The effects of repetitive head impacts on neuroimaging and biomarkers in college athletes

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THE EFFECTS OF REPETITIVE HEAD IMPACTS ON NEUROIMAGING AND FLUID BIOMARKERS IN COLLEGE ATHLETES

by

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I would like to thank my parents for providing me with the opportunity to be in the position that I am today. I would also like to thank my friends and family for supporting me every step of the way. I would like to thank Dr. Robert Stern for his expertise in the field of neurology and guidance throughout this process. I would also like to thank Dr. Oren Berkowitz for imparting his knowledge in the field of research.
Football safety has increased over time, in part due to improvements in equipment and body mechanics, but there are still inherent risks involved, including exposure to repetitive head impacts (RHI). Significant head impacts can result in a constellation of symptoms including nausea, vomiting, headache, dizziness, and amnesia, which typically assist in the diagnosis of concussion. However, it has been shown that subconcussive impacts may result in microstructural changes and physiological alterations in the brain. This is particularly concerning because athletes may be undergoing changes in the brain in the absence of outwardly visible symptoms. Poorer neurologic outcomes later in life have been associated with cumulative exposure rather than number of diagnosed concussions.

Accelerometers installed in helmets have shown that college football players may receive up to 1,850 head impacts throughout the course of one season. The concussion rate is obviously much lower, indicating there are a high number of head impacts per diagnosed concussion. Axons are especially susceptible to damage from RHI because of their extension throughout the nervous system. The subtle changes thought to result from RHI are not easy to measure, but several modalities have been proposed. These include diffusion tensor imaging (DTI), plasma tau protein, and King-Devick testing.
The proposed study will look to quantify cumulative head impact exposure in college football players prior to the start of a season and see if this has any impact on the variables. They will then participate in one season of football wearing helmet accelerometers to measure the number of head impacts sustained. Changes in the variables will be compared to non-contact sport college athletes. Data will be analyzed to determine if number of head impacts correlates with changes in variables and if prior head impact exposure has any effect on these changes.

Data obtained from this study will have significant implications in the field of head injury. It may strengthen the use of several markers of brain injury that could be utilized in the future. Additionally, the effects of cumulative head impact exposure and one season of head impacts will be thoroughly examined. This information can be provided to trainers, coaches, and athletes to further improve football safety.
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LIST OF ABBREVIATIONS

AD ............................................................... Alzheimer’s Disease
ALS ........................................................... Amyotrophic Lateral Sclerosis
ATP .............................................................. Adenosine triphosphate
CHII .............................................................. Cumulative head impact index
CSF .............................................................. Cerebrospinal fluid
CT ............................................................... Computed tomography
CTE .............................................................. Chronic traumatic encephalopathy
DTI ............................................................... Diffusion tensor imaging
FA ............................................................... Fractional anisotropy
HITS ............................................................. Head Impact Telemetry System
ImPACT ....................................................... Immediate Post-Concussion Assessment and Cognitive Test
MD ............................................................... Mean diffusivity
MRI ............................................................. Magnetic resonance imaging
NCAA ......................................................... National Collegiate Athletic Association
NFL ............................................................. National Football League
NMDA .......................................................... N-methyl-D-aspartate
PET ............................................................. Positron emission tomography
RHI .............................................................. Repetitive head impacts
TBI .............................................................. Traumatic brain injury
INTRODUCTION

Background

It has been estimated that 1.8 to 3.6 million sports-related traumatic brain injuries (TBI) occur each year, most often in football. This is likely an underestimate considering that some may be unrecognized by healthcare personnel or underreported by athletes. With approximately 3,000,000 youth participants in football, 1,100,000 high school football athletes, and 100,000 post high school football players in the United States, this is an area of great public health concern.

TBI can result in short term effects, such as headache, nausea, vomiting, sleep disturbance, and disruptions in visual and verbal memory, but head impact exposure has also been linked to neurological problems later in life, including mild cognitive impairment, depression, amyotrophic lateral sclerosis (ALS), Alzheimer’s disease (AD), and chronic traumatic encephalopathy (CTE). Indeed, athletes who report multiple concussions during their careers have significantly higher rates of emotional disruption, mood disorders, irritability, and memory loss.

Of particular concern is the idea that neurodegeneration may be linked to accumulation of subconcussive hits to the head rather than significant impacts resulting in a diagnosed concussion. Neuropathological evidence of CTE has been found in athletes who sustained thousands of head impacts but never had a recognized concussion. Data from accelerometers installed in the helmets of players shows that college football players may sustain between 244 and 1,850 head impacts throughout the course of a season, with a disparity in frequency and magnitude according to position played.
Montenigro et al. used accelerometer data from several published studies, along with self-reported playing histories, to create the cumulative head impact index (CHII). This formula estimates total head impact exposure by position throughout youth, high school, and college football.

The consequence of this is that individuals exposed to repetitive head impacts (RHI) may be experiencing microstructural changes in the brain in the absence of symptoms visible to physicians, trainers, coaches, and even the players themselves. Several modalities have been proposed as methods to potentially measure the effect of RHI. These include diffusion tensor imaging (DTI), which measures the diffusion of water molecules within the brain, expressed in fractional anisotropy (FA) and mean diffusivity (MD), as well as plasma tau, and King-Devick testing.

**Statement of the Problem**

The effects that RHI have on the structure and function of the brain, both in the short-term and long-term setting, are not well understood. Currently, there are no guidelines to accurately and effectively diagnose brain injury. The methods used to diagnose concussions are vague and subjective. Additionally, minimal data exists looking at risk factors for the deleterious effects of RHI. Some postulations include type of head impact, duration of exposure, position played, age at exposure, gender, cognitive reserve, and genetic predisposition. Further research is needed to explore risk factors for brain injury from RHI so that this risk may be mitigated.
Hypotheses

College football athletes scoring higher on the CHII will have decreased FA, increased MD, increased plasma tau, and increased (worse) K-D scores prior to the start of the season. After one season of college football, athletes will show decreased FA, increased MD, increased plasma tau, and increased K-D scores as compared to non-contact control athletes. There will be a positive correlation between number of head impacts and magnitude of change in each of the variables. Football players scoring lowest on the CHII prior to the start of the season will show the most pronounced changes in each of the variables.

Objectives

The effects of RHI have long been studied in boxers but recently the negative health consequences of participating in football have gained more attention. Football players are repeatedly exposed to head trauma just by the nature of the game. RHI can result in both short-term and long-term negative effects, which are poorly understood at present.

The purpose of this study is to examine the effects of just one season of football on brain imaging, blood biomarkers, and saccadic eye movements, all of which have the potential to provide insight into measuring brain injury following RHI. Additionally, accelerometer data from helmets will be used to further explore these findings and elucidate possible risk factors, such as previous exposure and number of head impacts, that predispose certain individuals to increased neurologic sequelae. This information
could be used to provide recommendations to colleges regarding the importance of decreasing the frequency of head impacts and possible ways to do so.

**Specific Aims**

- Determine if there is a correlation between baseline head impact exposure, as measured by the CHII, and FA, MD, plasma tau, or K-D scores, in college football players
- Examine whether or not one season of college football results in changes in FA, MD, plasma tau, or K-D scores, as compared to non-contact college athletes
- Measure the total number of head impacts received over the course of one season, in practices and games, and determine if a greater number of head impacts results in greater changes in FA, MD, plasma tau, or K-D scores
- Determine if prior head impact exposure predisposes football players to greater changes in the variables or offers a protective effect
REVIEW OF THE LITERATURE

Overview

Origins

The negative effects of repetitive blows to the head have been recognized for a long time. Harrison Martland first introduced the term “punch drunk” to describe a condition in boxers where they exhibited Parkinsonian symptoms or appeared intoxicated after repeated head trauma.\[18\] Martland asserted that the syndrome most often appeared in less skilled boxers who were more prone to absorbing hits to the head, presented in greater than 50% of fighters, and seemed to be an occupational hazard specific to boxing rather than other forms of cerebral injury.\[18\] Since that time, evidence of neurodegeneration has been found in other cohorts exposed to head trauma, including American football players, ice hockey players, soccer players, professional wrestlers, military veterans who experienced one or more blast injuries, epileptics who repeatedly hit their heads, and victims of abuse.\[8,19\]

History of Football Safety

Recently, much attention has been devoted to the negative consequences of playing football, but these damaging effects have been known for a long time as well. In 1894, a U.S. Navy midshipman and football player named Joseph Reeves adorned the first football helmet after being told by a physician that another blow to the head would cause a traumatic psychosis.\[20\] This and many of the initial football “helmets” were actually just leather caps and did not offer significant protection. In the 1930s a suspension system was introduced, but this actually increased the number of head injuries due to a greater incidence of head-to-head impacts.\[21\] In 1969, after 38 fatalities on the
football field, the National Operating Committee for Standards on Athletic Equipment (NOCSAE) was formed to ensure proper safety specifications and testing for helmets. The game of football has certainly gotten safer over time, and in 1990 there were no direct fatalities reported in the Annual Survey of Football Injury Research, the first time since the survey began in 1931. This survey, which looks at catastrophic injury in football in the United States, indicated there were 8 direct fatalities, defined as deaths attributed to the mechanics of football (i.e. head injury, spine fracture), in 2013. These all occurred in high school athletes, and no fatalities were reported in youth, college, or professional football. This represents a continuing downward trend. This improvement in safety is likely due to increased data collection, enhanced techniques and body mechanics, and developments in equipment.

**Concussion**

The brain sits within the skull surrounded by cerebrospinal fluid, and a blow to the head results in a jostling of the brain inside the skull. The sudden acceleration and deceleration of the brain relative to the skull results in a deformation of the brain, stretching neurons, shearing axons, and disrupting membrane integrity. This triggers a constellation of events referred to as the “neurometabolic cascade of concussion”. Initially, there is an influx of calcium and an efflux of potassium, disrupting membrane potential. At the same time, there is an abrupt release of excitatory neurotransmitters, such as glutamate, that attach to NMDA receptors and further disrupt membrane potential. In response, the Na-K pump works harder to restore membrane potential, requiring more ATP and thus increased glucose metabolism. This occurs concomitantly
with diminished cerebral bloodflow and therefore results in a cellular energy crisis.\textsuperscript{23}

Additionally, concussions may cause a neuroinflammatory response, production of free radicals, and disruption of neurofilaments and microtubules.\textsuperscript{3,23}

**Subconcussive Head Impacts**

While much of the focus of brain injury has consisted of concussion research, several studies have shown changes in DTI, fMRI, and cognitive testing in athletes exposed to RHI but with no clinically diagnosed concussion.\textsuperscript{9,12,24} One issue is that concussion symptoms vary widely across individuals, thus making them difficult to identify and diagnose.\textsuperscript{25} Another issue is that athletes, especially at higher levels as the stakes get higher and the competition more intense, may be hesitant to report symptoms.\textsuperscript{25} They may be aware that they will be taken out of the game, and are afraid to let themselves or the team down, or they may not be aware of the repercussions of repeated head trauma.

Although concussions are certainly important in looking at brain injury and have distinct physical manifestations, this uncertainty in diagnosis and reporting of concussions suggests it may be prudent to focus on cumulative head impact exposure. Several studies have showed associations with number of years played in football players and poorer outcomes. McKee and colleagues stated that more progressive staging of CTE pathology was significantly correlated with number of years played.\textsuperscript{19} Singh and colleagues found that hippocampal size was inversely correlated with number of years played.\textsuperscript{26}
**Axonal Injury**

Blood vessels and axons are particularly susceptible to damage from concussive and subconcussive hits due to their extension throughout the nervous system. Smaller, unmyelinated axons are more vulnerable to damage than are larger, myelinated axons.\(^{27}\) In general, the severity and distribution of axonal injury parallels the severity of the traumatic brain injury (TBI), with mild TBI causing only microscopic axonal damage and more severe TBI resulting in pronounced axonal injury.\(^{27}\) Pathological studies of athletes who died within 6 months of sustaining a concussion have shown extensive perivascular axonal injury, most prominently in the corpus collosum, fornix, subcortical white matter, and cerebellum, as well as marked perivascular astrocytosis and microgliosis.\(^{27}\) Previous studies of brain injury have shown microscopic petechial hemorrhages, axonal injury, and perivascular microglial clusters.\(^{27,28}\) Indeed, there is activation of microglia and astrocytes in response to brain injury. Microglia are immune cells of the brain that aid in repair and are typically inactive in healthy brains. They are neuroprotective in the acute setting but long-term activation is associated with neurodegeneration. Traumatic axonal injury causes the upregulation of surface antigens on microglia, which leads to the release of pro-inflammatory cytokines.\(^ {29}\) It is believed that these cytokines engage in crosstalk to activate astrocytes. Astrocytes are structural support cells for neurons. In response to brain injury, astrocytes function to increase the release of glial fibrillary acidic protein as well as further release cytokines. Continued activation of astrocytes leads to nitration of tau protein, and consequently hastened development of neurofibrillary tangles. It may also cause mutation of tau protein, resulting in aggregation of tau oligomers.\(^ {29}\)
**Tau Protein**

Tau proteins are proteins that normally function to stabilize microtubules. They are bound to tubulin and are predominantly found in axons. Following a traumatic brain injury, tau may be dissociated from tubulin, and is then abnormally phosphorylated, misfolded, aggregated, and cleaved, causing it to become neurotoxic. Tau hyperphosphorylation increases in response to brain injury due to an increase in activity of tau kinases as compared to tau phosphatases. Tau phosphatases function to maintain tau in a dephosphorylated state. When tau becomes phosphorylated, it is quickly ubiquitinated and more prone to develop into neurofibrillary tangles. Perivascular accumulations of abnormal tau have been found in recently concussed individuals. This appears to contribute to long-term neurodegeneration, as more extensive and widespread deposition of tau is associated with a more progressive stage of disease in CTE.

Of note, total tau is a marker of axonal injury and has the potential to be a useful clinical biomarker in the assessment of brain injury. It has been shown that tau is elevated in the cerebrospinal fluid (CSF) of Olympic boxers as compared to healthy controls and increases immediately following a bout. However, obtaining a sample of CSF requires a lumbar puncture, which is an invasive procedure and not feasible to perform on every individual that sustains a brain injury. Recent studies have demonstrated an increase in tau in the plasma of athletes exposed to repetitive head impacts, including Olympic boxers and concussed ice hockey players, which is a promising biomarker that deserves further exploration.
**Neuroimaging**

Another modality that can be utilized to measure the effect of RHI is neuroimaging. This includes positron emission tomography (PET) scanning, computed tomography (CT) scanning, magnetic resonance imaging (MRI), and diffusion tensor imaging (DTI). PET scanning allows for the visualization of metabolism in different regions of the brain using a glucose analog.\(^{31}\) Thus, it is useful in measuring brain metabolism while, for instance, performing a functional task, but is less useful in measuring the diffuse axonal injury thought to occur after RHI. CT scanning is useful for assessing the structural integrity of the brain, and can show common structural abnormalities in those exposed to repetitive head impacts, such as cavum septum pellucidum, enlargement of the ventricles, and atrophy of the cerebral cortex.\(^{19}\) However, this method is ineffective at visualizing diffuse axonal injury. Several forms of MRI may be useful in looking at brain injury, including high-resolution T1-weighted volumetric MRI and functional MRI, both of which have been employed in studies looking at the effects of repetitive head impacts.\(^{3,24,26}\)

Diffusion tensor imaging is a more sensitive imaging technique that can identify subtle changes in the white matter of the brain. This method uses MRI to measure the diffusion of water within brain tissue.\(^{20}\) In a normal brain, water is typically constrained to white matter tracts and moves directionally along axons.\(^{20,31}\) In response to a TBI, shearing forces damage axons, typically at the interface of gray and white matter.\(^{32}\) This damage to axons and their myelin sheaths causes an increase in total diffusion but hinders the directional movement of water.\(^{20,32}\) Fractional anisotropy (FA) is scaled between 0
and 1 and is used to quantify the diffusion of water. A value of 1 indicates complete anisotropic diffusion while a value of 0 indicates compromised white matter integrity. Mean diffusivity (MD) measures the overall magnitude of the diffusion of water molecules. There are numerous studies that show DTI changes in white matter in groups exposed to RHI. Most studies have shown a decrease in FA and an increase in MD in response to axonal damage. DTI changes are most commonly seen in the anterior corona radiata, the uncinate fasciculus, the corpus callosum, the inferior longitudinal fasciculus, the cingulum bundle, the hippocampus/fornix, the inferior fronto-occipital and corticospinal tracts, and the internal and external capsules. Many of these changes are unable to be seen on other forms of imaging, such as PET, CT, or MRI, suggesting that DTI may be superior in looking at axonal damage caused by RHI.

**King-Devick Test**

The King-Devick Test is a timed number-naming test that assesses saccadic eye movements. This is a surrogate measure of visual processing, attention, concentration, and language, all of which may be used as relative indicators of impaired brain function. It consists of four test cards, which can be in the form of physical index cards or through an electronic medium such as a tablet or computer. Subjects are asked to read numbers on the card from left to right as quickly as possible without making any errors. If an error is made, subjects are allowed to correct it; errors are only recorded if the subject does not correct it. There is one demonstration card followed by three cards of progressive difficulty. Times are recorded at the completion of each of the three test cards and the K-
D score is the sum of all 3 times. This is a quick and easy tool that can be used to assess visual tracking and attention, and may indicate the need for further testing.

**Head Impact Telemetry System**

Various studies have utilized the Head Impact Telemetry (HIT™) System (Simbex, New Lebanon, NH) to examine the magnitude, frequency, and location of head impact. This system employs the use of six accelerometers installed in helmets that provide components of linear and angular acceleration. The accelerometers are secured to the helmets in such a way to ensure they measure impact to the skull and not just movement of the helmet. The sensors can be programmed to turn on at a certain threshold, and then record and transmit data to a laptop on the sidelines. Typically, studies have used 10g, 14.4g, or 15g of acceleration as the threshold to record a hit. The device records 40 milliseconds of data, 8 ms prior to the trigger and 32 ms after. The development of this system has been invaluable in quantifying head impact exposure at all levels of football.

**Effects of Different Football Position**

Data from accelerometers installed in football helmets reveals that players may incur between 244 to 1850 head impacts throughout the course of a season. Frequency and magnitude of impacts differ by position played, attributed to different on-field responsibilities. Offensive and defensive linemen line up across from each other and hit each other from a short distance on nearly every play, thus incurring a high number of low-impact hits. Linebackers and defensive backs are more involved in open-field tackles, so they may have a greater force on each head impact but are not involved in
every play. Wide receivers and running backs are usually involved in high-velocity but infrequent head impacts. Quarterbacks are generally protected by their offensive line and rules that restrict severe hits on them. Special teams players such as punters and kickers rarely sustain head impacts, but the other players on special teams may collide with each other at full speed after running the length of the field. In a small study of 10 college football players by Bazarian et al., the 3 players with the greatest number of head impacts were offensive or defensive linemen. However, the players with the highest average linear and rotational acceleration per hit were linebackers. Larger studies conducted by Crisco et al. following three division III college football teams found the greatest number of head impacts in offensive linemen, defensive linemen, and linebackers, per game and over the course of a season. They found the greatest magnitude of impacts were in running backs and quarterbacks. Additionally, they found that most players sustained hits to the front of the helmet, while quarterbacks had a high proportion of hits to the back of the helmet. The authors postulated that this could be due to quarterbacks being hit from behind, such as when a defender comes from their “blind side,” or when they are tackled they hit the back of their head on the ground. Another retrospective study based on questionnaires administered to college football players found offensive linemen to have a significantly higher number of “dings” and symptoms of concussion, such as dizziness, headache, and seeing stars, as compared to other positions. Offensive linemen also reported more frequently returning to play while still having symptoms. A study that found decreased neurocognitive performance as well as impairment in fMRI activation in players without a clinically diagnosed concussion reported that this group was comprised
primarily of linemen. All of this is evidence that position played may be a significant risk factor in the development of neurologic dysfunction.

**Biomechanics of Head Impact**

Head impacts sustained in American football are most commonly of the translational acceleration-deceleration variety. Accelerometer data has shown that impacts are typically linear through the top-front of the helmet, transmitted to the frontal lobes, through the brain, and to the brainstem and cerebellum. There are two mechanisms implicated in traumatic brain injury, these are impact loading and impulse loading. Impact loading involves a direct blow through the center of mass of an object, which can result in extracranial injuries, such as contusion, lacerations, and skull fractures. On the other hand, impulse loading is proportional to change in velocity, such as the sudden movement of the brain inside the skull that can result in concussion. While helmets have undoubtedly decreased the effects of impact loading and blows to the outside of the head, there is no way to completely stabilize the brain inside the skull and therefore no way to completely eliminate impulse loading.

Another factor in the pathophysiology of brain injury are the different types of head impacts that are absorbed. There are two types of forces in head trauma, linear and rotational, but these are not mutually exclusive and almost every impact is a combination of the two. Accelerometers installed in helmets have allowed the measurement of these variables. As mentioned earlier, head impacts in football are typically linear acceleration-deceleration forces. This force is transmitted through the frontal lobes, into the brain, and down to the brainstem. In contrast, in boxing, most of the blows to the head come...
from the hook punch, wherein a force is applied to the side of the head causing it to rotate on a fixed axis. This causes excessive strain on the cerebellopontine angle and midbrain which may result in shearing of axons. It has been theorized that this may contribute to a different manifestation of symptoms, such as motor symptoms in boxers due to damage to the substantia nigra which is part of the midbrain. Linear front-to-back head injuries tend to resolve more quickly, whereas side-to-side injuries cause more damage, but the effects of these impacts on long-term neurodegeneration are not well known.

Existing research

Traumatic brain injury has acute damaging effects on brain metabolism, memory, and cognition, but also imparts increased risk of long-term neurologic sequelae. Particular attention must be paid to axonal injury and the types of impacts that cause the most damage. Various studies have examined changes in DTI, total plasma tau, and reaction time in individuals exposed to repetitive head impacts and others have studied accelerometer data from the helmets of football players.

Plasma Tau Protein

A 2013 study performed by Neselius and colleagues examined the relationship of serum biomarkers in 30 Olympic boxers as compared to 25 controls who were friends or relatives of the boxers in order to attain similar social and educational backgrounds. They collected blood samples from the boxers 1-6 days after a bout and then again after at least 14 days of rest with no head trauma. Blood levels of various potential biomarkers were measured, including tau, S-100B, GFAP, BDNF, and Aβ42. No significant differences were found in any of the biomarkers except for tau. There was a significant increase in
tau following a bout as compared to controls (mean ± SD, 2.46 ± 5.10 vs. 0.79 ± 0.96 ngL⁻¹, p=0.038). Additionally, levels of tau decreased in the boxers after at least 14 days of rest (2.46 vs. 1.43 ngL⁻¹, p=0.030).¹⁵ One of the limitations of this study was that there were no baseline measurements in the boxers, so levels of tau may have been higher than in controls even before a fight. Secondly, there was a relatively small sample size. Also, there was a wide range in the timing of the second blood draw (14-760 days), limiting the utility of this measurement. Lastly, the study involved Olympic boxers, who wear headgear and have more padding in their gloves, so this may not be generalizable to other sports.

Another important study on serum biomarkers was done by Shahim and colleagues in 2014 on hockey players from the Swedish Hockey League who sustained a concussion. They first obtained blood samples from 47 players from 2 teams as a baseline. Out of 288 players, 33 sustained a concussion during the season and 28 were included in the study. In the players who sustained a concussion, blood samples were drawn at 1, 12, 36, and 48 hours postconcussion as well as 144 hours after or whenever they returned to play symptom-free. This study looked at total tau, S-100B, and neuron-specific enolase. The median value of tau in concussed players was significantly higher than the median preseason value (10.0 pg/mL vs. 4.5 pg/mL, p=.001). Additionally, the authors found that levels of tau rose greatly during the first hour after concussion, then tended to decrease for 12 hours, and showed another peak around 36 hours. The concentration of total tau 1 hour after concussion correlated with the number of days it took for concussion symptoms to resolve.¹⁶ An advantage of this study is the blood
sampling at repeated intervals in concussed players, allowing researchers to track longitudinal changes in blood biomarkers. Some drawbacks to this study are that they only obtained baseline data from 2 teams, whereas they looked at concussion data from all players. It would be beneficial to track the changes in tau in individuals who were concussed from preseason to postconcussion. Additionally, they only included players with a diagnosed concussion; it would be interesting to look at overall head impacts causing changes in tau protein. Lastly, there was once again a relatively small sample size.

A 2015 study by Olivera and colleagues used this same tau assay method to examine the correlation between TBI and total plasma tau in military personnel from September 2012 to August 2014 who had been deployed within 18 months. Researchers administered questionnaires to participants to determine TBI exposure and the Neurobehavioral Symptom Inventory to assess postconcussive symptoms. 70 veterans who reported a history of TBI were included and 28 veterans with no self-reported TBI were included in the study. Of the 70 participants with self-reported TBI, 24 had a clinically confirmed TBI in the medical record. Plasma concentrations of tau were significantly elevated in the TBI group as compared to controls (mean ± SD, 1.13 ± 0.78 vs. 0.63 ± 0.48 pg/mL, p=0.03). Of the self-reported TBI cases, those with a TBI confirmed in the medical record had an increased concentration of tau as compared to those with only self-report (mean ± SD, 1.57 ± 0.92 vs. 0.85 ± 0.52 pg/mL, p=0.02). Those with 3 or more reported TBIs had higher levels of tau than those with less than 3 TBIs (mean ± SD, 1.52 ± 0.82 vs. 0.82 ± 0.60 pg/mL, p=0.008), and severity of
postconcussion symptoms correlated with total tau concentration in the self-reported TBI group \( r=0.37, \ p=0.003 \). One limitation to this study is that it involves self-reporting of TBI, which could be subject to recall bias, although the authors did try to counteract this by confirming with the medical record. Another is only one blood draw with significant variation in time from TBI, making it difficult to discern the relationship of the TBI to the changes in serum tau.

**Diffusion Tensor Imaging**

McAllister and colleagues performed a prospective cohort study on 80 college football and ice hockey athletes compared to 79 noncontact athlete controls looking at preseason to postseason changes in DTI and neurocognitive measures. All participants had imaging done and completed the California Verbal Learning Test-II as well as the Wide Range Achievement Test-4 prior to the season and after the season. Head Impact Telemetry System (HITS) was used to measure number of head impacts, peak linear acceleration, peak rotational acceleration, and HITsp, a measure based on peak acceleration, impact duration, and impact location. Athletes who sustained a concussion were excluded from the study. Contact athletes showed a significant increase in mean diffusivity in the corpus callosum whereas noncontact athletes had a decrease in mean diffusivity \( p=0.036 \). In addition, imaging changes were seen in the amygdala, cerebellar white matter, hippocampus, and thalamus. The magnitude of change in MD in the corpus callosum was associated with poorer performance on the measures of verbal learning and memory, indicating this axonal damage in the corpus callosum may result in diminished cognitive functioning. The exclusion of subjects with a concussion provided
an interesting look at the effect of repetitive subconcussive impacts but it would have also been intriguing to include these subjects as a comparison group. The authors note a limitation as DTI being adequate to track relatively small changes in a single subject, but physiologic motion and artifact across comparisons making it difficult to assess group changes. Additionally, the measures of postseason FA and MD were somewhat conflicting, with some increasing and some decreasing, hindering the reproducibility and applicability of these findings.

Bazarian and associates conducted a prospective cohort study where they enrolled 10 Division III college football athletes as well as 5 control subjects from the general student body and looked at changes in DTI, helmet accelerometer data, and clinical correlates. The investigators outfitted the athletes with helmets equipped with the HITS system and recorded various measures of head impact exposure in both games and practices throughout the course of a season. They obtained brain imaging at the beginning of the season, at the end of the season, and 6 months after a no-contact rest period. At these 3 points, subjects also underwent cognitive performance testing, balance testing, and had blood drawn to measure levels of S100B and apoA-I. No athlete suffered a clinically evident concussion during the study. Number of head impacts ranged from 431 to 1850. In general, offensive and defensive linemen accrued the greatest amount of head impact exposure; the athlete with the highest cumulative exposure was a center. As compared to controls, athletes experienced greater white matter changes, although some had increases in mean diffusivity (MD) while some had decreases in MD. For statistically significant results, athletes had a greater decrease in fractional anisotropy (FA) (p=0.024),
decrease in MD (p=0.017), and increase in MD (p=0.003) at the end of the season compared to the beginning of the season.\textsuperscript{9} These changes in white matter persisted 6 months after the season with no contact. Head impact correlations showed that a greater number of head hits with high rotational acceleration was associated with greater FA decrease. These findings were not associated with changes in cognitive testing or balance, indicating that these changes in white matter may be clinically silent. Some advantages to this study are the use of accelerometer data to measure frequency and magnitude of head impacts and the various clinical measurements to correlate to changes in brain imaging. Limitations of this study are its small sample size. Additionally, the use of non-athlete controls raises the question of whether changes in DTI were due to physical exertion rather than repetitive head impacts.

Davenport and colleagues conducted a study with many similarities to the proposed study. They enrolled 24 high school football players and tracked them throughout practices and games over the course of a season. All subjects underwent preseason and postseason DTI. They also underwent Immediate Post-Concussion Assessment and Cognitive Testing (ImPACT) preseason and postseason. All players were outfitted with the HITS. Investigators measured cumulative exposure, linear impacts, rotational impacts, and a combination of linear and rotational impacts. They also measured changes in fractional, linear, planar, and spherical anisotropy, as well as mean diffusivity (MD). Regression analyses were performed and showed a correlation between the combined linear and rotational impacts and all of the DTI measures. Significant correlations were also found for linear impacts and all DTI measures.\textsuperscript{12} No subjects in the
study suffered a diagnosed concussion during the season, indicating that subconcussive head impacts may result in changes in DTI. This is an exemplary study because they used accelerometer data from helmets to compare head impact exposure to changes in DTI. One of the biggest limitations to this study is the lack of a control group to use as a comparison. Thus it is difficult to say whether the changes in DTI imaging found in this study are directly related to repetitive head impacts.

Davenport and colleagues further went on to use the data acquired and publish another study looking at changes in diffusion kurtosis imaging (DKI). DKI is an expansion of DTI that essentially uses a greater number of gradient directions. The authors state that DTI is useful when diffusion is normal and Gaussian, but often times diffusion in the brain is naturally anisotropic and non-Gaussian. DKI allows for the measurement of deviation from a Gaussian distribution. The authors used a voxel-wide analysis to determine number of abnormal voxels (±2 SD from the mean) in mean kurtosis, axial kurtosis, and radial kurtosis. The number of abnormal voxels in all metrics was significantly associated with risk weighted exposure (RWE), a cumulative measure of frequency and severity of head impacts. It was not addressed whether these metrics increased or decreased. Limitations are similar to their previous study, small sample size and lack of a control group, although this is one of few studies linking cumulative head impact exposure to neuroimaging changes in individuals without a concussion.

The study of DTI changes has not been limited to football. Koerte and colleagues published a study on 25 male ice hockey players who were part of the Hockey Concussion Education Project (HCEP) in a Canadian Interuniversity Sports (CIS)
league. DTI scans were performed on all players prior to the season and after the season. 6 athletes were excluded due to either a preseason or postseason scan not being available. 2 athletes were excluded due to motion artifact on their scans. Thus, 17 hockey players had preseason vs. postseason DTI compared, of which 3 suffered a diagnosed concussion. All players were included in the analysis, which revealed significantly increased mean diffusivity, axial diffusivity, and radial diffusivity in the postseason scans. No difference was found in FA. Limitations of this study include small sample size and lack of a healthy control group. Also, there is no quantification of head impacts so one cannot determine whether head impacts played a direct role or if certain individuals are more susceptible to structural changes.

Soccer athletes are also exposed to repeated head impacts, either by collision with the ball during headers or, less commonly, other players. Lipton and colleagues conducted a retrospective cohort study where they compared heading exposure in soccer to differences in brain imaging and cognitive testing. 29 male and 8 female amateur soccer players were given a questionnaire to quantify heading in the previous 12 months and lifetime concussion history. They then underwent diffusion tensor imaging and cognitive testing and the data was analyzed. Participants had headed the ball between 32 and 5400 times over the previous year, with a median of 432. Images were reviewed by a neuroradiologist and revealed significantly decreased FA in three regions of the temporo-occipital cortex in participants with high heading exposure, defined as greater than 1096 headings in the past year. Memory performance was also significantly decreased at a threshold of 1800 headings. The authors postulate that this may indicate a limit at which
heading should be curtailed during a season, although some of the participants with less than 1800 headers still had decreases in FA or memory performance, suggesting that some individuals may be more susceptible to the damage of repetitive head impacts. This is a useful comparison study because soccer heading is similar in biomechanics to the linear head impacts incurred by offensive and defensive linemen, although football players wear helmets whereas soccer players do not. Another benefit of this study is the use of men and women, because most of the research on pathologic changes associated with repetitive head impacts has focused on men. Limitations of this study include its retrospective nature, which make it difficult to identify a causal relationship between head impact and changes in brain imaging and cognition. Another limitation is the use of questionnaires, which can be inaccurate and subject to recall bias, although the authors conducted a test-retest reliability substudy to establish the validity of this method.

While several neuroimaging techniques have strengths in looking at brain structure and function, DTI has proven to be particularly effective in measuring diffuse axonal injury, as evidenced by the referenced studies. DTI measures the diffusion of water molecules, which can be hindered by axonal damage, thought to be an important aspect of RHI. Previous studies have used various methodologies in looking at DTI, and the proposed study will look to combine and build upon the strengths of these studies.

**King-Devick Test**

The King-Devick Test has been validated in multiple studies, such as one that was performed by Galetta and colleagues. The authors of this study recruited 219 college athletes from various sports at one university, including varsity football, sprint football,
men’s and women’s soccer, and men’s and women’s basketball. All participants were administered the K-D Test prior to the season and after the season. Any athlete who sustained a concussion as determined clinically by the athletic trainers was immediately given the K-D Test. Ten athletes experienced a concussion, and their sideline K-D scores were significantly higher (worse) than their baseline score (median 46.9 s vs. 37.0 s, p=0.009). In the overall cohort, scores improved slightly from a median of 37.9 s to a median of 35.1 s, p=0.03. This most likely indicates a learning effect and is common among timed tests administered more than once. The test was given to the men’s basketball players mid-season following a scrimmage, and showed slight improvement (35.0 s post-scrimmage vs. 38.6 s baseline, p=0.0003), indicating that fatigue most likely did not contribute to the decreased performance of those with concussions. Although there were a small proportion of athletes with concussions (n=10), the difference in test time was significant.

Seidman and colleagues conducted another study examining the effectiveness of the King-Devick Test. The methodology of this study was similar to the previous one, however investigators enlisted 337 high school football players. All participants underwent K-D Testing pre-season and post-season, and any athlete diagnosed with a concussion underwent testing within 30 minutes. 9 athletes suffered a concussion during the course of the season. Sideline testing scored significantly higher (worse) than baseline (median 66.2 s sideline vs. median 47.1 baseline, p=0.003). Among non-concussed athletes, pre- and post-season testing showed a minimal improvement in scores, although this was not statistically significant (median 47.4 s pre vs. median 46.8 s post, p=0.73).
Additionally, data was broken down by position played, revealing that concussions were sustained by 3 offensive linemen, 2 defensive backs, 1 wide receiver, 1 linebacker, 1 running back, and 1 quarterback. There was once again a small cohort of concussed athletes, and the lack of a control group limits the generalizability of this study.

**Head Impact Telemetry System**

Talavage and associates conducted a study where they outfitted 24 high school football players with the HITS over the course of one season.\(^2\) Prior to the season, 23 athletes underwent a neurocognitive assessment known as ImPACT™ as well as functional MRI to establish a baseline. During the season, 11 players were chosen to undergo repeat ImPACT and fMRI. 3 of these players had suffered a diagnosed concussion, and the other 8 had either a high number of head impacts, at least one high magnitude impact, or neither as measured by the HIT System. As expected, the 3 players diagnosed with concussion exhibited decreased performance on the ImPACT as well as significant alterations on their fMRI. Unexpectedly, 4 of the 8 in the other group showed decreased performance on ImPACT testing and decreased activation in the dorsolateral prefrontal cortex (DLPFC) and cerebellum. This indicates that 4 out of the 8 players without a diagnosed concussion had altered cognition and brain physiology. Of note, this group experienced the highest number of head impacts as well as more high magnitude impacts to the top-front of the helmet, directly over the DLPFC. This group was also comprised mostly of linemen. Total number of head impacts in this study ranged from 226 to 1855. Although this was a small cohort, one of the advantages is that the investigators utilized multiple modalities of measuring changes in the brain, such as
cognitive testing and neuroimaging, and obtained accelerometer data to correlate with these changes.

Crisco et al performed an observational study where they measured the frequency and location of head impacts among different player positions using the HIT System.\(^\text{10}\) The authors enrolled 188 college football players from three Division I schools and tracked them in practices and games over the course of one season. The maximum number of head impacts for any one player on each team was 1022, 1412, and 1444. The median number of head impacts on the 3 teams was 257, 294, and 438. Overall, subjects received an average of 6.3 impacts per practice and 14.3 impacts per game. Linemen and linebackers received the greatest number of head impacts, with offensive linemen experiencing the greatest percentage of hits to the front of the helmet. All player positions received most hits to the front of the helmet, except for quarterbacks, who received the largest proportion to the back of the helmet. Although this was an observational study it simply and effectively measures frequency and location of head impacts in football and breaks this down into position played. The large sample size through the recruitment of three different teams adds to the strength of this study.

Montenigro and colleagues used accelerometer data from this study as well as others to create the Cumulative Head Impacts Index (CHII).\(^\text{11}\) This measure takes into account an athletes’ subjective self-report of playing history as well as objective accelerometer data from various studies at the youth, high school, and college level to estimate an athlete’s cumulative exposure to head impacts. Utilizing this measure, the authors reported a threshold after which there is a dose-response relationship between
CHII and neurologic impairment. The CHII was also more predictive of neurologic impairment than number of concussions, age of first exposure to football, and number of seasons played. A table showing how the CHII is calculated is included in Appendix A.

**Football Position Played**

Baugh and colleagues conducted a notable study where they examined factors related to head impacts, categorized by position played. The authors administered self-report questionnaires to 730 Division I college football players. The components of the questionnaire included diagnosed concussions, undiagnosed concussions, “dings”, concussion-related symptoms (i.e. dizziness, seeing stars, nausea or vomiting, headache, etc.), returning to play while symptomatic, intention to report symptoms, and frequency of full-contact practice. There were no significant differences in diagnosed concussions. However, offensive linemen reported significantly greater numbers of undiagnosed concussions, “dings”, dizziness, seeing stars, and headaches compared to other positions. Additionally, significantly more offensive linemen reported returning to play while symptomatic and participating in full-contact practice. This may indicate that offensive linemen experience greater numbers of unobservable symptoms, such as dizziness and headaches, and builds upon existing accelerometer data that shows greater frequency of low-magnitude head impacts in offensive and defensive linemen. An advantage of this study is its large sample size. However, there was a low response rate during recruitment. 110 teams were approached, and 10 agreed to participate, therefore there may be a selection bias in teams who chose to participate. Also, the use of self-report questionnaires may have been inaccurate and subject to recall bias.
As evidenced by the existing research, DTI, plasma tau, and King-Devick testing are effective methods of measuring changes in brain function in response to RHI. However, no studies to date have utilized all three of these variables on a large cohort. In addition, the use of accelerometer data in the proposed study will allow for precise measurement of head impact exposure and examine how this contributes to observed changes in the data.
METHODS

Study design

The proposed study will be a prospective cohort study looking for evidence of neurologic changes in football players based on both previous exposure and one season of football. In addition, investigators will measure accelerometer data to determine if head impacts directly relate to these changes.

Study population and sampling

The subjects will be all consenting members of the Harvard University men’s football team, a National Collegiate Athletic Association Division I program. The control group will be members of the men’s swim team, men’s golf team, men’s tennis team, and men’s track and field team, excluding high jumpers and pole vaulters. Although football players tend to have a distinct body composition, this control group is chosen based on similar demographics and athletic expenditure, and who will most likely not experience head impacts during a season. Control group athletes with a history of participation in contact sports or concussion will be excluded. Other exclusion criteria for all participants will be any history of neurologic illness, history of psychiatric illness, or contraindication to MRI scanning. Based upon findings from a DTI study performed by Koerte and colleagues,\textsuperscript{37} we estimate that we will need a sample size of 98 contact athletes to see a change in MD from $2.24 \times 10^{-3}$ to $2.36 \times 10^{-3}$ (SD $0.3 \times 10^{-3}$) preseason to postseason at a power of 80% and $\alpha$ of 0.05. Therefore, a control group of 98 noncontact athletes will also be recruited.
Exposure

In this study, exposure will be considered the summation of all head impacts sustained in practices and games throughout the course of a college football season. In addition, overall head impact exposure prior to the season based on number of seasons played will be analyzed.

Study variables and measures

All study participants will be administered a questionnaire at the beginning of the study to obtain demographic information, such as age, race, education level, height, and weight. Football players will be administered a questionnaire previously described by Montenigro and colleagues, which asks sports played, age at first exposure to football, levels of play (youth, high school, college), number of seasons at each level, number of years played, positions played at each level, and percentage of time spent in each position. This information will be used to calculate the cumulative head impact index (CHII) for all football players. All study participants will undergo diffusion tensor imaging (DTI) prior to the season. Statistical analysis of DTI is difficult, because of inter-subject variability, motion artifact, and subtle changes. Based on the review of the literature, studies have often used tract-based spatial statistics (TBSS), described in detail by Smith et al. Basically, all subjects’ FA or MD data is projected onto a mean skeleton, and then voxelwise cross-subject statistics are applied. This will allow us to obtain a mean FA and MD value for each subject. All study participants will undergo blood sampling prior to the season, to measure plasma total tau, as previously used by Shahim and colleagues. Lastly, all study participants will take the King-Devick Test,
which is measured by time to complete the test, as described by Galetta et al.\textsuperscript{4} Football players will be given helmets equipped with the Riddell Head Impact Telemetry (HIT\textsuperscript{TM}) System to measure the total number of head impacts throughout the season. After the athletes’ respective seasons, they will be brought back to once again undergo MRI scanning, blood drawing, and K-D testing, to obtain postseason measures of FA, MD, tau, and K-D scores.

**Recruitment**

Initially, the athletic director at Harvard University will be contacted via e-mail or phone to approve participation of the university in the study. Then, individual coaches of each team will need to be contacted, again via phone or e-mail. Lastly, informed consent will be obtained from all athletes and they will have the option to decline participation at any time.

**Data collection**

All individuals participating in the study will be invited to the medical campus prior to the season for individual clinical visits. Assuming we can perform about 5 clinic visits per day, with approximately 200 subjects, this process will take roughly 40 days. Due to the extent of data that needs to be collected, subjects will have testing done at different times of the day and sometimes weeks apart. This is discussed in the limitations section. At the visit, subjects will be given a questionnaire to obtain demographics and athletic history. They will then be administered the K-D test. Next, they will have blood samples drawn. Finally, an MRI scan will be performed. DTI processing will be performed as described in previous studies.\textsuperscript{9,12,13,37} A DTI sequence with 2 averages and
the following parameters will be performed: 60 noncolinear diffusion directions, repetition time 7015 msec, echo time 60 msec, matrix size 100 x 100, voxel size 2.2 x 2.2 x 2.2 mm, b-value = 0 and 700 sec/mm², and 70 slices. Football players will be outfitted with helmet accelerometers during the season, set to record hits that are over 14.4g. Accelerometer data will be collected throughout all practices and games. Athletes who sustain a concussion during the season will not be included in the primary outcome analysis, but will be included in a secondary analysis. At the end of the season, all participants will be invited to the medical campus for a second clinical visit. At this time, they will again undergo K-D testing, blood sampling, and an MRI scan. Once again, assuming 5 clinic visits per day, this process will take about 40 days after the conclusion of the season to complete.

**Data analysis**

Using preseason data, a regression analysis will be performed for the football player cohort only using CHII as the independent variable and FA, MD, tau, and K-D as the dependent variables. This will investigate whether cumulative head impact exposure prior to the season has any effect on the variables. Next, a 2 x 2 study design will be utilized with contact vs. noncontact athletes on one side and preseason vs. postseason on the other. This will be used for FA, MD, tau, and K-D, and an analysis of variance will be performed to look for relationships between these values. Then, a regression analysis will be performed with number of head impacts as the independent variable and deltas of FA, MD, tau, and K-D as the dependent variables. Finally, covariate adjustment will be performed for CHII to see if this has an effect on the change in variables. During all
regression analyses, investigators will control for the number of days prior to the season that testing was performed, number of days postseason that testing was performed, and time of day that testing was performed using covariate adjustment.

**Timeline and resources**

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<td>Fall 2016</td>
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<td>Spring 2017</td>
<td>- Obtain funding and resources</td>
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<tr>
<td></td>
<td>- Subject recruitment</td>
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<td>Fall 2017-Spring 2018</td>
<td>- Data collection prior to, during, and after athletic seasons</td>
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<tr>
<td>Summer 2018</td>
<td>- Data analysis</td>
</tr>
<tr>
<td>Fall 2018</td>
<td>- Manuscript submission for peer-review</td>
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</table>

Undertaking an ambitious study such as this one will require significant resources. Use of the Center for Biological Imaging at Boston University Medical Center will be required, where they have a 3 Tesla Phillips Acheiva MRI scanner. Five scans per day, seven days per week will be attempted in order to achieve the goal of 40 days of data acquisition. Research staff will be asked to assist on weekends. The principal investigator will be responsible for project oversight and sampling. Data collection and data entry will be performed by study staff. MRI scans will be performed and analyzed with the assistance of a neuroradiologist. Research assistants will be trained in phlebotomy, so that he or she may assist with multiple aspects of data collection at each visit. Data analysis will be performed with the help of a statistician. We will also need access to the laboratory assay of plasma tau, use of the King-Devick test, and use of Helmet Impact Telemetry system.
Institutional Review Board

The proposed study will involve the use of human subjects and interventions such as brain imaging and venipuncture. Thus, a detailed protocol and INSPIR application will be submitted to the Boston University Medical Center IRB for full-board review.
CONCLUSION

Discussion

Multiple studies have looked at the damaging effects that repetitive head impacts have on brain physiology, biomarkers, and cognition. An advantage of the proposed study is the combination of these measures on one cohort. No study that we are aware of to date has utilized this unique combination of variables. In addition, obtaining accelerometer data from the football players offers an objective measure of head impact exposure during the season and whether or not this has any implication on the variables. This may potentially identify a threshold at which neurologic changes become manifest. Administering a survey to determine prior head impact exposure is a simple addition that allows for further study of risk factors, i.e. whether or not previous exposure offers a protective effect or predisposes individuals to further damage. The use of collegiate level athletes will provide a high level of competition and sufficient head impact exposure to theoretically produce changes in the variables.

One obstacle that may be encountered is subject participation in the study. The athletics department and/or coaches may be hesitant to submit agreement to participate because this will be an added burden when they are focusing on the upcoming season. The athletes themselves may be hesitant to participate in the study because while a questionnaire and timed visual test are rather benign, going into an MRI machine and giving blood samples are not. However, this study design can be applied to any of the area universities to gather a sufficient sample size.
Several limitations to the proposed study must be noted. Examining football players from Harvard University, a high-level academic institution, may not be generalizable to all football players. These players may initially have a higher cognitive reserve that could skew the results. Additionally, given such a large sample size with significant data collection, it will take time to gather data at the conclusion of the season. Thus, some athletes will have testing done immediately after the season while some will have it done weeks later, possibly giving them time to recover from any effects of RHI. Additionally, data collection will require testing throughout the day, so subjects will have testing done at different times of the day. There may be fluctuations in DTI, plasma tau, and K-D scoring based on time of day rather than exposure to RHI. Statistical analysis will attempt to control for this.

This study will not draw any definitive conclusions between RHI and brain injury. The subtle changes in brain structure and physiology thought to occur in response to subconcussive head impacts are difficult to measure. The study will potentially show changes in DTI, which may be an indication of axonal injury within the brain. It will also look at changes in plasma tau, a potential biomarker of axonal injury. Lastly, it will look at K-D scores, a measure of saccadic eye movements that may indicate an impairment in brain function. While this study will not establish definitive causal relationships, it will undoubtedly build on existing research of these measures as potential indicators of brain injury and add to the accelerometer data already in existence.
Summary

It is well established that full contact sports that involve exposure to head impacts, such as American football, ice hockey, soccer, and lacrosse carry an inherent risk of neurologic sequelae. Concussion awareness has improved tremendously over time, among physicians, athletic trainers, parents, coaches, and athletes. However, the diagnosis of concussion is still an imperfect science. Recently, brain injury in American football has been garnering attention exponentially in the popular press and among the general population, but the scientific literature cannot maintain that pace. Much more research is needed in this area. Particularly concerning is the fact that evidence of brain damage has been found in athletes without a diagnosed concussion\(^3,8\) and number of years played has been directly correlated with later-life depression, apathy, executive dysfunction, and behavioral dysregulation.\(^\text{11}\) This study will look to quantify a history of overall head impact exposure, and determine if this has an effect on variables that have been previously presented as potential markers of brain injury. We will then track these variables over the course of one season, to look for acute changes. Finally, we will observe the number of head impacts to determine if there is a relationship between number of impacts and changes in these measures.

Clinical and/or public health significance

Head impact exposure has been linked to neurological impairment later in life, including depression,\(^6\) amyotrophic lateral sclerosis (ALS),\(^7\) Alzheimer’s disease (AD),\(^7\) and chronic traumatic encephalopathy (CTE).\(^8\) It is notable that all neuropathologically confirmed cases of CTE have had exposure to RHI, while not all those who sustain RHI
develop CTE. The reason that some individuals exposed to RHI develop neurodegenerative disease while others do not deserves further exploration. It may be beneficial to follow this cohort over time to look at any neurologic changes that develop and what risk factors or characteristics play a role in these changes.

The findings of the proposed study will have significant implications regarding head impacts in football, both in diagnosis of injury and in further research. Examining the correlation between cumulative head impact exposure and these sensitive neurologic markers may establish a greater sense of the damaging effects of RHI. Thus we may advise coaches and players to limit this exposure, for instance by decreasing the number of full-contact practices, and substituting non-contact drills. Likewise, we may find a threshold in number of head impacts at which the markers start to show changes in the acute setting. Teams that utilize helmet accelerometers may be able to monitor players’ exposure and sit them out for a period of time when this threshold is reached. In addition, the use of DTI, plasma tau, and K-D testing may strengthen their validity as potential markers of brain injury. These could be used in sideline testing, evaluating for return to play, and/or in future research.
APPENDIX A

Calculation of the Cumulative Head Impacts Index, reproduced from Montenigro *et al.*\textsuperscript{11}

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\text{Cumulative Head Impacts Index} = [A] + [B] + [C]
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## APPENDIX B

### List of Study Variables

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REFERENCES


CURRICULUM VITAE

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EDUCATION
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Master of Science, Physician Assistant Studies
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2012-2013 University of South Florida, Tampa, FL
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2006-2010 Boston University, Boston, MA
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EXPERIENCE
2013-2014 Pharmacy Technician
CVS Pharmacy, Whiting, NJ

2010-2012 Emergency Room Technician
Lahey Clinic, Burlington, MA

CERTIFICATION AND LICENSURE
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