDE?

FDA Application No:

Organizations

Type	Organization
Performing Organization	Indiana University

Funding Source

Funding Type	Code/Number	Sponsor Name	Sponsor Type	Prime Sponsor Name	Prime Sponsor Type
Unfunded					

Subjects

Subject	Count
Total	80

Areas of Research

Code	Description
000001	All Research Areas

Personnel

Person Name	Units		Role	Affiliate	Training Flag
Kawata, Keisuke	BL-KINE	KINESIOLOGY	PI	IU	Y
Coon, Sarah	BL-KINE	KINESIOLOGY	CO-PI	IU	Y
Wirsching, Angela	BL-KINE	KINESIOLOGY	CO-PI	IU	Y

Study Personnel

PersonName	Role	Affiliation	Training
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Gruber, Allison Hibbert	Key Personnel	IU	Y
Innis, Isaiah J	Key Personnel	IU	Y
Mirdamadi, Jasmine Lauren	Key Personnel	IU	Y
Newman, Sharlene D.	Key Personnel	IU	Y
Seigel, Courtney R	Key Personnel	IU	Y

Roles

Protocol Aggregator

User Name
Block, Hannah Justine
Kawata, Keisuke

Actions

Description	Comments	Action Date
Assigned to Agenda		12/18/2017
Submitted to IRB	Submitted to IRB	12/18/2017
Returned To PI	Open for re-review	12/18/2017
Submitted to IRB	Submitted to IRB	12/18/2017
Returned To PI	Open for pre-review	12/18/2017
Submitted to IRB	Submitted to IRB	11/17/2017
Protocol Created	ProtocolBase created	11/17/2017

Attachments

Description	Attachment Type	Last Updated	Updated By
Only the symptom checklist from SCAT 3 will be used in the study.	Data Collection Instrument	01/05/2017 12:41:32	kkawata
ICS - athletes	Informed Consent Statement	03/24/2017 09:51:06	kmumaw
ICS - controls	Informed Consent Statement	03/24/2017 09:51:06	kmumaw
stamped consent	Informed Consent Statement	03/24/2017 09:51:06	kmumaw
stamped ICS - controls	Informed Consent Statement	03/24/2017 09:51:06	kmumaw
REVISED3-Protocol for Soccer Heading Study	Protocol	03/24/2017 09:51:06	kmumaw
Screening For TMS	Recruitment Materials	12/07/2016 17:21:25	kkawata
Concussion History and Health Questionnaire	Recruitment Materials	12/07/2016 17:21:25	kkawata
recruitment email	Recruitment Materials	03/24/2017 09:51:07	kmumaw
Data Safety Monitoring Summary	Monitoring Report / AE Summary	12/18/2017 15:45:28	jilawall

Other Attachments

Description	Last Updated	Updated By
Supporting Literature 1:	12/12/2016 10:42:55	kkawata
Supporting Literature 2	12/12/2016 10:43:11	kkawata
Supporting Literature 3	12/12/2016 10:43:46	kkawata

Determinations

Determination	Date Assigned	Date Inactive	Status	Comments
Greater than Minimal Risk	03/24/2017		A	IRB-04

Amendment/ Renewal

Type	Version	Status	Created Date	Summary
Renewal	001	Submitted to IRB	11/17/2017	Application for the renewal of the study.

Amendment/Renewal Summary

Summary	Editable Modules
Application for the renewal of the study.	Add / Modify Attachments and Notes,Personnel Tab,

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IRB APPROVAL

This research project, including all noted attachments, has been reviewed and approved by the Indiana University IRB.

Study meets the criteria of approval for a period of: ☒ one (1) year ☐ other, _____

Reviewing IRB Committee: IRB-02
Level of Review: FULL

Authorized IRB Signature: _____ IRB Approval Date: 1/9/2018

Printed Name of IRB Member: Danielle Giltner on behalf of Michael Turik, MD

Renewal - Protocol Status & Summary

Protocol Number:	1610743422R001	**Submission Type:**	Renewal without Amendment
Title:	Effects of soccer heading on ocular-motor function, brain-derived blood biomarker, and neuronal activity	**Principal Investigator:**	Kawata, Keisuke
Report Printed:	12/18/2017		

- ID #350: Projected date of study completion:

  [X] **May 2019**

- ID #351: Select the appropriate status of the study.

  [X] **Open to Enrollment**
  [ ] Closed to Enrollment
  [ ] Data Analysis Only

  - ID #352: Choose the appropriate description.

    [X] **Enrollment of new subjects or review of records/specimens continues.**
    [ ] No subjects have been enrolled to date.

    - ID #359: Is the study temporarily suspended by the investigator or sponsor due to risk, safety, or compliance issues?

      [ ] Yes
      [X] **No**

      - ID #361: Does this study use records or specimens only (i.e. NO interaction with human subjects)?

        [ ] Yes
        [X] **No**

        - ID #356: Has the study completed at least one IRB renewal?

          [ ] Yes
          [X] **No**

        - ID #370: Since the beginning of the study, how many subjects have been enrolled?

          [X] **29**

          - ID #371: Since the beginning of the study, how many subjects failed screening?

            [X] **4**

          - ID #372: Since the beginning of the study, how many subjects have withdrawn from the study?

            [X] **1**

            - ID #369: State the reasons for subject withdrawal since the last review. Summarize the reasons for withdrawal in previous years.

☒ **He completed the first day (3 time points) but did not return to the second day (4th time point). He told us he lost his interest in the study.**

- ID #373: State the total number of ACTIVE subjects currently in the study.

  ☒ **0**

- ID #374: State the total number of subjects who have completed the study to date.

  ☒ **24**

- ID #24863: If necessary, provide further explanation regarding the number of subjects. (Enter N/A if none)

  ☒ **Our target is 80 subjects. Our subject enrollment took longer than expected as we needed to train graduate students from the beginning.**

- ID #377: Were any subjects consented using the short form written consent document?

  ☐ Yes
  ☒ **No**

- ID #400: Since the last IRB review, did any events occur that required prompt reporting to the IU IRB?

  ☐ Yes
  ☒ **No**

- ID #404: Since the last IRB review, did any events occur, involving an IU IRB-approved performance site that did NOT require prompt reporting to the IRB (e.g., minor protocol deviations, minor noncompliance)?

  ☐ Yes
  ☒ **No**

- ID #406: Is there a data safety monitoring plan for this study?

  ☒ **Yes**
  ☐ No

  - ID #407: Is there a Data Safety Monitoring Board (DSMB) or Data Monitoring Committee (DMC) for this study?

    ☐ Yes
    ☒ **No**

    - ID #25054: Summarize the data safety monitoring findings since the last IRB review, explain why findings are not available, or indicate that a summary has been uploaded to the Notes & Attachments tab.

      ☒ **A summary of the data safety monitoring findings is uploaded to the Notes and Attachment tab.**

- ID #408: Describe the progress of the research, including any preliminary observations and information about study results or trends.

Renewal - Protocol Status & Summary

☒ **There seem to be a small but detectable effects of subconcussive head impacts in eye movement function and brain-derived blood biomarker levels (as of now: CCL11), which have the potential to become clinical markers for brain trauma. However, we'll need more sample size to ensure our findings.**

- ID #409: Have subjects experienced any direct benefit(s) from their participation in the study?

☐ Yes
☒ **No**

  - ID #24864: Explain why subjects have not experienced benefit(s).

    ☒ **Besides monetary compensation, they do not receive any benefits that are tangible for subjects.**

- ID #411: Have any of the following occurred since the last IRB review? Choose all that apply.

☐ Literature publication which demonstrates a significant impact on the conduct of the study, or the well-being of subjects
☐ Audit from federal agencies which identified unanticipated problems involving risks to subjects or others or noncompliance
☐ Events which affected the validity of the data
☐ Increase in risk to subjects or others
☐ Increase in frequency or severity of adverse events
☐ Change in the risk to benefit assessment
☒ **None of the above**

Renewal - Changes & Amendments

Protocol Number:	1610743422R001	**Submission Type:**	Renewal without Amendment
Title:	Effects of soccer heading on ocular-motor function, brain-derived blood biomarker, and neuronal activity	**Principal Investigator:**	Kawata, Keisuke
Report Printed:	12/18/2017		

- ID #211: Review the information in the Protocol and Personnel tabs, and all attachments on the Notes & Attachments tab and confirm that no changes are needed. Choose all that apply.

☒ **No changes are needed.**
☐ This renewal includes changes to Personnel.
☐ This renewal includes an amendment.
☐ An updated Investigator Brochure or package insert is being submitted with this Renewal, but no other study documents are being changed as a result of the IB update.

Effects of soccer heading on ocular-motor function, brain-derived blood biomarker, and neuronal activity

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Table of Contents:

1.0 Background

In the United States, every year approximately 1.7 million people sustain a traumatic brain injury (TBI) with the majority (80% or 1.4 millions) of these injuries falling into the category of concussion or mild TBI (mTBI).[1] Concussion is classified as a mild form of traumatic brain injury, while changes in neural function following concussions are far from benign. Concussed athletes frequently experience lingering neurological symptoms, including headache, irritability, blurred vision, and confusion.[2] In respect to these symptoms, researchers have utilized various metrics to study brain damage, including brain-derived blood biomarkers, sensory systems, and neuroimaging. However, due to exceedingly high intra-group variability in the concussed cohort, to date there is no gold standard metric to gauge severity of brain damage following head impacts.[3]

One possible explanation of the high variability is due to subconcussive head impact, defined as an impact to the head that does not cause any symptoms of concussion, regardless of magnitude of the impact. It, however, has the potential to cause insidious effects in the brain if sustained repetitively over time.[4] An average athlete in contact sports experiences nearly a thousand subconcussive hits per season.[5] While the vast majority of research has focused on a single impact that elicits concussion (e.g., disorientation, headache), many athletes are frequently exposed to subconcussive head impacts prior to the concussive blow.[6, 7] Thus, it remains completely unknown whether neuronal damage and sensory deficit are caused by a single concussive blow, repetitive subconcussive impacts before the concussive blow, or both.

Ocular-motor System

Recently, the ocular-motor system has garnered significant scientific attention because of its sensitivity to various severities of brain injury.[8] The ocular-motor system orchestrates accommodation and vergence, and their concomitant adjustment enables individuals to visualize an object at various distance, direction, and speed.[9] Nearly 50% of the neural connections in the human brain, including cortical, cerebellar, and brainstem networks are involved in vision and eye movement functions. Since most head impacts cause the brain to bounce linearly and rotationally within the skull, almost 90% of concussed individuals exhibit some form of visual dysfunction.[10, 11] For example, a notable decline in saccades –rapid changes in eye orientation– has been observed in concussed boxers, football, and rugby players.[12, 13] Similarly, a near point of convergence (NPC), which is the closest point one can visualize an object before diplopia occurs, was impaired by 3-fold in concussed athletes and soldiers.[14, 15] Neuroimaging confirmed that these ocular-motor impairments were in concert with altered neuronal activity after concussion.[16]

Previously, for the first time we have demonstrated that repetitive subconcussive head impacts could significantly impair NPC in soccer[17] and football players.[18] More intriguingly, the NPC has **predicted concussions** before they occurred. In our football study, we tracked changes in NPC in 25 players, and 3 of 25 players were diagnosed with a concussion (Fig 1: unpublished). There was a biphasic increase in NPC: 1) acute increase after 1st full-gear practice (Pads-ON1) compared with pre-practice, and 2) on the day of concussion, *3 players showed abnormally high NPC before the practice*. Due to absence of concussion signs and symptoms, the players participated in the practice. Following moderate intensity of impacts, the 3 players became unconscious for < 30

seconds and diagnosed with a concussion by team athletic trainers. Despite it is preliminary, the ocular-motor function has the potential for a paradigm shift in concussion diagnosis. However, it is imperative to first understand the acute effects of subconcussive head impacts on the ocular-motor system.

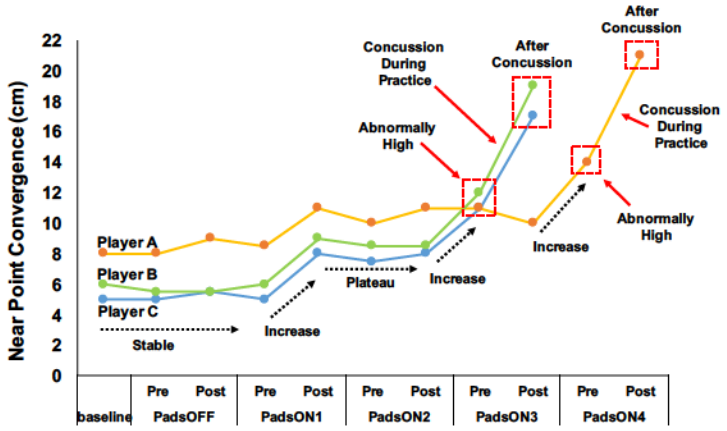

Fig 1. The near point of convergence (NPC) "predicted" concussion before it occurred. *Three players showed near identical trends. Acute NPC increase was observed after Pads-ON1 practice (full-gear practice), then remained high without normalized to baseline level, indicating slow recovery nature of the ocular-motor system. Player B and C sustained concussions during Pads-On 3 practice, and Player A sustained a concussion during Pads-On 4 practice. Both time, NPC before the practice was abnormally high (with no outward symptoms), suggesting particular vulnerability of the players on that day.*

Blood Biomarker

Importantly, identifying a biomarker that can detect **cumulative risk** for developing neurodegenerative disease is a paradigm shifting. A Particularly Interesting New Cysteine Histidine-rich (PINCH) protein is an adaptor protein involved in cell maintenance and survival of neuronal cells.[19] *PINCH is nearly undetectable in a healthy brain*. However, PINCH expression is upregulated as neuronal axons experience a high rate of strain forces, leading to disruption of tau –an axon stabilizing protein– from axonal microtubules (Fig 2). When tau disruption occurs, PINCH binds to tau and makes it difficult to degrade and clear from the brain.[20] Consequently, tau protein accumulates in the brain (Fig 2), as often observed in Alzheimer's disease and chronic traumatic encephalopathy (CTE).[21-23] If PINCH is detectable in the brain or even in the blood, neurons are mostly likely injured. Thus, this biomarker is potentially a groundbreaking because it carries an early sign of neurodegeneration.

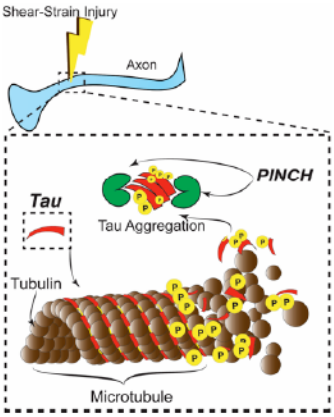

Fig 2. PINCH binds to disrupted tau and exacerbates tau aggregation. *P, phosphate. Figure modified from Kawata et al.*[23]

In the preliminary work, we first examined plasma PINCH in epilepsy patients, given that epilepsy is considered a form of neurotrauma due to neuronal hyper-excitability. We found that a robust expression of PINCH was detected in the blood of epilepsy patients while undetectable in healthy controls (Fig 3: in print).[24] Similarly, 10 out of 16 collegiate football players showed noticeable increases in plasma PINCH level at post-season compared with pre-season baseline (Fig 4: unpublished). Since none of 16 athletes were diagnosed with a concussion during the season, the increased level of plasma PINCH indicates the neuronal damage from cumulative subconcussive impacts.

Neuronal Activity

Peripheral diagnostic tools for brain injury have an immense clinical implication, however those methods are insufficient to truly gauge neuronal dysfunction after head hits. Electroencephalography (EEG) has produced promising results in patient with

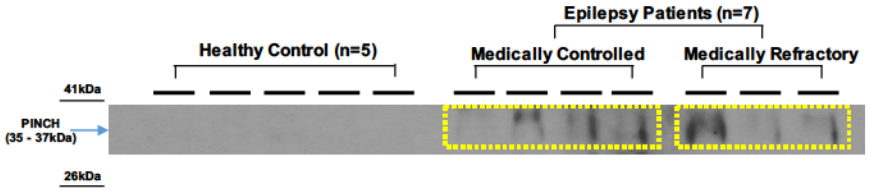

Fig 3. PINCH protein was detectable in mesial temporal epilepsy patients' blood. *Robust plasma expression of PINCH was observed in both medically controlled (no seizure) and refractory (transient seizure) epilepsy patients, while no PINCH in healthy controls.*

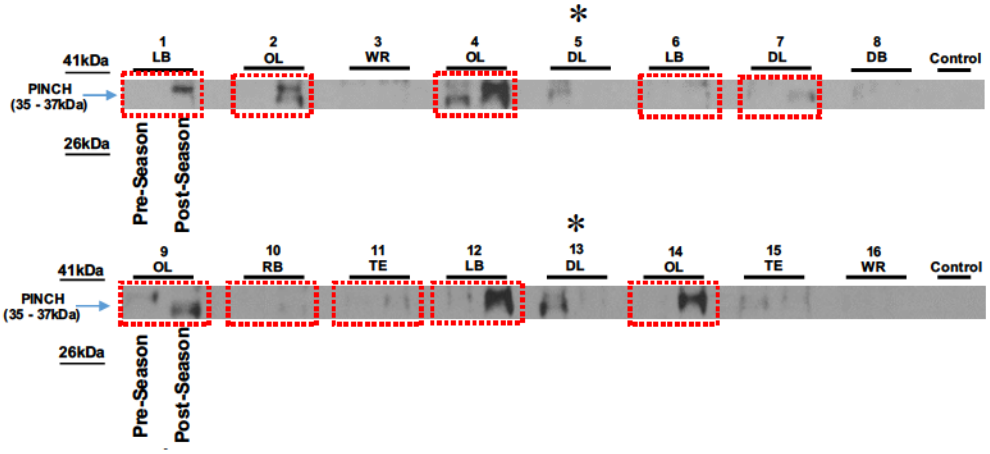

Fig 4. Post-season plasma PINCH was notably higher than pre-season baseline. *10 out of 16 players showed increase in plasma PINCH at post-season. * Player 5 and 13 did not play for the season due to surgery. Control: a subject with no history of head trauma. LB, linebacker; OL, offense lineman; WR, wide receiver; DL, defense lineman; DB, defensive back; RB, running back; TE, tight end.*

concussion, with reduced mean frequency of alpha, reduced power in the alpha and beta frequency bands, hypercoherence between frontal regions, and decreased gamma frequency.[25-29] In addition to EEG, transcranial magnetic stimulation (TMS) has garnered significant attention in scientific society recently, due to its non-invasive method to measure neuronal excitability. However, to date, there is virtually no literature demonstrating the effects of minor head impacts on neuronal activity using EEG nor TMS.

Soccer Heading Modality

Soccer heading is a common skill performed by soccer athletes during practice and games. An average collegiate soccer players perform about 500 headers during a single season and over 3000 during the course of a career [30, 31] Research has demonstrated the need for increased awareness of potential brain injury associated with heading the soccer ball with some suggesting that the cumulative effect of soccer head impacts over a career may lead to outcomes similar to sustaining multiple concussions.[32] Various studies have been conducted to examine the effect of an acute bout of soccer heading, including Dr. Kawata's previous laboratory at Temple University, with most authors reporting no significant effect of heading on the brain function measures of neuropsychological or balance performance (Table 1). We have tested collegiate soccer players post heading ball speed up to 50 mph with no significant alterations in common

clinical measures (e.g., balance error scoring system, signs and symptoms checklist). However, sensitive modalities like blood biomarker and ocular-motor testing begin to unravel subclinical perturbation caused by minor head hits if sustained repetitively.[17, 18, 33]

Table 1. Research studies examining the effect of an acute bout of soccer heading

Author	Subject	Method	Outcome
Patukian et al. (2000)	100 college athletes	20 minutes of practice heading	NSD in neuropsychological performance
Broglio et al. (2004)	40 college Athletes	20 headers in 20 minutes	NSD in postural control
Magnus et al. (2004)	10 college Athletes	20 headers in 5 minutes	NSD in balance
Schmitt et al. (2004)	31 college athletes	18 headers in 40 minutes	NSD in postural control, Symptoms increased (headache, vertigo, fatigue)
Mussack et al. (2003)	amateur athletes 61 heading group 58 active controls	55 min of heading practice Exercise	Significant increase in S-100B in heading versus active control group
Stalnacke et al. (2004)	44 professional athletes	Soccer game	Significant increase in S-100B, +Correlation between S-100B and # of headers
Otto et al. (2005)	adult athletes	Boxing Marathon Running Soccer Heading (16 mph)	Significant increase in S-100B Significant increase in S-100B NSD in S-100B
Haran et al. (2013)	16 college athletes	Soccer Heading (25 mph)	Significant change in postural control using virtual environment 24h post.
Zetterberg et al. (2007)	23 amateur soccer players 10 Non-athletic subjects	20 headings	NSD in S100B between pre vs. post and heading vs. control group.
Dorminy et al. (2015)	16 college soccer players	5 headings at 30, 40, 50 mph	NSD in S100B nor concussion symptoms after soccer heading at any speed.
Kawata et al. (2016)	20 college soccer players	10 headings at 25mph	Significant decrease in ocular-motor function immediately after and 24h post heading.
Hwang et al. (2016)	20 college soccer players	10 headings at 25mph	Significant change in vestibular function at immediately after headings but NSD in 24h post-heading.
Kaminski et al. (2007)	71 college soccer players	Pre-post Season	NSD in balance, neurocognitive performance
Kontos et al. (2011)	63 youth soccer players	Heading exposure groups High vs. Moderate vs. Low	NSD in neurocognitive performance between groups

2.0 Rationale and Specific Aims

Aim 1. Determine acute effects of subconcussive head impact on plasma PINCH and tau expression using controlled soccer heading model.

Hypothesis: Repetitive subconcussive head impacts will significantly increase plasma PINCH level at 24h-post heading, but plasma PINCH at 0h- and 2h-post heading will remain undetectable, while there will be a significant increase in serum tau level at immediately post-heading (0h and 2h) but normalize to baseline level at 24h-post heading.

After a concussion, a neural network is often disturbed due to axonal injury, observed via neuroimaging.[34, 35] Also, an acute increase in plasma tau has been observed in concussed athletes.[36] However, a half-life of tau protein in the blood is known to be short (~3h),[37] thus at 24h-post heading tau level is expected to normalize to the pre-heading baseline level. Recent preliminary findings showed that neural disturbance was notable in football players at post-season compared with pre-season baseline, as well as rugby players after a single rugby game compared with pre-game level.[38, 39] Although subconcussive impacts do not cause concussion-related symptoms,[18] neuronal axons are evidently experiencing pathological stress.[40] Repetitive subconcussive impacts likely to cause tau injury, and because PINCH continues to be over-expressed in the brain as long as tau is

damaged,[21, 22] a fraction of PINCH will be detectable in the peripheral blood stream via glymphatic system or disrupted blood-brain barrier.[37]

Aim 2. Determine acute effects of subconcussive head impact on dynamic gaze-target synchronization in a controlled soccer heading model.

Hypothesis: Repetitive subconcussive head impacts will significantly decrease saccadic velocity and smooth pursuit velocity gain, and increase gaze position error variability.

Soccer heading model is a safe and reliable method to study mild head impacts, with average impact magnitude of 14 *g*.[17, 33] To track a moving object, the brain must predict the trajectory of the moving target and maintain focus by concomitant adjustment of eye movement. We will employ the Eye-Sync system, which objectively measures important parameters, including smooth pursuit velocity gain, saccadic peak velocity, gaze position error variability.

Aim 3. Inspect subconcussive effects on changes in neuronal activity during resting default mode.

Hypothesis: Subconcussive impacts will induce a significant suppression of theta, alpha and beta waves (powers) in the frontal and occipital brain region during resting default mode, compared with baseline levels.

Since subconcussive impacts induce diffuse force to the brain, rather than a focal injury like contusion,[4] likely neuronal network is disrupted. Neuronal activity –delta, theta, alpha, beta powers– in the whole brain will be measured using electroencephalography (EEG) while resting.

Aim 4. Determine whether subconcussive head impacts induce physiological effects in primary motor cortex.

Hypothesis: Subconcussive impacts will significantly reduce primary motor cortex excitability compared with baseline levels.

To better characterize neuronal physiology after subconcussive impacts, we will employ transcranial magnetic stimulation (TMS) to detect subclinical, physiological change in the primary motor cortex (M1). Consistent with established protocol,[41] we will target the M1 representation of a finger muscle at baseline and each post-heading time point to compute the input/output (I/O) curve, which reflects M1 excitability.

3.0 Inclusion/Exclusion Criteria

Inclusion Criteria

For Soccer Cohort

1) being between 18 to 26 years of age
2) an active member of a soccer team (i.e., collegiate, intramural, club, professional)
3) at least 5 years of soccer heading experience.

For non-Athletic Control Cohort

1) Being between 18 to 26 years of age
2) Have never played organized sports
3) Have never been diagnosed with a concussion

Exclusionary criteria

For both Soccer and Non-Athletic Control cohorts

1) any head, neck, or face injury in the 1 year prior to the study (e.g., concussion, eye injury);
2) history of vestibular, ocular, or vision dysfunction (e.g., macular degeneration)
3) currently taking any medications affecting balance (e.g., antibiotics)
4) pregnancy
5) HIV
6) any neurological disorders (e.g., seizure disorders, closed head injuries with loss of consciousness greater than 15 minutes, CNS neoplasm, spinal cord injury/surgery, history of stroke)
7) hypertension, cardiac arrhythmia, or pulmonary disease
8) lower extremity injury that would prohibit normal walking
9) metal implants in the head
10) implantation of cochlear device, cardiac pacemaker, medical fusion device, intracardiac lines, or neurostimulator (e.g., DBS, epidural/subdural VNS)
11) history of severe injury to the bones, joints, or muscles in either arm

Session-specific exclusion criteria will include:
12) slept less than 4 hours before the 1st and 2nd test day (verified by the TMS screening questionnaire)
13) drank more than 3 alcoholic drinks or used recreational drugs 24 hours before the 1st and 2nd test day
14) drank more than 3 cups of coffee in an hour before test sessions
15) glasses are prohibited (contact lens are okay) for safety purpose for the heading intervention

4.0 Enrollment/Randomization

Potential participants will be recruited via listserv email to undergraduate students in the School of Public Health-Bloomington, and interested participants will contact (phone or email) and meet with the investigator to discuss the project and ask questions. The informed consent and Concussion History & Health History Questionnaire will be given to the potential candidates. Participants who meet the inclusion criteria and are free of exclusionary factors will advance to the testing procedures.

We will use simple randomization method for group assignments. Up on inclusion of the participants, based on dice roll they will be randomly assigned into one of three groups: Heading group (number of dice 1 or 2), kicking group (number of dice 3 or 4), or standing group (number of dice 5 or 6). After assigned to groups, based on flipping a two-sided coin, participants will be further assigned into either TMS (head) or EEG (tail) experiments.

Dr. Kawata (PI) will only know subjects' group assignment and ensure blinded experiment and data processing. Dr. Kawata will randomly assign participants into three groups and perform soccer heading/kicking/standing protocol, and draw blood. Ocular-motor testing and data processing will be performed by Ms. Coon and blood biomarker experiments will be performed by Ms. Wirsching. The EEG experiment will be conducted by Mr. Innis and analyzed by Dr. Newman. The TMS experiment will be performed by Ms. Mirdamadi and analyzed by Dr. Block. As noted, statistical analyses of all the data will be performed by Dr. Chen. These process will ensure single blind method.

5.0 Study Procedures

Research Design

We will use a repeated measures design. The independent variable will be time (pre-test, 0h post-test, 2h post-test, 24h post-test). Control groups (kicking and standing) will be used to analyze any effects from bodily damage, exercise, and normal changes in dependent variables over time. The dependent variables assessed over time will include symptom scores, ocular-motor function, blood biomarker concentration, brain waves, and cortical excitability. Impact kinematics will also be assessed during soccer heading.

The study consists of 4 data collection time points in 2-day period. The 1st collection - pre-heading baseline-, followed by soccer heading intervention. The 2nd collection, 0h-post-heading time point (immediately after headings). The 3rd collection, 2h-post-heading time point (2h after headings). The 4th collection, 24h-post-heading time point (2nd day). This study design will enable to test outcome measures over 3 acute phases (Figure 5). The 1st day will last 4.5 hours and 2nd day will last 1.5 hours. Since we propose to include a total of 60 subjects in the study, we estimate data collection and primary analysis of 60 subjects will last approximately 15 months.

In order to address the potential residual effects of previous soccer-related injury or head trauma, we will recruit additional 20 age- and gender-matched healthy-non-athletic controls, against subjects in the soccer heading group to validate whether baseline values of each parameter are comparable to a healthy non-athletic cohort. However, regardless of whether there will be a difference in baseline between soccer players and healthy-controls, the purpose of the current study is to determine acute subconcussive head impact effects in the athletic population. Thus, the changes between soccer players and healthy controls will not terminate the study.

Soccer Heading Protocol

When a subject who suffice inclusion criteria and free of exclusion criteria agree to participate in the study, he/she will be randomly assigned into one of three groups (heading, kicking-control, standing-control). A standardized and reliable soccer heading protocol will be used for the experiment. A triaxial accelerometer (Triax Technologies) embedded in a head-band pocket and positioned directly below the external occipital protuberance (inion) to monitor linear and rotational head accelerations. A JUGS soccer machine will be used to simulate a soccer throw-in with a standardized ball speed of 25mph across all 3 groups. The ball speed is similar to when soccer players make a long throw-in from the sideline to mid-field. Soccer players frequently perform this maneuver during practice and game. Subjects will stand approximately 40ft away from the machine to perform either the heading, kicking, or standing (Fig 5 & 6). Dr. Kawata's previous laboratory at Temple University has routinely employed the protocol and average linear head acceleration yielded from the header is 14.5g in which many of subconcussion studies in football and ice hockey have set a threshold of impact recording as 16g, indicating that impacts induced from our protocol are below the minimum magnitude recorded in other contact sports. Participants in the heading group perform 10 standing headers with 1 header per minute, whereas kicking control group performs 10 kicks. Standing control group will remain static while a soccer ball passes over the head. The subjects in the heading and kicking groups will be instructed to direct the ball back toward the JUGS soccer machine in the air, while the subjects in the standing group will remain static. The kicking and standing control groups will aid to

distinguish effects of subconcussive impact from exercise, bodily damage, and/or simply a daily variation of the outcome measures. The first phase of acute subconcussive effects will be measured at 0h-post heading, indicating immediate sensory perturbation and neuronal damage, followed by measurement of the second phase, 2h-post neural inflammatory response. Lastly, ocular-motor function coupled with neural activity at 24h-post heading will be measured to assess the third phase of subconcussive effects.

Procedure Outline

1. **Email will be distributed**
2. **Interested participants will contact PI (KK) to discuss Project**
3. **Participants meet with the PI to discuss the project and ask further questions.**
4. **Participants take informed consent form with them and return with signature if they are willing to participate in the study**

a. Pre-test	1. Symptom Assessment (Neurotrauma Lab; 5 mins, KK, SC, AW)
(~40 mins if TMS)	2. Ocular-motor assessment (Neurotrauma Lab; 10 mins, KK, SC)
(~1h if EEG)	3. Blood Draw (Exercise Biochemistry Lab; 5 mins, KK, SC, AW)
	4. TMS Assessment (Neuromotor Lab; 15-20 mins, HB, JM)
	4. EEG Assessment (EEG lab; 30-40 mins, SN, II)

Intervention: Soccer heading, kicking, or standing (10 mins)

b. 0h-Post-test	1. Symptom Assessment (Neurotrauma Lab; 5 mins, KK, SC, AW)
(~40 mins if TMS)	2. Ocular-motor assessment (Neurotrauma Lab; 10 mins, KK, SC)
(~1h if EEG)	3. Blood Draw (Exercise Biochemistry Lab; 5 mins, KK, SC, AW)
	4. TMS Assessment (Neuromotor Lab; 15-20 mins, HB, JM)
	4. EEG Assessment (EEG lab; 30-40 mins, SN, II)
c. 2h-Post-test	1. Symptom Assessment (Neurotrauma Lab; 5 mins, KK, SC, AW)
(~40 mins if TMS)	2. Ocular-motor assessment (Neurotrauma Lab; 10 mins, KK, SC)
(~1h if EEG)	3. Blood Draw (Exercise Biochemistry Lab; 5 mins, KK, SC, AW)
	4. TMS Assessment (Neuromotor Lab; 15-20 mins, HB, JM)
	4. EEG Assessment (EEG lab; 30-40 mins, SN, II)
1. 24h-Post-test	1. Symptom Assessment (Neurotrauma Lab; 5 mins)
(~40 mins if TMS)	2. Ocular-motor assessment (Neurotrauma Lab; 10 mins)
(~1h if EEG)	3. Blood Draw (Exercise Biochemistry Lab; 5 mins)
	4. TMS Assessment (Neuromotor Lab; 15-20 mins, HB, JM)
	4. EEG Assessment (EEG lab; 30-40 mins, SN, II)

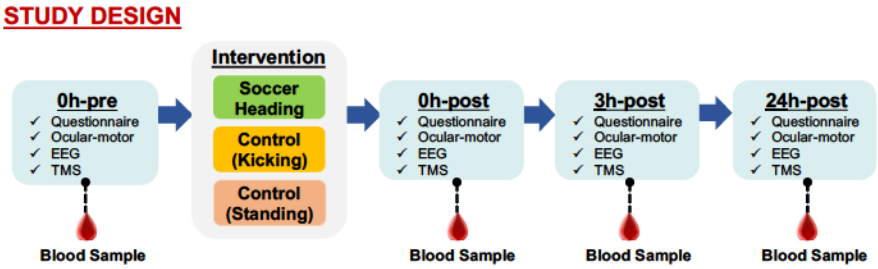

Fig 5. Study design. *3x4 repeated measures design will be used to assess acute effect of subconcussive head impact.*

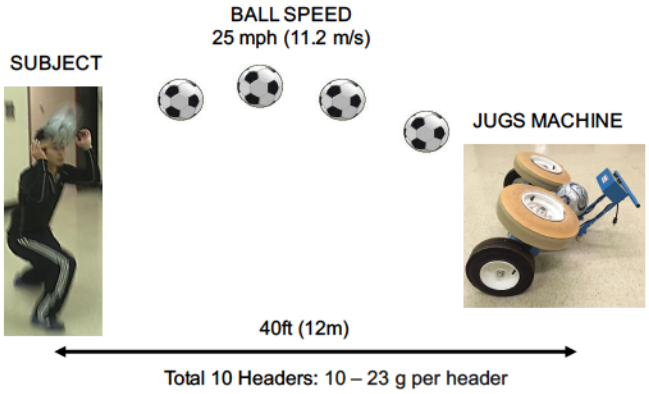

Fig 6. Mild head impact intervention, *previously used in our previous works (Hwang et al[33] and Kawata et al.[17]).*

Symptom Checklist

the post-concussion symptom scale, as a subset of the Sports Concussion Assessment Tool 3,[3] will be used as a method of assessing the presence and severity of symptoms. This paper-pencil symptom checklist consists of 22 symptoms with 7-point Likert scale per symptom to monitor subject's well-being. Participants will be instructed to report their current symptoms truthfully by circling symptom scores. Symptom scores will be manually transferred into an Excel spreadsheet for the future analyses. A tester or transferer will be blinded in regards to the group assignments and one's performance in other test parameters.

Blood Biomarker Assessment

The subject will be asked to lay on supine position with arm relaxed at 10-degree flexion. Antecubital vein blood draws will be performed each test session to help determine serum biomarker concentrations. A certified phlebotomist will thoroughly clean the antecubital fossa with an alcohol swab and draw 4 ml of whole blood into serum vacutainer tubes with a 21G butterfly needle. A Total of 16 ml blood for the study (4 ml x 4 time points). For non-athletic control subjects, 1 blood sample of 4 ml will be drawn. After the blood draw, the subject will use gauze to maintain direct pressure for 3 mins and Band-Aid will be provided. The whole blood will be centrifuged at 3,000 revolutions per minute for 20 minutes at 4°C after 40 mins of coagulation. The serum will then be divided and transferred into 1 mL cryovials and flash frozen and stored at -80°C in Exercise Biochemistry lab.

Ocular-Motor Assessment

The subject will then perform eye-movement task using the EYE-SYNC headset. This visual-tracking protocol has been replicated and validated in a number of concussion and sleep deprivation studies,[42-47] however to our knowledge this study for the first time will unravel subconcussive effects. Prior to testing, a Snellen chart will be used to verify that the subject has a normal or corrected-to-normal vision (minimum 20/30). The subject will be seated in a normally lit room and stabilize the headset with two hands while the elbows placed on the desk. The visual stimulus will be presented using a 120-Hz frame rate LCD screen in the headset and binocular eye movements will be tracked by a single camera secured in the headset. The test stimulus consists of a red circular target, 0.5° diameter in a visual angle with a 0.2° black dot in the center. The target moves in a circular clockwise trajectory of 10° radius at 0.4 Hz, with the target speed corresponding to 25°/s. The entire testing sequence will last approximately 3 mins consisted of a calibration and 2 consecutive test runs. Calibration of the eye position is conducted by having the subject fixate on a target presented at eight locations on the circular path of the test stimulus and one additional fixation point at the center of the circular path. The fixation target was presented at these nine locations in a randomized order. Each of the two test runs consists of 6 cycles of circular movement corresponding to 15 s in duration per test run. The subject will be instructed as "follow the movement of the target as closely as possible."

Transcranial Magnetic Stimulation Assessment

After eye movement tasks, a subset of subjects (n=10) per group will participate in a transcranial magnetic stimulation (TMS) experiment, while the different subset of subjects (n=10) per group will participate in an electroencephalography experiment. These pilot studies are to assess subconcussive effects on neural activities. TMS will be used to assess physiological change in the primary motor cortex after subconcussive impacts. TMS works by electromagnetic induction: a current passed briefly through a coil of wire held over the scalp induces a magnetic field around the coil which, in turn, induces an electrical current in the underlying nerve cells on the surface of the brain. We will place electrodes in the hand to assess the cortical representation of an index finger muscle, first dorsal interosseous (FDI).

During TMS application, both subject's arms will be relaxed on a pillow. Subjects will wear goggles that are tracked by a Brainsight neuronaviation system, allowing us to register the subject's head to the dimensions of a reference structural MRI. The hot spot for FDI will be determined at the beginning of each session and stored in Brainsight so that all stimulations within a session are applied at the same site. TMS above a certain threshold induces current in the cortex that activates neurons within the stimulated area. This results in a muscle twitch or motor evoked potential (MEP), measured with electromyography (EMG). Electrodes will be placed over the muscle belly and tendon, with a single common ground electrode over the ulnar styloid process of the target arm. EMG recording will be amplified, band-pass filtered (10-1000 Hz), and sampled at 5000 Hz. MEP amplitude reflects the excitability of the stimulated area. The slope of the input/output (I/O) curve, computed from MEP amplitudes at a range of stimulation intensities, will be examined to determine the changes in corticospinal excitability at each time point. Including preparation steps, the TMS experiment will last approximately 15 mins.

Electroencephalography Assessment
For EEG experiment, we will employ a standard 62-channel electro-cap with Ag/AgCl electrodes mounted to measure brain waves during default resting mode. To this end, the subject will be seated with eyes closed for 10 mins to ensure at least 2-min artifact free neural activity data. The 62 channels will be collapsed into four topographical regions of interest (frontal, central, parietal, and occipital) in each hemisphere (left and right). The EEG data will be processed offline using EEGLAB 5.03 software. Including preparation steps, the EEG experiment will last 30-40 mins.

6.0 Reporting of Adverse Events or Unanticipated Problems involving Risk to Participants or Others

Reporting Adverse Events
Keisuke Kawata, Ph.D. (primary investigator) will serve as the data safety monitor. The data safety monitor will monitor adverse event data, oversee procedures designed to protect the privacy of subjects, and coordinate the reporting of the outcome of any investigation of an adverse event. In the event of an adverse event, Dr. Kawata will report and cooperate with the IRB in any necessary investigation.

Adverse Events Associated with Soccer Heading
There is a risk of inducing symptoms such as headache, blurred vision, and disorientation during soccer heading testing, however ten soccer headers with mild head acceleration are commonly incorporated in soccer practice, and certainly soccer players utilize heading skill to direct a soccer ball for passing and scoring during practice and game. An average soccer player performs 500 headers over an entire season.[30, 31] It is extremely unlikely to elicit concussion-like symptoms (headache, nausea, disorientation, blurred vision) from headings with mild head acceleration (Avg. 14.5g). The study protocol has been replicated in multiple laboratories, producing invaluable data to progress traumatic brain injury research. Moreover, our preliminary study in football players have shown that a player sustained 39 head hits with the total magnitude of 1,200*g* during a single practice, but with no change in symptoms.[18] In order to minimize the risk of adverse events from soccer heading, we will only include soccer players who regularly play soccer and perform soccer headings.

Adverse Events Associated with Blood Draw
Complications of antecubital venous blood draws include bruising, local infection, phlebitis and injury to structures near the cubital vein. All of these complications are uncommon. To minimize these risks, Keisuke Kawata, Ph.D., (a certified phlebotomist) will perform the blood draws using single-use needles, tube holders and test tubes. The skin of the antecubital region will be cleaned with alcohol to further reduce the risks of infections. Following each blood draw the participant will remain seated while maintaining direct pressure against the site of the needle insertion. After each blood draw, the participant will be given a Band-aid to cover the puncture site. When the participant returns for each test session the certified phlebotomist will check the

puncture sites for any signs of complications (which would be referred for an additional medical examination).

Following the 48 hour post-test, the participant will be reminded to contact the research staff if they have any pain or discomfort associated with the procedure.
Pain and dizziness/fainting are other potential risks associated with antecubital venous blood draws. During the blood collection, the participants will be placed in a supine position. Prior to sitting up the certified phlebotomist will talk to the participant to confirm that the individual is comfortable. Once the participant is in a seated position they will sit for a few minutes to ensure that they are not feeling dizzy or faint. Ice will be offered at the end of each testing session to minimize any pain.

Adverse Events Associated with Ocular-motor testing
During performing ocular-motor tasks, participants may experience a transient headache and dizziness. Because the ocular-motor testing runs for 15 seconds and repeated twice, we believe that it is rare to elicit symptoms in the short period of testing. Based on the large-scale normative data (n=50,000) acquired by SynkThink Inc. among sports athletes and military servicemen, no one has claimed abnormal symptom due to the ocular-motor testing.

Adverse Events Associated with Electroencephalography (EEG)
There is minimal risk associated with EEG. An EEG measures the electrical activity in the brain (brain waves) using electrodes (small metal discs or sensors) placed on the head with gel. The test is non-invasive and no potential harm in neural structure nor metabolic balance, and the test does not hurt. The gel used to put the discs on the head is sometimes sticky and the discs may scratch the scalp. There may be a slight discomfort associated with sitting still in one position for 10-20 minutes. The likelihood of participant experiencing electric discharge from the EEG equipment is extremely small. No adverse events have been reported as a result of the EEG experiment.

Adverse Events Associated with Transcranial Magnetic Stimulation (TMS)
There is minimal risk associated with TMS. In particular, we are using magnetic brain stimulation due to it being well-tolerated, versus direct electrical stimulation of the brain, which is very painful. TMS briefly activates nerve cells in a small area on the surface of the brain. The stimulator device uses the principle of 'electromagnetic induction' to produce a weak electric current on the surface of the brain. While the physical risk is almost none (assuming they have completed the TMS Checklist), there is the potential for the subject to have an adverse psychological reaction to the stimulation techniques. The possible side effects as it has been mentioned in literature are: seizure induction (rare), syncope (epiphenomenon), transient headache, transient neck pain, transient toothache, transient paresthesia, and transient hearing changes. Based on safety literatures on paired-pulse, there is no extra risk in paired pulse comparing to the single pulse. There is no reason to believe that single pulse TMS, itself, poses any hazard. Extensive use in Dr. Block's previous laboratory at Johns Hopkins School of Medicine has not resulted in any difficulties with the device that could pose a hazard to participants. TMS does not appear to pose any hazard to the brain beyond that of electric stimulation, which has been in clinical use for decades. The procedure appears to be safe and without any side effects. Some of the original subjects have been stimulated many

thousands of times. Some people may experience a mild headache from the stimulation. A headache, if it occurs, is usually mild and only lasts a few minutes beyond the end of the test. However, participants with a history of a migraine or other types of severe or frequent headaches will be excluded from the experiment.

7.0 Study Withdrawal/Discontinuation

Data collection will require subjects to participate in 4 test sessions. Subjects may withdraw from the study at any time. A subject may also be withdrawn from the research without his/her consent if, for example, he/she 1) comes to the 1st and/or 2nd day with intoxication, 2) sustained injury prior to the 1st and 2nd day, 3) self-reported sleep deprivation for the 1st and/or 2nd day, 4) sustained a head injury prior to the 1st and 2nd day. If the subject completed for example entire 1st day procedure (3 test sessions) and unable to participate in the 2nd day (4th test session), the subject will be reimbursed hourly for the 1st day.

For non-athletic control cohort, we will evaluate and account for the soccer players' baseline, which may be influenced by a history of repetitive heading and sports participation. This group will participate in a single test session that lasts approximately in 1.5 hours. During the session, blood draw (4mL, about 1 teaspoon), measure eye movements using an ocular motor headset, and measure the brain activity using transcranial magnetic stimulation and electroencephalography. A subject may be terminated for the study participation if he/she 1) comes to the test session with intoxication, 2) sustained injury between screening and the day of test session, 3) self-reported sleep deprivation on the day of test session.

8.0 Statistical Considerations

Power analysis

According to our previous studies [Kawata et al. (2016)[17] and Hwang et al. (2016)[33]] and preliminary findings (Fig 4), the effect size is estimated to be 0.55 and the standard deviation is approximately 0.6. To achieve a power of 0.80 with $p<0.05$ as significance level, 18.68 subjects per group are suggested for the studies (Formula on the right). Although we did not have any drop-outs in aforementioned previous studies, the study would need 20 subjects per group to be able to interpret the data with any degree of confidence regarding the effects of subconcussive head impacts on outcome measures. Since we are recruiting 10 subjects per group for TMS and EEG for pilot studies, subsequent power analyses will be performed following data collection to determine whether we will need additional sample size or not.

$$n = \frac{Z_{\alpha} \quad Z_{1-\beta} \quad SD}{\frac{2(1.96 + 0.8416)^2 \ (0.6)^2}{(0.55)^2}}$$

Effect Size

Data analysis

Data analysis will be conducted by Dr. Zhongxue Chen, associate professor of biostatistics and director of biostatistics counseling center. To maintain the purity of statistical analyses, Dr. Chen will be blinded to group assignments by simply given the data set indicating Group (A, B, C). However, for our statistical model of longitudinal assessment, time points (pre, 0h-post, 2h-post, 24h-post) will be revealed to Dr. Chen.

Our primary interest is to identify the changes in biomarker expression, ocular-motor, and neural function after subconcussive head impacts in the heading group; hence, each parameter at post-heading time points (0h, 2h, and 24h) will be compared with pre-heading baseline. Each time point of the control groups (kicking and standing) will aid to interpret whether or not changes in the heading group are due to subconcussive impact, exercise, bodily hit, or simply a daily variation. To this end, a series of mixed effects regression models (MRM) with random intercept will be used for all aims.[48] The first MRM will focus on the within-group pattern of changes in biomarker expressions (PINCH and Tau), ocular-motor performance (smooth pursuit velocity gain, phase error, and gaze position error), neural activity (delta, theta, alpha, and beta powers), and motor cortex excitability (I/O curve) in 3 groups across the duration of the study – 4 time points. Next MRM will focus on between-group analyses on the variables (heading, kicking, standing) against changes in the biomarker levels, ocular-motor function, and neural function. Predictor variables include time, group, and time by group interaction. All analyses will be conducted with SAS and significance will be set at $p < 0.05$.

Study Endpoint

Throughout the study, we set the study endpoint based on subjects' well-being measured via symptom checklist or verbal claim to researchers. Because the mild nature of head impact, we expect to observe a subtle change in our sensitive outcome variables after soccer headers. To date, there is virtually no scientific evidence available to set study endpoints using ocular-motor, blood biomarker, and neuronal activity, we will guide the study based on symptoms, meaning that if a subject experiences any concussion-related symptoms following blood draw, soccer heading, ocular-motor testing, EEG, and TMS, his or her participation will be terminated.

Symptom

When a subject experiences any symptoms related to concussion (headache, nausea, blurred vision, disorientation, fogginess, sensitivity to light and noise, etc.), his/her participation will be terminated. However, there has been no participant thus far reported concussion-related symptoms due to soccer heading, blood draw, nor ocular-motor performance.

Blood Biomarker

Presently, there are no biomarker concentration levels that can serve as a cut-off point to diagnose concussion or subconcussion, while in more severe traumatic brain injury, astrocyte-derived biomarker (S100B and Glial Fibrillary Acidic Protein) have known to be useful in prognosis of brain injury and its outcome.[49-51] The proposed study tests neuron-enriched blood biomarkers (PINCH and tau proteins), where theoretically detecting these markers in the blood indicates neuronal damage in the brain. Our preliminary work on PINCH (Fig 4) illustrates that season-long exposure to subconcussive head impacts in football players induced increases in PINCH protein, while PINCH remains undetectable in 14 out of 16 players. However, there is no scientific evidence thus far demonstrating the association of PINCH/tau proteins in the blood and brain damage. The study design we employ allows us to test the ambiguous notion whether head impacts may cause the increase in PINCH and/or tau in the blood and possible association with neural activity measured via TMS and EEG.

Neuronal Activities

Altered neuronal functions will be measured via TMS and EEG. As noted in the hypotheses under Specific Aims, following subconcussive head impacts, we expect to observe a transient suppression of theta, alpha and beta waves (powers) in the frontal and occipital brain region measured via EEG, indicating temporary neuronal perturbation due to head impacts. Similarly, using TMS, we will target the M1 representation of a finger muscle at baseline and each post-heading time point to compute the input/output (I/O) curve, which reflects M1 excitability. If the subject experiences any adverse symptoms which are similar to concussion-related symptoms (headache, nausea, blurred vision, disorientation, fogginess, sensitivity to light and noise, etc.), he or she will terminate the study participation.

9.0 Privacy/Confidentiality Issues

All participant information, and even the fact that an individual is in the study, is considered confidential. Confidentiality will be assured in this study through several mechanisms. During interviews and treatments, the investigators and study coordinator will ensure physical privacy by conducting interviews in a closed room. Subjects will be assigned a subject number to help make data anonymous. The participant's Protected Health Information will be used for research purposes only. No names will accompany any data that is used for publication. To reduce the risk of confidentiality loss, electric data collected during the study will be stored on the university server and data collection sheets will be stored in a locked file cabinet in a locked room. The data will be stored indefinitely for data quality purpose for potential investigation after publishing the data. Only study personnel will have access to the data. All members of the research team are certified through the CITI program. Individual subject results will not be shared with the participants or their agents. Data analysis and publication will not include any identifying information.

Data Management

For symptoms, blood biomarker, and ocular-motor function, we will utilize spreadsheet to organize data and store in the IU Box account. Microsoft excel does not have range-checks system, however when we run statistical analysis using Statistical Analysis System (SAS), which can identify outlier and appropriate range for acquired data.

Symptom

Symptom scores will be manually transferred into an Excel spreadsheet for the future analyses. A tester or transferer will be blinded in regards to the group assignments and one's performance in other test parameters. The Excel spreadsheet will be saved in the university secured Box account, which will be backed up weekly.

Ocular-motor function

The eye-movement data acquired via the EYE-SYNC headset will be automatically saved in an Excel spreadsheet. The Excel spreadsheet will be saved in the university secured Box account, which will be backed up weekly.

Blood Biomarker
Biomarker expressions will be measured via Simoa HD-1 Analyzer and the data will be transferred into an Excel spreadsheet by the tester.

EEG
The EEG data will be processed offline using EEGLAB 5.03 software, which has a built-in range-check system. The data will be backed up weekly.

TMS
The TMS data will be captured and processed using Brainsight software (Rogue Research), where outliers can be identified by a built-in range-check system.

10.0 Follow-up and Record Retention

Since we propose to include a total of 80 subjects in the study, we estimate data collection and primary analysis of 80 subjects will last approximately 15 months. The electric data collected during the study will be stored on the university server, and data collection sheets will be stored in a locked file cabinet in a locked room. The data will be stored indefinitely for data quality purpose for potential investigation after publishing the data.

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