The Pennsylvania State University

The Graduate School

SUSCEPTIBILITY VERSUS RESILIENCY TO CONCUSSIVE INJURY AND HEAD ACCELERATION EVENTS IN ATHLETICS

A Dissertation in

Kinesiology

by

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Submitted in Partial Fulfillment

of the Requirements

for the Degree of

Doctor of Philosophy

May 2020

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Abstract

Involvement in sport has been an integral part of American culture, both as a social entity as well as a developmental one. However, despite the ever-growing popularity of sport, major concern has been raised recently regarding the safety of contact sports. By nature, contact sports often involve exposure of the head to potentially damaging forces. Sports-related concussion and repetitive exposure to impacts have become areas of growing public concern, especially given their potential link to long-term neurodegenerative damage. While this area of study has been burgeoning more recently, much is still unknown about these injuries and their long-term effects. More specifically, there have been questions raised regarding why, in individuals exposed to the same sport over the same periods of time, some individuals are functionally and clinically resilient to deficits while other individuals are susceptible to functional and structural damage from head injuries. Therefore, the overall goal of this dissertation was to examine, using multiple modalities, why some individuals may be more susceptible and some more resilient to brain changes after sports-related concussion or to repetitive exposure of head acceleration events. Individually, Chapter 2 was a systematic examination of the current literature regarding clinical/cognitive, imaging, and biomarker outcomes over the course of a single season of participation in contact sports focusing on athletes' college-aged and younger. Chapter 3 examined the influence of an individual athlete's genetics on past history of sport-related concussion. Chapter 4 examined various clinical and cognitive outcomes over the course of a single season of football participation and, in addition, compared reproducibility between two seasons and how governing body rule changes might indirectly affect outcome. Chapter 5 examined the role of the cervical spinal cord in exposure to repetitive head acceleration events using diffusion tensor imaging. Jointly, these chapters serve to further the understanding of 1) how different modalities can be used to study head injuries in an efficient, yet clinically and

functionally relevant way, and 2) how to begin to identify and distinguish factors that may differentiate individuals in regard to their susceptibility or resiliency to injury. The concept of susceptibility versus resiliency requires further study before concrete answers are determined, but this dissertation contributes to the growing body of work on the topic and how individual factors can be examined to best protect athletes and keep sports safe.

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Preface

This thesis was written specifically to fulfill dissertation requirements. Part of this work has either been submitted for publication or accepted in full for publication.

Chapter 2: This work was been submitted for publication at this time.

Chapter 3: This work has been accepted as: **Walter** A, Herrold AA, Gallagher VT, Lee R, Scaramuzzo M, Bream T, Seidenberg PH, Vandenbergh D, O'Connor K, Talavage TM, Nauman EA, Slobounov SM, Breiter HC. KIAA0319 Genotype Predicts the Number of Past Concussions in a Division I Football Team: A Pilot Study. J Neurotrauma. 2019 Apr 1; 36(7):1115–1124. PMID: 30351182

Chapter 4: Part of this work has been submitted for publication at this time.

Chapter 5: This work has been submitted for publication at this time.

Acknowledgements

This dissertation would not have been possible without the help of many people. I would like to first sincerely thank my advisor, Dr. Slobounov, for his valuable mentorship over my graduate school experience. He has been an amazing advisor and it has been a pleasure to be able to be his student and learn from him. I am honored to have been a part of his lab and cannot thank him enough for his mentorship and support over the years.

I would also like to extend my sincere thanks to the rest my committee: Dr. Peter Seidenberg, Dr. Wayne Sebastianelli, Dr. Peter Arnett, and Dr. John Miller. Their mentorship and feedback over the years has been extremely valuable in my learning experience and I cannot thank them enough for all of their help and support throughout this process.

In addition, I would like to acknowledge and thank all of the members of the concussion lab: James Wilkes, Katie Finelli, Michael Galantino, Tim Benner, Brian Johnson, and all of the many undergraduate students over the years. You have been such a crucial part of this experience and I cannot thank you enough for your support and friendship during this time.

Additionally, I want to thank the Penn State athletes who participated in this research as well as our collaborators from athletics: Tesa Johns, Andy Mutnan, Renee Messina, Madeleine Scaramuzzo, Tim Bream, and Scott Lynch. I am so appreciative of your continued support and help over the years.

Lastly, all of this would not have been possible without the continuous love and support of my friends and family. I am so appreciative of all of your encouragement, support, and belief in me throughout this process.

Chapter 1: Introduction

Participation in and enjoyment of sport has been a large part of culture in the United States. It is estimated that 45 million children participate in youth sports,[1] 8 million children continue sport at the high school level, and approximately 480,000 continue at the college level.[2] Involvement in sport has proven to provide numerous physical, mental, and social benefits ranging from reduced body fat and increased bone health, to decreased symptoms of depression and anxiety, to better social skills and self-esteem.[3,4] However, despite the numerous benefits of sport, over the past few decades there has been growing concern surrounding the overall brain health of participating athletes.

Contact sport athletes, in particular, are exposed to high numbers of impacts to the head and are at increased risk for sustaining head injuries, such as mild traumatic brain injury (mTBI). mTBI, or sports-related concussion (SRC), is defined as a traumatic brain injury induced by biomechanical forces with common features including a) a direct blow to the face, head, neck, or body with force transmitted to the head; b) rapid onset of short-lived impaired neurological function that resolves spontaneously; and c) a range of clinical symptoms that may or may not involve the loss of consciousness.[5] Annually, 1.6 to 3.8 million individuals are diagnosed with a concussion, yet despite this prevalence, it is thought that this may be an underrepresentation as 1 of 3 concussions are not reported or diagnosed.[6] Given its prevalence, more attention has been devoted to this area of research over the past decade, yet much is still unknown regarding these injuries. SRC are diagnosed by medical providers typically using the individuals' subjective reporting of symptoms since the vast majority of computerized tomography and magnetic resonance imaging findings are negative.[7] Treatment generally involves a period of 24 – 48 hours of cognitive rest which is then followed by gradual return to sport and academics.[5] The

large majority of individuals recover from the injury within 7 – 10 days;[8] however, there is a growing population (10 – 20%) whose recovery is more delayed and develops into post-concussion syndrome.[9]

At this time, there is little evidence regarding the biomechanical threshold necessary to produce injury. Studies have shown that various linear and rotational accelerations result in injury.[10–12] However, the physiological processes after injury are much more well defined. Giza and Hovda's seminal paper[13] outlined the cascade of physiological dysfunction after a force applied to the head. The brain undergoes a coup-contrecoup motion within the skull whereby the forces applied to the tissues of the brain cause a well detailed cascade. Briefly, there is immediate depolarization which leads to mass ionic influx of cations K^+ and Ca^{2+}. This causes an ionic imbalance resulting in the Na^+-K^+ ATP pumps working overtime. Following, a cascade of dysfunctions occurs including further oxidative phosphorylation, calcium sequestration in the mitochondria, and glucose imbalance. These physiological changes are thought to be the cause of specific clinical correlates.[14]

Typically, symptoms after a SRC are categorized into four groups: physical, cognitive, emotional, and sleep. Symptoms can range in timing from short-term (acute) to long-term (chronic) and can include, but are not limited to: loss of consciousness, nausea/vomiting, feelings of fogginess, headache, problems sleeping, behavior changes, deficits in concentration and memory, and oculo-motor deficits.[15,16] Various combinations of symptoms can be present after injury and premorbid status has shown to affect both risk[17] and recovery of SRC.[18] Risk of concussion has been associated with prior concussion history, participation in collision sports for men, participation in soccer for women, being female, age, and previous history of migraine (for a review see [17]). More well studied are premorbid characteristics and their role in recovery,

however, these findings are very mixed (for a review see [18]). Another confounding factor in recovery is the involvement of the neck and cervical spinal cord. Whiplash-associated disease (WAD) and SRC have similar mechanisms and symptom overlap, yet often this is overlooked in the diagnosis and management of SRC.[19]

More recently, there has been a shift in public concern from SRC solely to the accumulation of impacts to the head in contact sports. An inherent part of many contact sports involves forces being applied to the head repetitively throughout practice and game settings. These repetitive impacts have been called subconcussion, subconcussive impacts, receptive head impacts, and repetitive head acceleration events, among others. In this dissertation, head acceleration events (HAEs) will be used.

HAEs were initially defined as cranial impacts that did not result in known or diagnosed concussion based on clinical findings[20] and this definition has now evolved into "the transfer of mechanical energy to the brain with sufficient force to injure axonal or neuronal integrity, but not resulting in clinical symptoms."[21] It is assumed that participation in contact sports, such as football, rugby, soccer, lacrosse, hockey, and boxing, involves inherent exposure to HAEs. Similar to SRC, the threshold of impacts necessary to cause brain damage is unknown. It is theorized that it is the cumulative effect, rather than the maximum force, that is concerning and damaging.[22]

Part of the concern surrounding HAEs is their potential link to neurodegenerative diseases including Alzheimer's disease, chronic traumatic encephalopathy (CTE), and amyotrophic lateral sclerosis (ALS).[23,24] CTE, a neurodegenerative disease marked by tau accumulation, has been recently diagnosed in many former contact sport players, at many levels of experience ranging from high school to professional athletes.[25] The marked degeneration

present, in combination with the clinical symptoms reported, has increased concern regarding the safety of contact sports. Unfortunately, at this time, CTE can only be diagnosed upon autopsy, so many questions continue to remain surrounding its link to contact sports.

The study of HAEs is a growing field and most of the studies are observational, cohort studies done on single contact sport teams. In addition, findings from these studies are mixed as clinical metrics and imaging metrics often provide contradictory findings regarding participation in contact sports. Given the new nature of the field, little can be said regarding the threshold needed to qualify as brain injury and the diagnosis or treatment needed to address the damage. Additionally, the physiology of HAEs is challenging to study. It is assumed that the physiology would resemble the physiological cascade seen after SRC, however it has not been proven. Furthermore, the link between HAEs and SRC is complex and under great debate. Given the same exposure to HAEs, some individuals will sustain SRC while others will never be diagnosed with a SRC. While this highlights the subjective nature of diagnosing a SRC, it also highlights a unique concept which this dissertation will explore – the idea of susceptibility versus resiliency.

The overall goal of this dissertation was to examine why, in athletes who participate in various contact sports or even the same contact sport, some individuals are diagnosed with a SRC while others remain seemingly clinically "symptomless". Furthermore, why, when exposed to a similar number of HAEs, do some individuals develop deficits over time while others remain clinically "symptomless"? These questions have important clinical implications as the ability to currently identify athletes at risk for SRC or deficits after exposure to HAEs is lacking. The hope is that by answering these questions an understanding of how to protect the athlete, especially his or her brain, is gained while maintaining contact sports as an integral part of sport participation and development.

This question is extremely broad and will take many iterations of studies to fully answer. The overall goal of this dissertation, therefore, was to begin to understand the differences regarding susceptibility and resiliency to injury and to guide future work exploring this concept. Individually, each chapter examines the concept using various research methodologies with the same population: males participating in Division I college football.

Chapter Two was a systematic review completed to gain insight into current literature on subconcussive impacts/HAEs in child and college-aged athletes. More specifically, it focused on short term (i.e. a single season of participation) outcomes utilizing imaging measures, biomarker measures, and clinical/cognitive measures.

Chapter Three was a candidate gene study on the potential genetic link to previous concussion history in a cohort of college-age American football players.

Chapter Four was a two-part study examining the influence of a single season of American football on various functional outcomes. Data was collected during two separate football seasons to examine reproducibility of results and how coaching, medical, and regulatory decisions can indirectly influence outcome.

Chapter Five was an examination of the white matter integrity of the cervical spinal cord, using diffusion tensor imaging, after a single season of American football participation.

United, these chapters serve to provide a multimodal examination of susceptibility and resiliency to injury, particularly in a short time frame – a single season of participation in American football.

Chapter 2: The Accumulation of Subconcussive Impacts on Cognitive, Imaging, and Biomarker Outcomes in Child and College-Aged Athletes: A Systematic Review

Abstract

Objective: Examine the effect of subconcussive impact accumulation on cognitive/functional, imaging, and biomarker outcomes over the course of a single season, specifically in contact sport athletes at collegiate level or younger.

Design: Systematic review following PRISMA guidelines and using Oxford Center for Evidence-Based Medicine 2011 Levels of Evidence and Newcastle Ottawa Assessment Scale.

Data Sources: PubMed MEDLINE, PsycInfo, SPORT-Discus, Web of Science

Eligibility criteria for selecting studies: Original research in English that addressed the influence of subconcussive impacts on outcomes of interest with minimum preseason and postseason measurement in current youth, high school, or college-aged contact sport athletes.

Results: 796 articles were initially identified and 48 articles were included in this review. The studies mostly involved male football athletes in high school or college and demonstrated an underrepresentation of female and youth studies. Additionally, operationalization of previous concussion history and concussion among studies was very inconsistent. Major methodological differences existed across studies, with ImPACT and diffusion tensor imaging being the most commonly used modalities. Biomarker studies generally showed negative effects, cognitive/functional studies mostly revealed no effects, and advanced imaging studies showed generally negative findings over the season; however, there was variability in the findings across all types of studies.

Conclusion: This systematic review revealed growing literature on this topic, but inconsistent methodology and operationalization across studies makes it challenging to draw concrete

conclusions. Overall, cognitive measures alone do not seem to detect changes across this timeframe while imaging and biomarker measures may be more sensitive to changes following subconcussive impacts.

Introduction

Subconcussive impacts, or impacts less severe than those causing a concussive injury, have become a burgeoning area of study due to their potential accumulative effect and link to future neurological impairments. They have been defined as impacts that involve the transfer of mechanical energy to the brain with sufficient force to injure axonal or neuronal integrity but do not result in clinical symptoms.[1] Subconcussive impacts are especially common among athletes involved in contact sports; however, since they do not result in clinical symptoms or diagnosis,[2] they are difficult to diagnose or manage. The concern regarding these impacts has increased as contact sport athletes can accumulate large numbers of impacts over a career and even a single season.[3,4]

Many studies suggest that there may be an accumulative effect of repetitive impacts, altering advanced MRI findings and blood biomarker levels, and affecting cognitive processes; contact or collision sports, in particular, have the highest risk for accumulation of these subconcussive impacts. Previous studies have shown that in a single season, football players at the high school and collegiate level can experience on average 600 and 1000 impacts across the season, respectively,[2] while collegiate women's soccer players can experience 600 impacts across the season.[5] However, these exposures can vary on a multitude of factors including age, level of play, previous concussion history, sport played, and premorbid status.

Previous reviews examining subconcussive impacts on the short-term health of athletes[6] and, specifically in male youth and high school-aged football players,[7] have not been systematic

in nature and have not been able to draw any concrete conclusions about the effect on athlete health. Additionally, there is still controversy over the link between subconcussive impacts and effects on overall brain health.[8,9] Although recent literature has begun to identify possible accumulative effects of subconcussive impacts, many questions still remain regarding the nature and mechanism of these events. Notably, three main questions surrounding these impacts include: (1) the true definition, both physiologically and symptomatically/clinically; (2) the threshold, in terms of intensity and frequency, necessary to cause an accumulation of damage, and (3) the effect of these impacts on both short-term and long-term overall brain health. It is anticipated that a systematic scan of the literature will produce a richer understanding on the commonalities in how researchers examine subconcussive impacts, while also providing an opportunity to aggregate findings across domains of study.

Accordingly, this systematic review aims to examine the current knowledge on subconcussive impacts and their effect on cognitive and physiological processes over a short time frame. Specifically, does participation in a single season of contact and collision sport alter imaging findings, blood, urine, or saliva biomarker levels, or cognitive test results at postseason in currently participating child and college-aged athletes (under 23 years old), as this age timeframe encompasses a large range of brain development. By examining the existing literature and revealing core trends across the evidence base, this review aims to identify the current status of knowledge on this topic, especially as it relates to short-term health outcomes.

Methods

Search Terms and Databases: Guidelines for the reporting of systematic reviews (PRISMA; Preferred Reporting Items for Systematic Review and Meta-Analyses[10]) were followed for this review. We sought articles reporting on the association between subconcussive impacts and key

measures representing outcomes of interest. Based on this question, two groupings of keywords were used: head injury (e.g., subconcussive, subconcussion, repetitive head impact, head acceleration event) and outcome (e.g., clinical, imaging, biomarkers). Using a comprehensive list of keywords, a literature search was conducted on May 28, 2019 using the following databases: PubMed MEDLINE, PsycInfo, SPORT-Discus, and Web of Science for articles published in English, in humans, and with no date restriction. The PubMed MEDLINE search (Supplemental File 1) was adapted for other databases. A librarian with expertise in systematic reviews reviewed all search strategies.

Study Selection: After the search was run, articles were identified for screening (Figure 1). Two authors (AEW, JRW) independently and blindly screened all citations in the title/abstract stage, excluding studies based only on: 1) foreign language, 2) wrong population, 3) wrong publication type, or 4) wrong outcome. These simplified criteria were used to capture all possibly relevant articles and ensure that enough information was accessible to determine inclusion based on the criteria and operational definitions presented in Table 1. Any disagreements on articles to review were discussed between the two reviewers and consensus was reached. Following this screening process, citations that met the criteria moved to full-text review (all articles reviewed here are presented in Supplemental File 2). Articles were selected based on detailed criteria presented in Table 1 and a third reviewer (SMS) would have reviewed any disagreements on articles to be included in the review; however, there were none.

Figure 1. PRISMA Flow Chart for Studies Included in the Review.

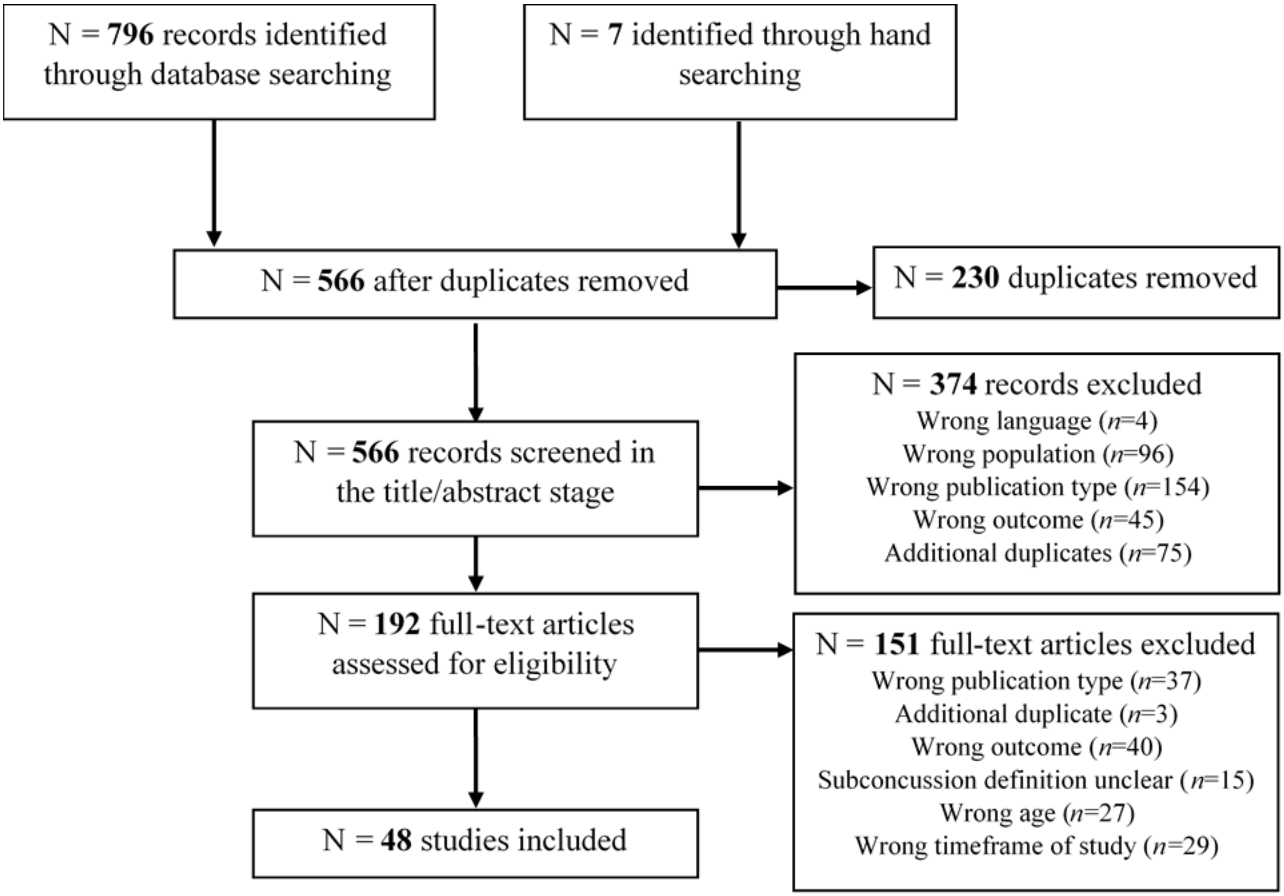


Table 1. Inclusion and exclusion criteria and operational definitions of terms

Domain & Operational Definition	Inclusion Criteria	Exclusion Criteria
Language	English	Not in English
Publication Type	Peer reviewed journal articles	Dissertations/theses, communications, conference abstracts, review articles

Population: ***Sport defined as:*** ***Contact/collision****: Football, rugby, soccer, lacrosse, field hockey, ice hockey, basketball, boxing, wrestling, martial arts/combat sports* ***Non-contact****: swimming, diving, volleyball, track and field, cross country, ice skating, skiing*	Athletes currently involved in an organized sport, and at minimum a group involved in a contact sport	Military or animal studies; former athletes
Age	Current participation in sport in youth (<13 years old), high school-aged (<18 years old) and college-aged athletes (<23 years old)	Professional or amateur athletes over college-age
Length of Study	At least one season with, at minimum, a pre and post measure	One bout/one game/one session; no pre and/or post measure included
Subconcussive Definition: *involving the transfer of mechanical energy to the brain at enough force to injure axonal or neuronal integrity, but not be expressed in clinical symptoms.*[1]	Includes subconcussion as a main or secondary outcome of interest of the study.	No concussion/post injury testing (or if so, as a subset of the study) *If concussion as an injury is involved in the study it needs to be either:* *(a) History of concussion (as a predictor, mediator, etc.)* *(b) A separate group used for analysis*
Measures: *See specific details below*	Modalities of cognitive/balance, imaging, or biomarkers (see below for details).	
Clinical (Cognitive/Balance) Measures: *Domains not limited to: memory, attention, executive functioning, verbal or math ability, problem solving, perception, balance, reaction time that can be done in a clinical setting*	Not limited to: ImPACT, SCAT, VR, King-Devick, ANAM, CVS, any other common neuropsychological test, BESS, Romberg, use of forceplate, computer reaction time, full body reaction time	
Imaging Measures: *Any conventional MRI or fMRI sequence*	Not limited to: rsfMRI, fMRI, ASL, DTI, SWI, DWI, MRS	

Biomarker Measures: *Any blood, urine, or saliva biomarker*	Not limited to: IL-#, TNF, SBDP, s100B, GFAP, NSE, NGB, Tau, BDNF, NF-L, UCH-L1, miRNA	

Abbreviations: SCAT= Sport Concussion Assessment Tool; VR= virtual reality; ANAM= Automated Neuropsychological Assessment Metrics; CVS= Concussion Vital Signs, BESS= Balance Error Scoring System; MRI= magnetic resonance imaging; fMRI= functional magnetic resonance imaging; rsfMRI= resting state functional magnetic resonance imaging; ASL= arterial spin labeling; DTI= diffusion tensor imaging; SWI= susceptibility weighted imaging; DWI= diffusion weighted imaging; MRS= magnetic resonance spectroscopy; IL= interleukin; TNF= tumor necrosis factor; SBDP= spectrin breakdown products; GFAP= glial fibrillary acidic protein; NSE= neuron-specific enolase; NGB= neuroglobin; BDNF= brain-derived neurotrophic factor; NF-L= neurofilament light; UCH-L1 = ubiquitin carboxyl-terminal hydrolase isoenzyme L1; miRNA = microRNAs;

Data Extraction Process: Data Extraction Process: For included articles, two authors (AEW, JRW) performed the data extraction and rated each article for both level of evidence and methodological quality. Pilot coding was done to determine consistency between reviewers and create a standardized process before beginning duplicate extraction. Data was extracted into a standardized table and for each article that was included, the level of evidence was rated with the Oxford Centre for Evidence-Based Medicine 2011 Levels of Evidence[11] and the methodological quality was assessed using the Newcastle Ottawa Assessment Scale.[12] The Oxford Centre for Evidence-Based Medicine 2011 Levels of Evidence rates on a scale of 1 to 5 and the Newcastle Ottawa Assessment Scale (NOS) rates studies in three categories – selection, comparability, and outcome – for a maximum of 9 stars.

Results

The search initially identified 796 article records, which ultimately resulted in a total of 48 studies included in the review (see Figure 1 for article selection process). Initial title and abstract screening resulted in 192 articles that were assessed in the full-text stage. An additional 151 articles were excluded for wrong publication type (*n*=37), duplicate (*n* =3), wrong outcome (*n* =40), unclear subconcussive definition/focus (*n* =15), wrong age (*n* =27), or wrong timeframe

(*n*=29). This resulted in 41 studies to be included in the review. Hand searching from bibliographies resulted in an additional seven studies, totaling 48 studies.

Data were extracted and are summarized in Table 2 with full extraction presented in Supplemental File 3. The levels of evidence using Oxford Centre for Evidence-Based Medicine 2011 Levels of Evidence and methodological rating using the NOS are presented in Supplemental File 4 for all studies included in this review. The ratings for level of evidence were 2 (2.1%), 3 (91.6%), or 4 (6.3%) and average NOS methodological rating was 5.2 stars out of 9.

Table 2. Overview of studies included in the review (n=48).

First Author, Year citation number	**Contact Group**: Sample size (n=X); sex; sport	**Comparison Group**: Sample size (n=X); sex; sport	**Measure used**: (0) clinical, (1) imaging, (2) biomarker	Specific measurement information
Youth				
Bahrami, 2016 [13]	n=25 (M; football)	NA	1*	DTI
Campolettano, 2018 [14]	n=34 (M; football)	NA	0	BESS, Force Plate (COP)
Janda, 2002 [15]	n=75 (55M, 20F; soccer)	NA	0	verbal learning (WRAML), digit span, SDMT, verbal learning delay
Jennings, 2015 [16]	n=59 (M; football)	n=28 (M; baseball)	0	Child SCAT-3
Munce, 2014 [17]	n=15 (M; football)	NA	0	postural stability, KD, ImPACT, PCSS
Rose, 2019 [18]	n=134 (M; football; 70 youth, 64 HS)	NA	0*	MSVT, TOVA, WISC-V, WASI-II, ChAMP, TMT, CogState, SWAN, SDQ, VOMS, SCAT-3, BESS
Rose, 2019 [19]	n=166 (M; football; 70 youth, 96 HS)	NA	0*	MSVT, TOVA, WISC-V, WASI-II, ChAMP, TMT, CogState, SWAN, SDQ, VOMS, SCAT-3, BESS

High School

Abbas, 2015 [20]	n=22 (M; football)	n=10 (M; swim, tennis, cross country, golf, baseball)	1*	rsfMRI
Abbas, 2015 [21]	n=10 (M; football)	NA	1	rsfMRI
Bari, 2018 [22]	n=63 (40M, 23F; football, soccer)	n=27 (14M, 13F; cross country, swimming, track, tennis, basketball, softball)	1*	MRS
Bazarian, 2012 [23]	n=10 (M; football, ice hockey)	n=5 (1F, 4M; uninjured and orthopedic injured)	0, 1	DTI, ImPACT
Breedlove, 2012 [24]	n=52 (M; football)	NA	1*	rsfMRI
Brett, 2017 [25]	n=626 (428M, 198F; variety)	n=145 (35M, 110F; variety)	0	ImPACT
Chun, 2015 [26]	n=34 (M; football)	n=9 (M; non-collision)	1*	DTI
Davenport, 2014 [27]	n=24 (M; football)	NA	0, 1*	DTI, ImPACT
Davenport, 2016 [28]	n=24 (M; football)	NA	0, 1*	DKI, ImPACT
Forbes, 2016 [29]	n=105 (F; soccer)	n=105 (F; soccer (history of concussion))	0	ANAM
Gong, 2018 [30]	n=17 (M; football)	NA	1*	DKI
Joseph, 2018 [31]	n=16 (M; football)	NA	0, 2*	CCAT, K-D, BESS, SCAT-3, SAC, blood sample (NF-L, Tau, GFAP, SBDP, UCH-L1)
Kaminski, 2008 [32]	n=393 (F; soccer)	NA	0	ANAM; concussion checklist

Kaminski, 2007 [33]	n=47 (F; 21 college, 26 HS; soccer)	n=24 (F; soccer)	0	WDST, HVLT, BESS
Kuzminski, 2018 [34]	n=17 (M; football)	NA	0, 1*	DTI, NP assessment metrics
Myer, 2016 [35]	n=30 (M; football)	n=32 (M; football (wore a collar))	1*	DTI, SWI
Myer, 2018 [36]	n=35 (F; soccer)	n=40 (F; soccer (wore a collar))	1*	DTI
Rose, 2019 [18]	n=134 (M; football; 70 youth, 64 HS)	NA	0*	MSVT, TOVA, WISC-V, WASI-II, ChAMP, TMT, CogState, SWAN, SDQ, VOMS, SCAT-3, BESS
Rose, 2019 [19]	n=166 (M; football; 70 youth, 96 HS)	NA	0*	MSVT, TOVA, WISC-V, WASI-II, ChAMP, TMT, CogState, SWAN, SDQ, VOMS, SCAT-3, BESS
Svaldi, 2015 [37]	n=16 (M; football)	n=10 (M; non-collision)	1*	fMRI, CVR
Svaldi, 2017 [38]	n=14 (F; soccer)	n=12 (F; basketball, track, cross country, swimming, softball, gymnastics)	1*	fMRI, CVR
Vogelpohl, 2017 [39]	n=55 (M; football)	n=33 (M; baseball or no participation in organized sport)	0	ImPACT
Wahlquist, 2019 [40]	N=217 (F; soccer)	NA	0	ANAM, PCSS
Yuan, 2017 [41]	n=30 (M; football)	n=32 (M; football (wore a collar))	0, 1*	N-back verbal working memory fMRI
Yuan, 2018 [42]	n=25 (F; soccer)	n=32 (F; soccer (wore a collar))	0, 1*	N-back verbal working memory fMRI

Yuan, 2018 [43]	n=21 (M; football)	n=21 (M; football (wore a collar))	1*	DTI
College				
Bazarian, 2014 [44]	n=10 (M; football)	n=5 (M; nonathletes)	0, 1, 2*	ImPACT, BESS, DTI, ApoA1, S100B, antiS100Bab
Caccese, 2019 [45]	n=38 (15M football; 23F soccer)	NA	0*	TG, SAC, BESS, CRT, KD, IMPACT
Champagne, 2019 [46]	n=26 (M; football)	NA	1	fMRI
Gysland, 2012 [47]	n=46 (M; football)	NA	0*	ANAM, SAC, SOT, BESS, GCS
Kaminski, 2007 [33]	n=47 (F; 21 college, 26 HS; soccer)	n=24 (F; soccer)	0	WDST, HVLT, BESS
Marchesseault, 2018 [48]	n=15 (M; lacrosse)	NA	0*	Stroop, CTMT
Marchi, 2013 [49]	n=10 (M; football)	NA	0, 1, 2	ImPACT, BESS, DTI; S100B, antiS100Bab
Mayinger, 2018 [50]	n=19 (M; football)	n=5 (NR; general population)	0, 1	ImPACT, DTI
McAllister, 2012 [51]	n=214 (81%M; football, ice hockey)	n=45 (93%M; track, crew, nordic skiing)	0*	ImPACT, NP battery
McAllister, 2014 [52]	n=80 (64M, 16F; football, ice hockey)	n=79 (56M, 23F; track, crew, nordic skiing)	0, 1*	DWI, CVLT-II, WRAT-4
Miller, 2007 [53]	n=76 (M; football)	NA	0	ImPACT, SAC
Miyashita, 2017 [54]	n=36 (M; lacrosse)	NA	0*	BESS
Murray, 2018 [55]	n=14 (M; football)	n=14 (3M, 11F; cheerleading)	0*	postural stability
Oliver, 2018 [56]	n=35 (M; football)	NA	2	serum NF-L, plasma tau

Papa, 2018 [57]	n=23 (M; football)	n=30 (15M, 15F; general population)	0, 2	miRNA, SAC, VR
Reynolds, 2017 [58]	n=63 (M; football, lacrosse, soccer)	n=30 (M; NR)	1	rsfMRI
Slobounov, 2017 [59]	n=23 (M; football)	NA	1*	rsfMRI, DTI, SWI, ASL
Volberding, 2014 [60]	n=42 (M; football)	NA	0	GSC, ImPACT

For more details of each study, see Supplemental File 3.
(*) use of accelerometer during study
Abbreviations: M = male; F = female; NA = not available; fMRI= functional magnetic resonance imaging; DTI= diffusion tensor imaging; BESS= Balance Error Scoring System; COP= center of pressure; SDMT= Symbol Digit Modalities Test; SCAT-3= Sport Concussion Assessment Tool – 3rd Edition; K-D= King-Devick Test; PCSS= post concussion symptom scale; MSVT= Medical Symptom Validity Test; TOVA= Test of Variables of Attention; WISC-V= Wechsler Intelligence Scale for Children; WASI-II= Wechsler Abbreviated Scale Intelligence, 2 Ed; ChAMP= Children and Adolescent Memory Profile; TMT= Trail Making Test; SWAN= Strengths and Weaknesses of Attention-Deficit/Hyperactivity-symptoms and Normal-behaviors; SDQ= Strengths and Difficulties Questionnaire; VOMS= Vestibular/Ocular Motor Screening; rsfMRI= resting state functional magnetic resonance imaging; MRS= magnetic resonance spectroscopy; DKI= Diffusion kurtosis imaging; ANAM= Automated Neuropsychological Assessment Metrics; CCAT= Axon Sports Computerized Cognitive Assessment Tool; NF-L= neurofilament light; GFAP= glial fibrillary acidic protein; SBDP= spectrin breakdown products; UCH-L1 = ubiquitin carboxyl-terminal hydrolase isoenzyme L1; WDST= Wechsler Digit Span Test; HVLT= Hopkins Verbal Learning Test; NP = neuropsychological; SWI= susceptibility weighted imaging; CVR= cerebrovascular reactivity; TG= Tandem Gait; SAC= Standardized Assessment of Concussion; CRT= Concussion Recognition Tool; SOT= Sensory Organization Tool; GSC= graded symptom checklist; CTMT= comprehensive trail making test; DWI= diffusion weighted imaging; CVLT-II= California Verbal Learning Test-II; WRAT-4= Wide Range Achievement Test; miRNA = microRNAs; VR = virtual reality; ASL= arterial spin labeling

Overall, studies varied in general methodological approach (Figure 2) and specific method used. For clinical metrics, ImPACT (13/48; 27%) and BESS (10/48; 20.8%) were the most commonly used measures with widespread use of other cognitive and functional metrics in studies (Figure 3). Within imaging metrics, DTI was the most commonly used (12/48; 25%), again with sporadic use of other sequences including rsfMRI (*n*=5), fMRI (*n*=4), CVR (*n*=2), ASL (*n*=1), SWI (*n*=1), DWI (*n*=1), DKI (*n*=1), and MRS (*n*=1). Biomarker studies were more limited and had a low-frequency, high-variety of blood biomarkers under study including: NF-L

(*n*=2), Tau (*n*=2), GFAP (*n*=2), SBDP (*n*=2), UCH-L1 (*n*=2), S100B (*n*=2), antiS100Bab (*n*=2), ApoA1 (*n*=1), and miRNAs (*n*=1).

Figure 2. Distribution of Studies by Type Across Age

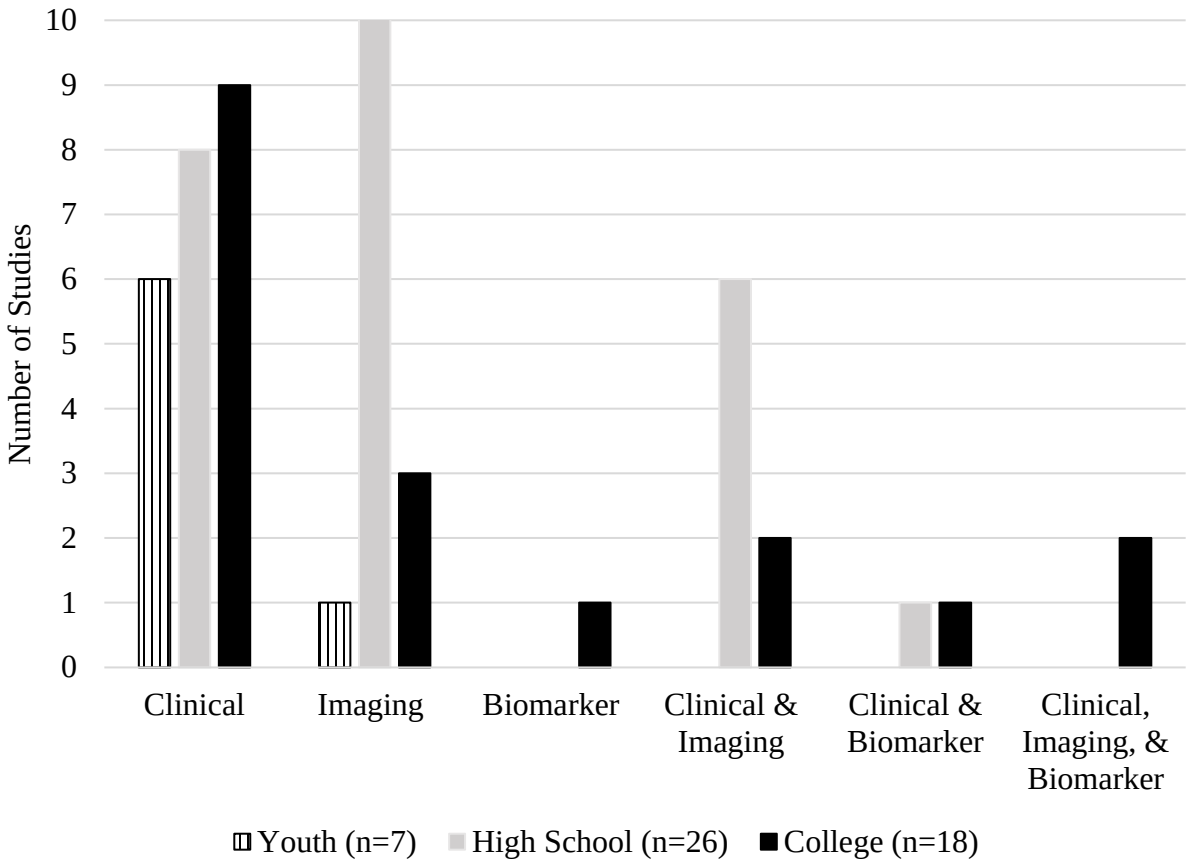
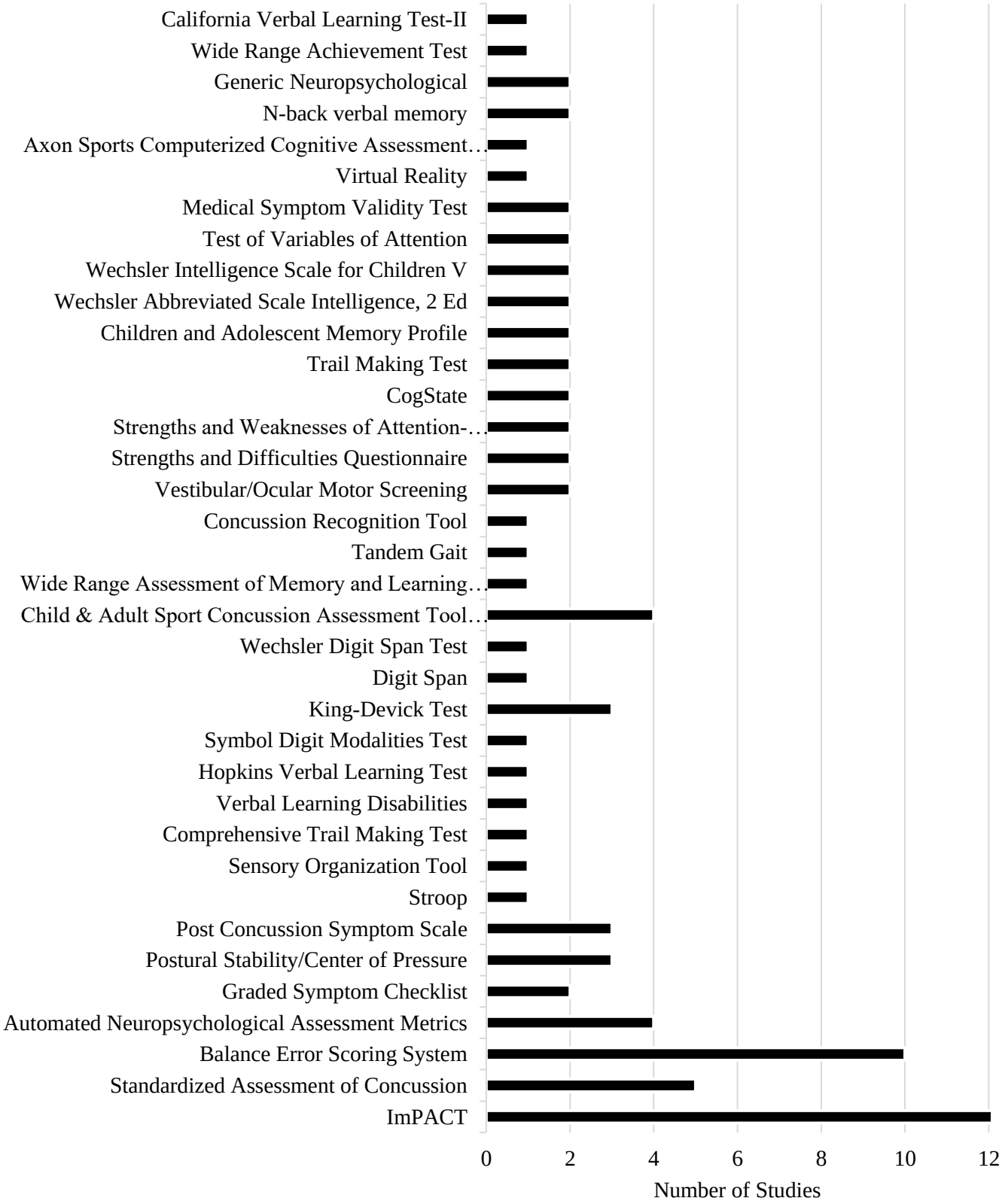


Figure 3. Frequency of Clinical Test Used Across All Studies (n=41)



Descriptions of Studies by Age

There were 7 studies included that focused on child athletes (<13 years old). The majority of studies (*n*=6) only used clinical (cognitive) measures,[14–19] while the remaining study used imaging, specifically DTI.[13] Sample sizes ranged from 15–166 (median: *n*=67) for the cognitive studies and *n*=25 for the imaging study. The cognitive methods used varied greatly. Lastly, two of the cognitive studies had mixed cohorts of youth and high school aged athletes.[18,19]

There were 26 studies included that focused on high school aged athletes. Eight of the studies only used clinical measures,[18,19,25,29,32,33,39,40] 11 only used imaging measures,[20–22,24,26,30,35–38,43] 1 used a combination of clinical and biomarker measures,[31] and 6 used a combination of clinical and imaging measures.[23,27,28,34,41,42] It should be noted that 3 of the clinical studies included mixed cohorts: 2 with youth and high school[18,19] and 1 with high school and college.[33] Sample sizes ranged from 10–63 (median: *n*=22) for imaging studies, 47–626 for clinical studies (median: *n*=67), and 10–30 for studies using a combination of methods (median: *n*=15). The modalities used varied greatly with DTI being the most common imaging metric and ImPACT being the most common cognitive metric.

There were 18 studies included that focused on college-aged athletes. Nine of the studies used clinical measures only,[33,45,47,48,51,53–55,60] 3 used imaging only,[46,58,59] 1 used biomarkers only,[56] 2 used clinical and imaging,[50,52] 1 used clinical and biomarker,[57] and 2 used a combination of all three methods.[44,49] Again, it should be noted that one of the cognitive studies included a mixed cohort of both high school and college-aged athletes.[33] Sample sizes ranged from 14–214 for cognitive studies (median: *n*=42), 23–63 for imaging studies (median: *n*= 26), and 10–80 for studies using a combination of methods (median: *n*=19). The methods used in the

studies varied greatly with DTI being the most common imaging metric and ImPACT being the most common clinical metric.

Descriptions of Studies by Sex

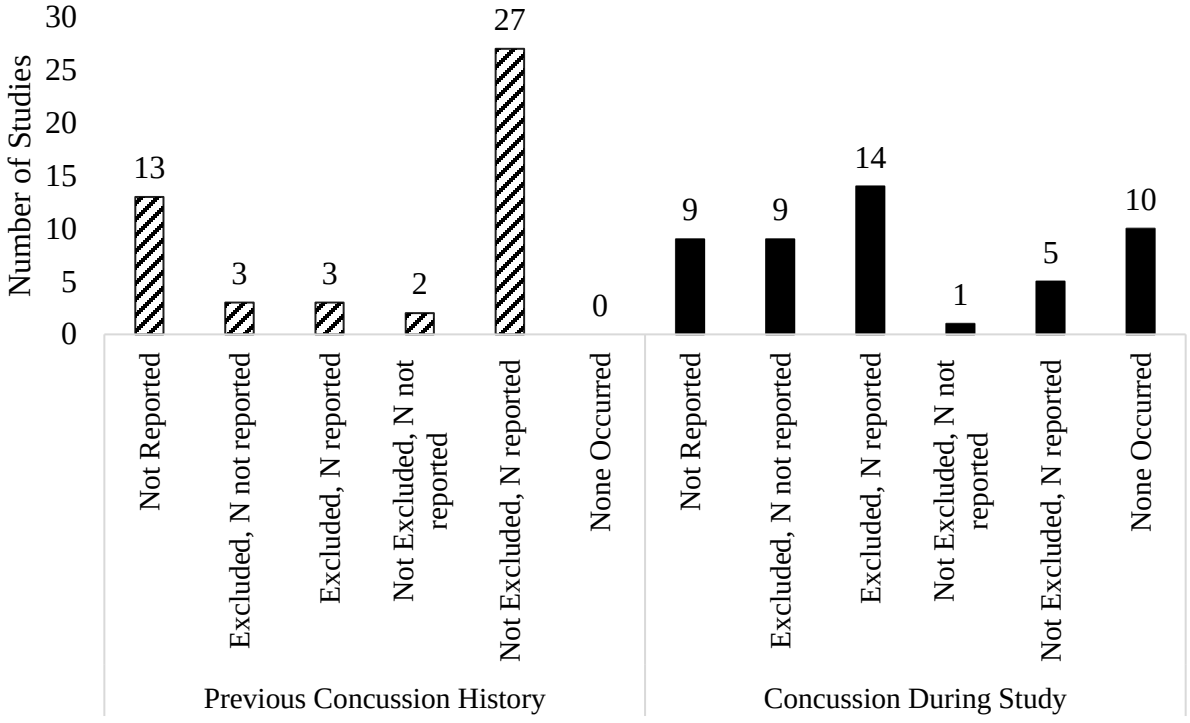
In terms of sex, the majority of studies were on male athletes, specifically those participating in football. Females were included in a total of 13 of 48 studies (27.1%); however, there were only 7 (14.6%) female specific studies, all on soccer athletes. For youth-aged athletes, there was one study that included females;[15] however, it was a mixed sample of males and females and reported no sex-specific statistics. For high school-aged athletes, there were two studies that were mixed samples of males and females[22,25] and seven studies that were female specific, using soccer players.[29,32,33,36,38,40,42] For mixed sample studies of males and females, there were significant differences reported between sexes. Bari and colleagues[22] found different regions of the brain with significant changes in glutamate concentration using MRS (females had an increase in primary motor cortex and males had a decrease in dorsolateral prefrontal cortex) in postseason measures while Brett and colleagues[25] showed females had higher ImPACT scores at both preseason and postseason time points compared with males. Female specific studies used either cognitive/functional metrics (*n*=4) or imaging metrics (*n*=3). Imaging studies showed negative changes from preseason to postseason using DTI[36] and fMRI[38,42] while the clinical studies had no significant changes from preseason to postseason.[29,32,33,40]

For college-aged, there were three studies with mixed samples of males and females[45,51,52] and one study that was female specific, using soccer players.[33] It should be noted that the one study[33] examined both high school and college soccer players. For mixed samples, there were no significant differences found[45] or no sex-based statistical analyses were run.[51,52]

Descriptions of Studies by Concussion History and Concussion During the Season/Study

When controlling for previous concussion history and concussion during the season or study, there was great variability in how researchers approached these variables (Figure 4). For previous concussion history, the majority of studies (27 of 48; 56.3%) did not exclude participants with a history of concussion but did report the number of participants in the sample with a history of previous concussion. When a concussion occurred during the season, or when enrolled in the study, there was more variability with most studies excluding the athlete and reporting how many concussions occurred (14 of 48; 29.2%), stating none occurred (10 of 48; 20.8%), not reporting any information (9 of 48; 18.8%), or excluding the participants, but not reporting the number (9 of 48; 18.8%).

Figure 4. Number of Studies (n=48) Reporting on Previous Concussion History and Concussion During Study



Overview of Results for Studies Involving a Biomarker Measure

There was little overlap in the type of blood biomarker studied. One study examined miRNAs, small non-coding RNA molecule involved in RNA silencing and post-transcriptional regulation of gene expression, and found that there were higher levels of circulating miRNAs in athletes at both preseason and postseason relative to controls.[57] Additionally, there were preseason to postseason changes in miRNA levels, but only in athletes with baseline SAC scores of 28–30 and not in those with baseline scores <28. Lastly, virtual reality scores were negatively correlated with miRNA levels across the season.[57]

Two studies examined Tau, involved with stabilizing microtubules and axonal disruption, with varying results. Joseph and colleagues[31] found increased levels of Tau postseason compared to preseason, while Oliver and colleagues[56] found that Tau decreased at time points during the season but returned to baseline level after the season. Additionally, the study by Joseph and colleagues[31] found non-significant increases in NF-L, GFAP, SBDP and a significant increase in UCH-L1, a multifunctional protein exclusively expressed in neurons in the brain, at postseason compared to preseason. Oliver and colleagues[56] however, found increased levels of NF-L, involved in the axoskeleton and intracellular transport, throughout the season.

One study found increases in antiS100Bab levels across the season that also correlated with increases in DTI levels of mean diffusivity, increases in impulse control scores, and increases in BESS score (indicating worse performance;[49]). Additionally, when comparing ApoA1, S100B, and antiS100Bab with DTI metrics, it was found that ApoA1, a measures of high density lipoprotein involved in lipid metabolism, preseason and postseason levels correlated with decreases in mean diffusivity values, S100B preseason and postseason levels correlated

with decreases in fractional anisotropy values, and antiS100Bab preseason and postseason change score correlated with increases in mean diffusivity values.[44]

Overview of Results for Studies Involving an Imaging Metric

The studies involving imaging measures had fairly varied results, even within the same sequence. These results focus on preseason to postseason imaging metric changes in the contact group only. DTI, the most common imaging metric, revealed varying responses. In terms of FA values, no changes,[34,59] increases in level,[23,26,42,50] and decreases in level[13,26,44] were all reported at postseason. Decreases[23,35,36,43] and increases[44] in MD were also reported as were decreases in RD and AD.[35,36,43] Additionally, decreases in FA were correlated with increased head impact exposure[13,34] as well as blood biomarker levels or cognitive functioning.[34] The significant changes from preseason to postseason for all other sequences are summarized in Table 3.

Table 3. Summary of significant findings from studies involving an imaging measure by first author's last name and year.

Measure		Decrease at Postseason Compared to Preseason	Increase at Postseason Compared to Preseason
rsfMRI			
	DMN	Abbas, 2015a Abbas, 2015b	Abbas, 2015a Abbas, 2015b Breedlove, 2012 Slobounov, 2017
	ReHO		Reynolds, 2017
fMRI			
	BOLD		Yuan, 2017, Yuan, 2018a Champagne, 2019
	CVR	Champagne, 2019 Svladi, 2015 Svaldi, 2017	
MRS			
	Glx	Bari, 2018 (males)	Bari, 2018 (females)
SWI		Slobounov, 2017	
ASL			Slobounov, 2017
DKI			
	MK	Gong, 2018	Davenport, 2016
	MD		Gong, 2018
DWI (MD)			McAllister, 2014

Overview of Results for Studies Involving a Cognitive Measure

The large majority of studies that involved a cognitive measure found either no change from preseason to postseason or improved scores from preseason to postseason. There were also mixed findings within studies using the same metrics. Traditional neuropsychological measures (including WRAML, Digit Span, SDMT, verbal learning delay, MSVT, TOVA, WISC-V/WAIS-IV, WASI-11, ChAMP, TMT, CogState, SWAN, SDQ, CVLT-II, WRAT-4, HVLT, WDST, Stroop) showed no significant changes from preseason to postseason.[15,18,19,33,48,52] CTMT was one of the only traditional neuropsychological metric to show a significant improvement in score, with scores increasing postseason.[48] CVLT-II also showed improvement in scores postseason compared to preseason, however, with contact sport athletes doing significantly

worse than noncontact athletes.[51] Significant findings from more commonly used clinical tests are presented in Table 4.

Table 4. Significant findings (from preseason to postseason) from studies involving a clinical measure by first author's last name and year.

	Significant Improvement in Score	No Significant Change Over Time	Significant Decline (Worsening) in Score
ImPACT *Goal: computerized neurocognitive test*	Brett, 2017 Volberding, 2014 McAllister, 2012 Miller, 2007 Munce, 2014	Caccese, 2019 Mayinger, 2018	Vogelpohl, 2017
King-Devick *Goal: assess saccadic eye movements*	Munce, 2014 Joseph, 2017	Caccese, 2019	
SAC *Goal: assess mental status*	Miller, 2007	Gysland, 2012 Joseph, 2017 Caccese, 2019	
ANAM *Goal: assess cognitive domains*		Forbes, 2016 Wahlquist, 2019 Gysland, 2012 Kaminski, 2008	
BESS *Goal: asses postural stability*	Gysland, 2012	Campolettano, 2018 Rose, 2019a Rose, 2019b Joseph, 2017 Caccese, 2019	Kaminski, 2007 Miyashita, 2017
COP/Postural Stability *Goal: assess postural stability*		Campolettano, 2018 Murray, 2018 Caccese, 2019	Munce, 2014
Child SCAT-3/SCAT-3 *Goal: evaluate concussion*	Jennings, 2015	Rose, 2019a Rose, 2019b	
SOT *Goal: assess visual, proprioceptive, and vestibular function*			Gysland, 2012
Reaction Time	Munce, 2014	Caccese, 2019	
CCAT *Goal: aptitude test*	Joseph, 2017		

ANAM measures attention, concentration, reaction time, memory, processing speed, and decision-making. ImPACT measures attention, concentration, reaction time, memory, and processing speed.

Discussion

The purpose of this systematic review was to examine the influence of subconcussive impacts on cognitive/functional outcomes, neuroimaging outcomes, and biomarker outcomes over the course of a single season in athletes. Specifically, it examined preseason to postseason changes in youth, high school, and college-aged athletes currently participating in a contact sport to identify the current state of the literature and help guide future directions and studies.

The 48 articles included in this review covered a wide range of methodological approaches with both clinical assessment and advanced research tools used. The majority of studies were in high school (*n*=26; 54.2%) or college-aged athletes (*n*=18; 37.5%) with a limited number of studies examining youth-aged (*n*=7; 14.6%) or female athletes (*n*=14; 29.2%).

Youth-aged athletes represent an important population, especially if they are just beginning exposure to contact sports. This age group could provide valuable information about the initial effects of subconcussive impacts and how age could be a mediator to effects seen, particularly on imaging data. Brain development, including myelination, and grey and white matter, is greatly changing during the age ranges in this review,[61–63] so a better understanding of the influence of age, or stage of brain development, on outcome is needed. However, due to the limited number of imaging studies and the variety of clinical tests used and their mixed findings, no concrete conclusions about the influence of exposure to subconcussive impacts on age can be made.

In terms of sex, the large majority of studies involved males in football (*n*=39; 81.3%), or females in soccer (*n*=13; 27.1%), while studies specific to females only made up a small percentage of total studies (*n*=7; 14.6%). At this time, few conclusions can be drawn regarding the influence of subconcussive impacts by sex over the course of a season. In female specific studies, there were more negative findings when an imaging metric was used; however, clinical

tools showed no change over the course of the season. Additionally, when comparing sexes, many studies did not report sex-based statistical analyses, making it challenging to draw conclusions. Of the few that did report differences, these studies highlight the varying results that can occur, especially when studying participation in different sports.[22] Given that sex differences exist within imaging metrics, cognitive ability, and biomarker levels,[64–69] having more female specific studies will allow for better generalizability of results. Additionally, studies are lacking comparing sexes within the same sport operating under equivalent rules making this topic difficult to elucidate. Although such an approach will be challenging, it will provide a better understanding of sex differences within exposure to subconcussive impacts.

This review also begins to highlight how research deals with two important confounding variables: previous concussion history and concussion during the course of a study. Research has shown that there are different reactions within outcomes based on an individual's previous concussion history,[70–72] and if a concussion occurs during the season, a more severe change in outcome will likely be seen. While this review focuses on exposure to subconcussive impacts, these two variables have the ability to confound findings and there was little consistency in how studies approached these variables. Many analyses in the studies included in the review make no mention of controlling for, or even considering, these variables in their analysis. This could contribute to the mixed findings that occur within the same modality. Future studies should aim to report, or control, these variables during analysis. Such consistency will help determine the effect on outcomes and whether including previous concussion history and concussion during the season as covariates is necessary. Additionally, other learning and psychological diagnoses such as ADHD, learning disability, depression, anxiety, etc., varied greatly in their reporting. Only 6 of 48 studies (12.5%) reported exclusion criteria regarding these topics and only an additional 3

of 48 (6.25%) reported participant demographics on these diagnoses if individuals were not excluded. The remaining studies (39 of 48; 81.3%) made no note of these diagnoses, potentially confounding results presented especially given the known link to outcome on some of these diagnoses. Given the evidence of the effects of premorbid diagnoses on outcome variables of interest, the findings from these studies should be interpreted with caution.

Another issue highlighted by this review is the use of a comparison or control group. The majority of studies (*n*=25; 52%) did not use any sort of comparison group. Those studies that did include a comparison group used other study populations. All biomarker studies with a comparison group used the general population while most imaging and cognitive studies used non-contact sport athletes. The lack of comparison groups is concerning, again as many other factors could explain potential changes seen in modalities. Comparison groups ensures the same methods and technologies were used which cannot be assumed with groups from other studies. For more rigorous studies, the use of one or two comparison groups is critical to fully understand the influence of subconcussive impacts on outcome changes as compared to other factors, like age, sex, or exercise and training regimen per say.

The biomarker studies in this review showed variability within results. All studies (*n*=5) involved blood biomarkers, which demonstrated negative outcomes, as shown by increases in level at the postseason time point; however, within these studies, there were conflicting results on certain biomarkers. The only biomarkers studied that had potential for identification of the effects subconcussive impacts – miRNA, Tau, UCH-L1, S100b/antiS100Bab, ApoA1 – should be explored further. One possible confound to consider in studying biomarkers is that they can be elevated by exercise alone[73–75] or may not be brain specific.[76] Overall, biomarkers were positively correlated to subconcussive impacts, as well as, negatively correlated to cognitive and

imaging findings postseason suggesting a deleterious effect overall. This highlights the importance of using multiple modalities within a subconcussive impact study, as biomarkers alone may not be sensitive enough.

Another critical issue is the timeframe for collection of the biomarker sample. It is possible that the timeframe of interest in this review, an entire season, is too long to detect meaningful clinical changes or that these levels can self-modify. The use of biomarkers for pre- to post-session or game,[77–79] concussion,[80] or TBI[81] seems to be more sensitive to detecting changes. Due to the inconsistencies of these findings, the sensitivity of biomarkers for subconcussive impacts at this time is still unknown.

Overall, most imaging studies showed changes postseason using various sequences. However, there was variability on findings within the same technique. DTI was the most commonly used MRI sequence across studies and most of these studies were done in male football players with results varying within individual measurements (i.e. FA, MD). These findings seem to align with results presented in a similar systematic review uniquely on DTI that highlighted varied findings, mostly likely due to methodology and analysis techniques.[82] A study by Bari and colleagues[22] highlights how sex and sport played are important considerations, as males in football and females in soccer had differential reactions postseason using the same metric. Additionally, there are limited numbers of studies using other sequences, such as SWI, ASL, MRS, so reproducibility should be considered, especially given the differential reactions seen within metrics like DTI. Overall, in most studies, imaging measures revealed changes preseason to postseason suggesting imaging may be a technique with more specificity in detecting the influence of subconcussive impacts within this timeframe.

Studies involving cognitive measures had the most inconsistent results. There was variability in measurement tool used and like imaging studies, there was inconsistency of findings within the same measure. Overall, studies showed no change in mean scores from preseason to postseason or had an improvement in mean score preseason to postseason. There were, however, a limited number of studies that showed declines in cognitive function preseason to postseason. This suggests that there could be a practice effect (with improvements simply reflecting this), that the measures are not specific enough to consistently detect change, or that the cognitive processes under study are not affected by exposure to subconcussive impacts. Additionally, this difference could be explained by analysis methods in that these studies used mean changes in scores rather than reliable change which could influence the results, as individual variability over time will be masked by the mean score change (see [83] for an example of this).

When cognitive measures were used in combination with imaging or biomarker outcomes, more negative findings emerge at postseason. Several studies found an association between declines in cognitive ability and decreases in biomarker level or negative changes in imaging sequences. This suggests that cognitive measures, when used in the preseason to postseason timeframe, might not be beneficial when used alone but instead would be more beneficial when used in conjunction with other physiological measures.

This review highlights a few shortcomings of the published research in this field. There is a glaring lack of studies on youth-aged and female athletes. Many studies are also flawed because they lack a meaningful comparison group, have confounding variables (i.e. concussion), and/or lack standardization in methodology. An additional complicating factor in these studies is that they assume that participation in contact sports means exposure to subconcussive impacts.

While many studies used accelerometers or counted impacts to the head, there was inconsistency with the word choice used to describe those impacts (subconcussion, impact, head acceleration event, repetitive head impact, repetitive subconcussive impact, exposure event, etc.) and often a definition of subconcussion is not cited in these studies. A recent review[84] highlights this issue in the field regarding the varying possible definitions of subconcussive impact as they found 42 unique terms all referring to 'subconcussion' while only 37.5% of studies included a definition. Risk of exposure to these impacts is assumed to be higher in sports like football, rugby, boxing, and soccer making generalization of findings challenging and perhaps not possible. The large majority of studies in this review focus on these higher risk groups of athletes. However, it is unknown if the externally applied force to the head impacts the brain the same way – body size, neck strength, individual brain size, connective tissue, etc. – could all influence how force transmits to the brain and warrants further study.

Limitations of Study

This review only included studies with a preseason and postseason measure in current athletes of college age or younger (8–23 years old). This eliminated other populations, including older amateur, professional, or former athletes, thereby not examining the effects of a lifetime of exposure on findings of interest. Additionally, pre- to post-session or game findings were not reported in this review, potentially affecting the understanding of methodologies that are more sensitive to immediate effects of repetitive impacts.

Conclusions

This systematic review highlights variability across studies in this field and the need for standardization in future studies. Overall, cognitive measures alone do not reveal meaningful changes during a single season of contact sports, while both biomarker and imaging measures in combination may reveal more negative associations between subconcussive impacts and outcome measures. The variety of assessment methods included in this review suggests that highly-specific measures are needed to completely understand the influence of subconcussive impacts. A combination of multimodal methodologies would be most beneficial, especially when including a cognitive assessment. By using a more challenging cognitive measure or including physiological measures, we can start to better examine the effect of subconcussive impacts within contact sports. At this time, no concrete, evidence-based conclusions can be drawn about the influence of subconcussive impacts on overall athlete health or well-being.

Acknowledgements: Dr. Christina Wissinger for her help in optimizing the search strategy used in this review.

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Supplemental Material

Supplemental File 1:

PubMed MEDLINE Search Strategy:

Head Injury Terms:

"subconcussive" [TW] OR "subconcussion" [TW] OR "sub concussion" [TW] OR "sub-concussion" [TW] OR "sub-concussive" [TW] OR "sub concussive" [TW] OR "head acceleration event" [TW] OR "head acceleration events" [TW] OR "repetitive head impact" [TW] OR "repetitive head impacts" [TW] OR "repetitive subconcussive impact" [TW] OR "repetitive subconcussive impacts" [TW] OR "exposure event" [TW] OR "exposure events" [TW]

AND

Outcome Terms:

"Cognitive" [TW] OR "Cognition" [mesh] OR "Cognition" [TW] OR "Cognitions" [TW] OR "Cognitive Function" [TW] OR "Cognitive Functions" [TW] OR "Neurobehavioral Manifestations" [mesh] OR "Neurobehavioral Manifestations" [TW] OR "Neurobehavioural Manifestations" [TW] OR "Cognitive Symptom" [TW] OR "Cognitive Symptoms" [TW] OR "Neurobehavioral Manifestation" [TW] OR "Neurobehavioural Manifestation" [TW] OR "Cognitive Manifestations" [TW] OR "Cognitive Manifestation" [TW] OR "Neurobehavioral Signs and Symptoms" [TW] OR "Neurobehavioural Signs and Symptoms" [TW] OR "Multimodal Imaging" [mesh] OR "Multimodal Imaging" [TW] OR "Hybrid Imaging" [TW] OR "Magnetic Resonance Imaging" [mesh] OR "Magnetic Resonance Imaging" [TW] OR "NMR Imaging" [TW] OR "MR Tomography" [TW] OR "NMR Tomography" [TW] OR "Steady-State Free Precession MRI" [TW] OR "Steady State Free Precession MRI" [TW] OR "Zeugmatography" [TW] OR "Chemical Shift Imagings" [TW] OR "Chemical Shift Imaging" [TW] OR "Proton Spin Tomography" [TW] OR "Magnetization Transfer Contrast Imaging" [TW] OR "MRI Scans" [TW] OR "MRI Scan" [TW] OR "fMRI" [TW] OR "Functional MRI" [TW] OR "Functional MRIs" [TW] OR "Functional Magnetic Resonance Imaging" [TW] OR "Spin Echo Imaging" [TW] OR "Spin Echo Imagings" [TW] OR "Arterial Spin Labeling" [TW] OR "Diffusion Tensor Imaging" [mesh] OR "Diffusion Tensor Imaging" [TW] OR "Diffusion Tractography" [TW] OR "Neuroimaging" [mesh] OR "Neuroimaging" [TW] OR "Brain Imaging" [TW] OR "Functional neuroimaging" [mesh] OR "Functional neuroimaging" [TW] OR "Functional Brain Imaging" [TW] OR "Functional Brain Imagings" [TW] OR "Multimodal Imagings" [TW] OR "Hybrid Imagings" [TW] OR "Magnetic Resonance Imagings" [TW] OR "NMR Imagings" [TW] OR "Magnetization Transfer Contrast Imagings" [TW] OR "Functional Magnetic Resonance Imagings" [TW] OR "Diffusion Tensor Imagings" [TW] OR "Brain Imagings" [TW] OR "microRNAs" [mesh] OR "microRNAs" [TW] OR "MicroRNA" [TW] OR "miRNAs" [TW] OR "Micro RNA" [TW] OR "miRNA" [TW] OR "Primary MicroRNA" [TW] OR "Primary miRNA" [TW] OR "pri-miRNA" [TW] OR "pri miRNA" [TW] OR "stRNA" [TW] OR "Small Temporal RNA" [TW] OR "pre-miRNA" [TW] OR "pre miRNA" [TW] OR

“Biomarkers” [mesh] OR “Biomarker” [TW] OR “Biomarkers” [TW] OR “Biologic Markers” [TW] OR “Biologic Marker” [TW] OR “Biological Marker” [TW] OR “Biological Markers” [TW] OR “Laboratory Markers” [TW] OR “Laboratory Marker” [TW] OR “Serum Markers” [TW] OR “Serum Marker” [TW] OR “Surrogate Endpoints” [TW] OR “Surrogate End Points” [TW] OR “Surrogate End Point” [TW] OR “Surrogate Endpoint” [TW] OR “Clinical Markers” [TW] OR “Clinical Marker” [TW] OR “Viral Markers” [TW] OR “Viral Marker” [TW] OR “Biochemical Marker” [TW] OR “Biochemical Markers” [TW] OR “Immune Markers” [TW] OR “Immunologic Markers” [TW] OR “Immune Marker” [TW] OR “Immunologic Marker” [TW] OR “Surrogate Markers” [TW] OR “Surrogate Marker” [TW] OR “Accelerometer” [TW] OR “Accelerometers” [TW] OR “Accelerometry” [mesh] OR “Accelerometry” [TW] OR “Psychological Reaction” [TW] OR “Psychological Reactions” [TW] OR “Cognitive Ability” [TW]

Supplemental File 2: All articles screened at the full-text stage.

Abbas, K. (2016, 2017). *Effects of concussive and repetitive subconcussive injury in high school football athletes using resting state FMRI* (Ph.D.). Purdue University.

Abbas, K., Shenk, T. E., Poole, V. N., Breedlove, E. L., Leverenz, L. J., Nauman, E. A., et al. (2015). Alteration of default mode network in high school football athletes due to repetitive subconcussive mild traumatic brain injury: a resting-state functional magnetic resonance imaging study. *Brain Connectivity*, *5*(2), 91–101. https://doi.org/10.1089/brain.2014.0279

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Alosco, Michael L., Tripodis, Y., Fritts, N. G., Heslegrave, A., Baugh, C. M., Conneely, S., et al. (2018). Cerebrospinal fluid tau, A beta, and sTREM2 in Former National Football League Players: Modeling the relationship between repetitive head impacts, microglial activation, and neurodegeneration. *ALZHEIMERS & DEMENTIA*, *14*(9), 1159–1170. https://doi.org/10.1016/j.jalz.2018.05.004

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Supplemental File 3. Complete extraction for all studies in the review (n=48).

First Author, Year	Contact Group: Sample size (n=X); sex; sport	Comparison Group: Sample size (n=X); sex; sport	Level of Play	Previous concussion history	Concussion during the season	Measure used: (0) cognitive, (1) imaging, (2) biomarker	Specific measurement information (length of study[a])	Main Results
Abbas, 2015a	n=22 (M; football)	n=10 (M; swimming, tennis, cross country, golf, baseball)	High School	Not excluded (n=9 contact; n=0 noncontact)	None occurred	1*	rsfMRI (pre, in, post)	At pre and post season, no significant DMN connections in noncontact group. Contact group had a higher number of significant DMN connections at pre, in1, in2, and post time points than noncontact group. Temporal variations were associated with changes in mechanical loading over the season: contact group significantly more hyperconnected than noncontact group at pre, post, and during the season sessions.
Abbas, 2015b	n=10 (M; football)	NA	High School	Not excluded (n=5)	None occurred	1	rsfMRI (pre, in, post)	Number of DMN connections had variability within season. In-season connections were significantly reduced at in1 and significantly increased at in2. Post season connections were significantly reduced at all sessions except post2.
Bahrami, 2016	n=25 (M; football)	NA	Youth	Excluded (n=4)	Excluded (n=2)	1*	DTI (pre, post)	Significant linear relationship between cumulative head impact exposure and decreases in FA in left IFOF and right SLF. Significant correlation between decreases in FA of right SLF terminal and cumulative head impact exposure and decreases in FA of left IFOF terminal and cumulative head impact exposures.

Bari, 2018	n=63 (40M, 23F; football, soccer)	n=27 (14M, 13F; cross country, swimming, track, tennis, basketball, softball)	High School	Not excluded (n=22 contact; n=9 noncontact)	None occurred	1*	MRS (contact: pre in, post; noncontact: pre, post)	In female soccer players, in M1, a significant increase in concentration of Glx at post compared to preseason. In male football players, DLPFC had a significant decrease in concentration of Glx at in2 vs pre, in1, post 2. Males had a significant increase in ratio tCho:tCR in the DLPFC in2 compared with pre, in1, and post2. Females had a significant increase in ratio Glx:tCr in M1 at in2 and post1 compared with pre.
Bazarian, 2012	n=10 (M; football, ice hockey)	n=5 (1F, 4M; uninjured and orthopedic injured)	High School	Excluded if concussion <1 month before study (n=NR)	Not excluded (n=1)	0, 1	ImPACT, DTI (pre, post)	Controls had a small percentage of WM voxels that had significant pre to post FA changes. Contact players had FA percentage changes between control level and the concussed individual. Contact group had higher percentage of WM voxels with significant changes in FA and MD (3x control level). Contact group had significant differences in cognitive performance compared with controls with visual motor speed and reaction being significantly higher in the subconcussive group.
Bazarian, 2014	n=10 (M; football)	n=5 (M; nonathletes)	College (DIII)	Not excluded (n=1)	None occurred	0, 1, 2*	ImPACT, BESS, DTI, ApoA1, S100B, antiS100Bab (pre, post, 6 months after)	Athletes had greater WM changes in FA and MD from pre to post, specifically decreases in FA and increases in MD colocalized to the corpus callosum. There were greater voxel changes in FA and MD from pre to 6 months after season. WM was not associated with BESS or ImPACT at post or 6 months post. From pre to post and to 6 months post there were decreases in FA with increases in serum ApoA1. There were increases in FA with decreases in S100B at post, decreases in MD with decreases in S100B at pre, increases MD with increases ApoA1 at pre.
Breedlove, 2012	n=52 (M; football)	NA	High School	Not excluded (n=NR)	Not excluded (n=7)	1*	rsfMRI (pre, post)	Of 116 ROIs, 33 had significant regressions related to changes in fMRI to impacts sustained, particularly in the parietal and occipital lobes.

Brett, 2017	n=626 (428M, 198F; variety)	n=145 (35M, 110F; variety)	High School	Excluded (n=NR)	Excluded (n=NR)	0	ImPACT (pre, post)	Gender differences at pre and post timing on composite scores with females doing better than males. All composite scores improved at post testing with visual motor speed significantly increasing in the noncontact group. In contact sports, females did significantly better than males on verbal memory and visual motor at both pre and post.
Caccese, 2019	n=38 (15M, football; 23F, soccer)	NA	College (DI)	Not excluded (W: 0.6±1.1; M: 0.5±0.9)	Excluded (n=NR)	0*	TG, SAC, BESS, CRT, KD, IMPACT (pre, post)	Pre to post season scores increased in both groups but no significant difference between groups at time points. Significant association between impacts and visual memory and TG for women's soccer. Significant association between impacts and KD for football. Increased exposure led to worse performance.
Campolettano, 2018	n=34 (M; football)	NA	Youth	NR	None occurred	0	BESS, Force Plate (COP) (pre, post)	BESS showed no significant difference pre to post. Force plate/COP showed no significant difference pre to post and BESS was not associated with COP metrics.
Champagne, 2019	n=26 (M; football)	NA	College	Not excluded (n=14)	Excluded (n=4)	1	fMRI (pre, in, post)	Group DMN connectivity was more pronounced throughout the season in L hemisphere. DMN CBF significantly decreased post vs pre and vs in. Significantly lower CVR at in and post vs pre.
Chun & Mao, 2015	n=34 (M; football)	n=9 (M; non collision)	High School (2 teams)	NR	None occurred	1*	DTI (pre, in, post)	Control group had no significant changes in ROI averages and skeletonized FA. There were significant changes in FA in contact group pre and post season. Team 1 had increases in FA in superior corona radiata left and superior fronto occipital fasciculus right. Team 2 had decreases in FA in retrolenticular part of internal capsule right.
Davenport, 2014	n=24 (M; football)	NA	High School	Exclude past 6 months (n=1)	Excluded from study (n=2)	0, 1*	DTI, ImPACT (pre, post)	RWEcp significantly associated with changes in DTI scalars. Regression showed a significant relationship between RWEcp and FA, especially linear anisotropy. Post – pre ImPACT verbal memory change score and DTI correlated

								significantly with a decrease in ImPACT and the number of abnormal voxels for each DTI scalar.
Davenport, 2016	n=24 (M; football)	NA	High School	Concussion within past 6 months (n=1)	Excluded (n=2)	0, 1*	DKI, ImPACT (pre, post)	Significant relationship between risk weighted cumulative exposure probability and mean kurtosis. No significant relationship between DKI metrics and ImPACT.
Forbes, 2016	n=105 (F; soccer)	n=105 (F; soccer (history of cx))	High School	Included (n=105 average 1.3 previous)	Excluded (n=NR)	0	ANAM (SRT, CPT, MTH, MSP, STN) (pre, post)	No significant difference between groups on any cognitive measures.
Gong, 2018	n=17 (M; football)	NA	High School	NR	Excluded (n=1)	1*	DKI (pre, post)	Deep gray matter had significant parametric percentage changes pre to post in MK and MD. Specifically, a significant decrease in MK in the thalamus and a significant increase in MD in thalamus and putamen. Cortical regions had significant decreases in MK in the left paracentral, right pars triangularis, right inferior parietal lobe, right cuneus, and right rostral middle frontal cortices. There was a significant increase in MD in the left rostral middle frontal cortices.
Gysland, 2012	n=46 (M; football)	NA	College (DI)	Excluded past 3 months (n=NR)	excluded (n=NR)	0*	ANAM, SAC, SOT, BESS, GCS (pre, post)	Regression equations were not significant for any ANAM test modules, SAC total score, or GCS score. Regression equation was significant for SOT with number of years being a significant predictor for overall composite equilibrium balance changes scores pre to post season. BESS scores improved pre to post and number of impacts, cumulative magnitude of impacts, number of concussion were significant predictors of change in BESS total error score.

Janda, 2002	n=75 (55M, 20F; soccer)	NA	Youth	Not excluded: 12% of children (n=7)	NR	0	verbal learning (WRAML), digit span, SDMT, verbal learning delay (pre, post)	No significant differences on any measure pre to post season.
Jennings, 2015	n=59 (M; football)	n=28 (M; baseball)	Youth	NR	Excluded from study (n=3)	0	Child SCAT-3 (SAC, BESS, FTN) (pre, post)	Significant difference in cognitive orientation and coordination scores (preseason significantly higher in noncontact; both groups higher at postseason vs preseason). Postseason immediate memory scores significantly lower in noncontact group.
Joseph, 2017	n=16 (M; football)	NA	High School	Not excluded (n=6)	Excluded from study (n=3)	0, 2*	CCAT, KD, BESS, SCAT-3, SAC, blood sample (NF-L, Tau, GFAP, SBDP, UCH-L1) (pre, in, post)	Significantly increased levels of Tau and UCH-L1 at the end of season. Significant improvement in KD test time and processing speed.
Kaminski, 2007	n=47 (F; 21 college; 26 HS soccer)	n=24 (F; soccer)	High School & College (DI)	Not excluded (college n=7; HS n=1; control n=2)	Excluded from study (n=NR)	0	WDST, HVLT, BESS (pre, post)	No significant correlations between number of headers and changes in scores on all outcomes. College soccer players' BESS scores were significantly worse than HS and controls. WDST and HVLT had no significant differences pre to post.

Kaminski, 2008	n=393 (F; soccer)	NA	High School	Not excluded (0.3 ± 0.6)	Excluded from study (n=2)	0	ANAM; concussion checklist (pre, post)	Symptom checklist showed no changes pre to post. ANAM scores increased nonsignificantly post vs pre. No relationship between number of headers and any ANAM measure.
Kuzminski, 2018	n=17 (M; football)	NA	High School	Not excluded (n=6)	Not excluded (n=1)	0, 1*	DTI, NP assessment metrics (pre, post)	No changes in FA across the season had significant differences from zero. 2 ROIs had an interaction with the number of impacts (cingulum hippocampus and fornix stria terminalis): a negative change in FA with an increase in number of impacts. Decreases in FA in fornix tria terminalis correlated with decreases in visual memory scores.
Marchesseault, 2018	n=15 (M; lacrosse)	NA	College (DIII)	NR	NR	0*	Stroop, CTMT (pre, in, post)	CTMT had significant increases in score from pre to mid and mid to post and between pre and post. Stroop had no significant differences at any time point.
Marchi, 2013	n=10 (M; football)	NA	College (DIII)	Not excluded (n=1)	Not excluded (n=NR)	0, 1, 2	ImPACT, BESS, DTI; S100B, antiS100Bab (pre, post, 6 months after)	Change in mean diffusion correlated most significantly with increases in auto-antibody (ab) post levels. Significant correlation between BESS and ab (increase in level, decreases in scores).
Mayinger, 2018	n=19 (M; football)	n=5 (NR; general population)	College (DIII)	Not excluded (n=3)	None occurred	0, 1	ImPACT, DTI (pre, post, 6 months after)	No significant group differences in cognitive performance at any time. There was a significant pre to post increase in FA in left parietal lobe and a significant decrease in FA in the same cluster from post to 6 months after. Using TBSS, there was a pre to post increase in trace in the brainstem and left temporal lobe and a decrease in trace from post to 6 months after in a different but overlapping brainstem area.

McAllister, 2012	n=214 (81%M; football, ice hockey)	n=45 (93%M; track, crew, nordic skiing)	College (DI)	Not excluded (control no history; contact NR)	Excluded (n=NR)	0*	ImPACT, NP battery (pre, post)	The contact group had a significantly better visual memory score on ImPACT than noncontact group. In the NP battery, preseason there were no between group differences except letter fluency (noncontact group had higher scores). Pre to post there was improvement on PASATC and CVLT with contact improving on PASATC and noncontact improving on CVLT.
McAllister, 2014	n=80 (64M, 16F; football, ice hockey)	n=79 (56M, 23F; track, crew, nordic skiing)	College (DI)	Not excluded (control no history; contact NR)	Excluded (n=5 contact; n=NR control)	0, 1*	DWI, CVLT-II, WRAT-4 (pre, post)	Main effect of group for MD in the corpus callosum. Postseason FA and MD were different across groups in the amygdala. More changes in MD in the corpus callosum were worse than predicted (>1.5SD) at post CVLT-II in a subgroup of athletes. There were no overall significant changes in CVLT-II or WRAT-4.
Miller, 2007	n=76 (M; football)	NA	College (DIII)	Not excluded (n=17)	Excluded (n=4)	0	ImPACT, SAC (pre, in, post)	There was a significant increase in SAC total score from pre to post and mid to post. ImPACT had a significant improvement on visual memory pre to post and reaction time pre to mid and pre to post.
Miyashita, 2017	n=36 (M; lacrosse)	NA	College (DI)	NR	Excluded (n=1)	0*	BESS (pre, post)	Number of errors pre to post differed significantly. There was a significant increase in errors on double left foam, tandem foam, total number of errors on firm, and total number of errors on foam. There was a significant correlation between the number of total errors on foam and linear acceleration.
Munce, 2014	n=15 (M; football)	NA	Youth	Not excluded (n=0)	Excluded (n=2)	0	postural stability, KD, ImPACT, PCSS (pre, post)	Postural stability eyes closed significantly improved compared to pre. KD was significantly faster during postseason. ImPACT reaction time significantly improved pre to post season.
Murray, 2018	n=14 (M; football)	n=14 (3M, 11F; cheerleading)	College (DI)	Not excluded (control no history; FB 0.5±0.8	NR	0*	postural stability (pre, post)	No significant interactions for group by time on any measure.

Myer, 2016	n=30 (M; football)	n=32 (M; football (wore a collar))	High School	NR	NR	1*	DTI, SWI (pre, post)	There was a significant pre to post decrease in MD, AD, and RD in WM regions in non-collar group. There were no changes pre to post on DTI in the collar group. Larger pre to post DTI changes in non-collar group within corpus callosum, internal and external capsule, and other WM areas (cingulum, corona radiata, post thalamic radiation, superior longitudinal fasciculus).
Myer, 2018	n=35 (F; soccer)	n=40 (F; soccer (wore a collar))	High School	NR	Not excluded (n=3)	1*	DTI (pre, post & subset: pre, post, 6 months after)	Pre to post had no significant differences between groups pre or post in all DTI metrics. Within group, significant changes pre to post in non-collar group with decreases in MD, AD, and RD. No significant changes in collar group. For subset (n=11), pre to post to 6 months after, no significant group differences on DTI changes between groups. Collar group showed no significant pre to post or post to 6 months after. Non-collar group had significant decreases in MD, RD from pre to post and significant increases in MD, AD, and RD from post to 6 months after.
Oliver, 2018	n=35 (M; football)	NA	College (DIII)	NR	Excluded (n=NR)	2	NF-L, Tau (pre, in, post)	At pre, tau was significantly lower in starters compared with nonstarters. Tau decreased throughout season but returned to pre levels at post1 (starters) and post2 (nonstarters). No significant difference between starters and nonstarters at any time. For NF-L, levels increased at in1 versus pre in both groups. Starters had higher levels throughout.

Papa, 2018	n=23 (M; football)	n=30 (15M, 15F; general population)	College (DI)	Not excluded (n=18)	Not excluded (n=2)	0, 2	miRNA, SAC, VR (pre, post)	Athletes had elevated levels of circulating miRNAs pre and post compared to controls. SAC scores of 28-30 vs <28 had differing levels with higher pre levels miRNA in those with <28. Pre vs post season in athletes was significant for SAC 28-30, not for <28. VR scores decreased as miRNA concentration increased pre to post.
Reynolds, 2017	n=63 (M; football, lacrosse, soccer)	n=30 (M; NR)	College (DI)	Not excluded (FB=19; lacrosse=7; soccer=6; control=0)	Excluded (n=3)	1	rsfMRI (pre, post)	Mass univariate analysis TFCE voxel analysis for spatially homogenous pre to post differences in ReHO and DC showed a 9 voxel cluster increase of ReHo in posterior superior temporal sulcus for contact group. There was a 2 voxel cluster increase in ReHo in the left superior parietal lobe in control group. Football also had a significant class accuracy for ReHO but not DC.
Rose, 2019a	n=134 (M; football)	NA	Youth & High School	Not excluded (n=30)	Excluded (n=1)	0*	SCAT3, VOMS, SWAN, SDQ, MSVT, TOVA, WISC-V/WAIS-IV, WASI-II, TMT, CHAMP, CogState Battery, BESS (pre, post)	Concussion history not associated with worse performance on preseason measures. Those with a history had better performance on TOVA, RT, Cogstate attention and working memory, and BESS. Age and ADHD predicted worsening on multiple change scores.
Rose, 2019b	n=166 (M; football)	NA	Youth & High School (2 seasons)	Not excluded (n=44)	NR	0*	MSVT, TOVA, WISC-V/WAIS-IV, WASI-11, ChAMP, TMT, CogState, SWAN, SDQ, VOMS, SCAT-3, BESS, NPC (pre, post)	Minimal changes over time, most scores stayed the same or increased. Only processing speed decreased from pre to post. For a subset over 2 seasons, no changes in scores but age and ADHD predicted worse scores.

Slobounov, 2017	n=23 (M; football)	NA	College (DI)	Not excluded (n=8)	None occurred	1*	rsfMRI, DTI, SWI, ASL (pre, post)	rsfMRI: significant changes in functional connectivity pre to post in right ICC, left ICC, left hippocampus. SWI: decreased signal in at least 8 of 55 cortical regions after the season. ASL: significant global (cortex) and regional (post central gyrus) increase in CBF after the season.
Svaldi, 2015	n=16 (M; football)	n=10 (M; non collision)	High School	Not excluded (n=NR)	None occurred	1*	fMRI (CVR) (control: pre, post; football: pre, in, post)	Controls showed no difference pre to post but 6 of 10 had decreases in CVR at post. Contact group had significant deviations from pre to in1 in all lobes. Overall decreases in CVR in 14 or 16 at in1; 10 of 16 at in2; and 8 of 16 at post.
Svaldi, 2017	n=14 (F; soccer)	n=12 (F; basketball, track, cross country, swimming, softball, gymnastics)	High School	Not excluded (contact n=3; controls n=4)	None occurred	1*	fMRI, CVR (pre, in, post)	Whole brain showed no differences between groups at any time. Contact had significant shifts in the season and post vs controls with distribution of whole brain Beta weights localized to fronto-temporal areas of the brain. Changes recovered by post3.
Vogelpohl, 2017	n=55 (M; football)	n=33 (M; baseball or no participation in organized sport)	High School	Not excluded (n=2 contact)	Excluded from study (n = 3 contact)	0	ImPACT (pre, in, post)	Significant group by time interaction effect for verbal memory composite scores, with football during the postseason scoring significantly lower than low contact group.
Voldberding, 2014	n=42 (M; football)	NA	College (DI)	NR	Excluded from study (n=NR)	0	GSC, ImPACT (pre, post)	GSC had no significant change pre to post season; ImPACT had a significant increase in verbal memory and reaction time postseason.
Wahlquist, 2019	n=217 (F; soccer)	NA	High School (4 seasons)	Not excluded (n=30)	NR	0	ANAM, PCSS (pre, post)	Subtle decreases throughout testing period, no significant differences between seasons compared to baseline.

Yuan, 2017	n=30 (M; football)	n=32 (M; football (wore a collar))	High School	NR	NR	0, 1*	N-back verbal working memory fMRI (pre, post)	BOLD signal activation significantly increased at post vs pre in non-collar group but not in collar group. Significantly larger pre to post changes in activation in non-collar group compared to collar. Areas of less activation changes in collar group included precuneus, inferior parietal cortex and DLPFC. No significant changes in N-back accuracy or time.
Yuan, 2018a	n=25 (F; soccer)	n=32 (F; soccer (wore a collar))	High School	Not excluded (contact n=2; collar n=4)	NR	0, 1*	N-back verbal working memory fMRI (pre, post)	No pre to post changes for N-back accuracy or response time. Pre showed significant activation differences in anatomical loci including cingulate gyrus, dorsolateral and ventrolateral prefrontal cortex, lateral and medial premotor cortex, medial posterior parietal lobe, inferior parietal lobe, thalamus, and cerebellum. No significant differences between collar and non-collar group at pre. Pre to post showed no significant changes in the collar group. In the non-collar group there were significant changes in precentral gyrus, frontal gyrus, SMA, cingulum, superior occipital lobe, post central gyrus, superior and inferior parietal lobe, precuneus, angular gyrus. Pre to post increases in activation correlated with decreases in accuracy on N-back in the non-collar group.

Yuan, 2018b	n=21 (M; football)	n=21 (M; football (wore a collar))	High School (2 seasons)	Not excluded (contact n=1; collar n=3)	NR	1*	DTI (pre, post)	DTI revealed no differences between groups at pre. There were significant pre to post decreases in MD, AD, and RD in the non-collar group and no significant pre to post changes in the collar group. There were significantly greater pre to post increases in FA and decreases in MD, AD, and RD in the non-collar group especially in the corpus callosum, internal capsule, superior and posterior corona radiata, posterior thalamic radiation, superior longitudinal fasciculus. From post to pre of season 2, DTI patter flipped with decreases in FA and increases in MD, RD, and AD. These were still significantly different from baseline pre levels. Collar group changes were not significant at any time over the 2 seasons.

(*) = use of an accelerometer during the study

[a]Time frame of study: Pre = before the season; in = during the season; post = at the end of the season

Abbreviations: M = male; F = female; NA = not available; NR = not reported; fMRI= functional magnetic resonance imaging; DTI= diffusion tensor imaging; BESS= Balance Error Scoring System; COP= center of pressure; SDMT= Symbol Digit Modalities Test; SCAT-3= Sport Concussion Assessment Tool – 3rd Edition; K-D= King-Devick Test; PCSS= post concussion symptom scale; MSVT= Medical Symptom Validity Test; TOVA= Test of Variables of Attention; WISC-V= Wechsler Intelligence Scale for Children; WASI-II= Wechsler Abbreviated Scale Intelligence, 2 Ed; ChAMP= Children and Adolescent Memory Profile; TMT= Trail Making Test; SWAN= Strengths and Weaknesses of Attention-Deficit/Hyperactivity-symptoms and Normal-behaviors; SDQ= Strengths and Difficulties Questionnaire; VOMS= Vestibular/Ocular Motor Screening; rsfMRI= resting state functional magnetic resonance imaging; MRS= magnetic resonance spectroscopy; DKI= Diffusion kurtosis imaging; ANAM= Automated Neuropsychological Assessment Metrics; CCAT= Axon Sports Computerized Cognitive Assessment Tool; NF-L= neurofilament light; GFAP= glial fibrillary acidic protein; SBDP= spectrin breakdown products; UCH-L1 = ubiquitin carboxyl-terminal hydrolase isoenzyme L1; WDST= Wechsler Digit Span Test; HVLT= Hopkins Verbal Learning Test; NP = neuropsychological; SWI= susceptibility weighted imaging; CVR= cerebrovascular reactivity; TG= Tandem Gait; SAC= Standardized Assessment of Concussion; CRT= Concussion Recognition Tool; SOT= Sensory Organization Tool; GSC= graded symptom checklist; CTMT= comprehensive trail making test; DWI= diffusion weighted imaging; CVLT-II= California Verbal Learning Test-II; WRAT-4= Wide Range Achievement Test; miRNA = microRNAs; VR = virtual reality; ASL= arterial spin labeling; SWI= susceptibility weighted imaging
DMN = default mode network; FA = fractional anisotropy; IFOF= inferior fronto-occipital fasciculus; SLF= superior longitudinal fasciculus; M1= primary motor cortex; Glx= glutamate & glutamine; DLPFC= dorsolateral prefrontal cortex; Cho= choline; Cr= creatine; WM= white matter; MD=mean diffusivity; DMN= default mode network; CBF= cerebral blood flow; ROI= region of interest; RWEcp= combined-probability risk-weighted cumulative exposure; TBSS= tract based spatial statistics; MK= mean kurtosis; NP= neuropsychologic; std dev= standard deviation; RD= radial diffusivity; TFCE= threshold free cluster enhancement; ReHO= regional homogeneity; DC= degree centrality; ICC= inferior colliculus

Supplemental Table 4. Quality Rating of each Article in the Review (N=48) using the Oxford Centre for Evidence-Based Medicine 2011 Levels of Evidence (Oxford) and the Newcastle Ottawa Assessment Scale (NOS).

First Author	Year	Oxford Rating	Selection	Comparability	Outcome	NOS Rating	First Author	Year	Oxford Rating	Selection	Comparability	Outcome	NOS Rating
Abbas	2015	3	3	0	3	******	Marchi	2013	3	2	1	2	*****
Abbas	2015	3	2	0	3	*****	Mayinger	2018	3	3	0	3	******
Bahrami	2016	3	2	0	2	****	McAllister	2012	3	3	1	2	******
Bari	2018	3	3	0	2	*****	McAllister	2014	3	3	0	3	******
Bazarian	2012	3	2	0	3	*****	Miller	2007	4	2	0	3	*****
Bazarian	2014	3	3	0	2	*****	Miyashita	2017	3	2	0	3	*****
Breedlove	2012	3	2	0	3	*****	Munce	2014	3	2	0	2	****
Brett	2017	3	2	1	3	******	Murray	2018	3	3	0	2	*****
Caccese	2019	3	2	0	2	****	Myer	2016	2	3	1	3	*******
Campolettano	2018	3	2	0	3	*****	Myer	2018	3	3	0	2	*****
Champagne	2019	3	2	1	2	*****	Oliver	2018	3	2	0	2	****
Chun & Mao	2015	3	3	0	3	******	Papa	2019	3	2	1	3	******
Davenport	2016	3	2	1	2	*****	Reynolds	2017	3	3	0	2	*****
Davenport	2014	3	2	1	2	*****	Rose	2019	3	2	1	3	******
Forbes	2016	3	3	1	3	*******	Rose	2019	3	2	0	3	*****
Gong	2018	3	2	0	3	*****	Slobounov	2017	3	2	0	3	*****
Gysland	2012	3	2	0	2	****	Svaldi	2015	3	3	0	3	******
Janda	2002	3	2	0	3	*****	Svaldi	2017	3	3	0	2	*****
Jennings	2015	3	3	0	2	*****	Vogelpohl	2017	3	3	1	2	******
Joseph	2017	3	2	0	3	*****	Voldberding	2014	3	2	0	2	****
Kaminski	2008	3	2	0	2	****	Wahlquist	2019	3	2	0	2	****
Kaminski	2007	3	3	1	2	******	Yuan	2018	2	3	1	3	*******
Kuzminski	2018	3	2	0	3	*****	Yuan	2018	3	3	1	2	******
Marchesseault	2018	3	2	0	2	****	Yuan	2017	2	3	0	3	******

Chapter 3: KIAA0319 Genotype Predicts the Number of Past Concussions in a Division I Football Team: A Pilot Study

Abstract

This candidate gene study evaluated the relationship of a past history of concussion with single nucleotide polymorphisms (SNPs) in nine genes in a small cohort (N = 87) of a nationally ranked Division I football team. Genes and SNPs studied were selected based on their published connection to brain injury and brain development, as well as impulsivity. We used multinomial logistic regression analysis (MLRA) to quantify how well genotype predicted the number of previously diagnosed concussions (three categories: none, one, two or more), while covarying race and number of years participating in football. The rs4504469 SNP for KIAA0319 was the only locus that significantly predicted number of previously diagnosed concussions ($p = 0.005$, meeting Bonferroni correction for multiple comparisons). The KIAA0319 results raise the hypothesis that having the CT or TT genotype of KIAA0319 may be predictive of a lower incidence of previously diagnosed concussion. This finding raises a number of hypotheses for future pre-clinical research, particularly whether alterations in neural organization related to KIAA0319 rs4504469 lead to reduced susceptibility for lasting head trauma, or greater resilience in the face of repeated subconcussive injury.

Introduction

A better understanding of factors that influence susceptibility and resilience to concussion, or mild traumatic brain injury (mTBI), are needed given the associations between concussion and serious neuropsychiatric sequelae such as mood disorders and neuropathologies such as chronic traumatic encephalopathy (CTE).[1,2] There is tremendous variability within the sports concussion literature on outcome and recovery,[3–7] raising the need for developing a framework for

understanding susceptibility and resilience in the context of this injury. Examining the role of genetics in brain injury is a relatively new area that may hold promise for better understanding risk factors and recovery patterns following concussion.

While our current understanding of how genetics affect concussion outcomes is still in its infancy, a limited body of literature has started to examine specific genes that may be involved in the repair/recovery process following brain injury. The *apolipoprotein E* (*APOE*) gene and the *APOE-ε4* allele, in particular, is the most widely studied with respect to its potential role in recovery and outcome following TBI,[8–10] or headache and self-reported symptoms in concussed collegiate athletes.[11] A number of studies, however, have failed to detect associations between *APOE* and TBI or TBI-related outcomes, indicating the need for further study.[12–14] Other genes that may be connected to the repair/recovery process after concussion include *brain-derived neurotrophic factor* (*BDNF*) and the *serotonin transporter gene* (*5-HTT*).[10,15,16]

Part of the reason for the dearth of literature is that even among populations at high risk of incurring a concussion, such as Division I athletes, the overall incidence rates of diagnosed concussions are relatively low (i.e., 7.6% of all collegiate football injuries https://www.ncaa.org/sites/default/files/NCAA_Football_Injury_WEB.pdf), and there is concern about the potential for underreporting of injury, making more comprehensive gene identification approaches such as Genome-Wide Association (GWAS) challenging. We accordingly took a candidate gene approach, with an *a priori* selection of genes based on specific constructs including not only the two genes previously studied in concussion samples (i.e., *APOE-ε4* and *BDNF*), but also seven other genes that are related to brain or neuronal injury and development. These seven other genes included: *Atypical Chemokine Receptor 1* (*DARC),*[17] *Poly(ADP-Ribose)Polymerase 1* (*PARP-1*),[18,19] *Neuroglobin* (*NGB*),[20,21] *Interleukin 6 (IL-6),*[22]

KIAA0319,[23,24] as well as those that are implicated in impulsive behavior (i.e., *Catechol-O-Methyltransferase (COMT)*[25]; *Tryptophan Hydroxylase 2 (TPH2)*[25]), which might increase the risk for concussion (please see Table 5 for summary, and detailed comments in Methods).

In this *pilot* study, we determined whether genotype for single nucleotide polymorphisms (SNPs) in 9 candidate genes predicted the number of previously diagnosed concussions in the combination of two cohorts of Division I football players. We used multinomial logistic regression analysis (MLRA) to assess the accuracy of predicting 0, 1, or 2+ medically confirmed and documented concussions. After correction for multiple comparisons, we found that only *KIAA0319* genotype significantly predicted the number of previously diagnosed concussions for the combined cohorts. Trend effects, however, were detected for the *APOE-ε4* carrier, *BDNF*, *COMT* and *DARC*.

Methods

Participants and procedures: A total of 87 Penn State University (PSU) collegiate football players participated in this study, collected initially as a cohort of 23 subjects in 2015 (Cohort 1) whose neuroimaging was published in Slobounov et al., 2017.[26] One year later, a follow-up cohort was collected with 64 subjects (Cohort 2), not including any of the subjects previously tested in Cohort 1. Missing data reduced our sample by one to two subjects for Cohort 2, depending on the measure. Given the overall low numbers of subjects in Cohort 1 and Cohort 2, all subjects were pooled and their results analyzed together. All subjects finished research procedures in one research visit, during the athletic season. All subjects provided informed consent, as approved by the PSU Institutional Review Board (IRB#4078), consistent with the Declaration of Helsinki.

Participants completed a standard self-report demographics form that collected age, race, years of play, and football team position. Player position was annotated as "speed" vs. "non-speed" according to the criteria put forth by Lehman[27] and used to confirm consistency across the two Cohorts. Consistent with the Lehman[27] criteria, kickers, punters, and long snappers (n=7) were not included as either "speed" or "non-speed."

Participants reported to research staff the total number of times that they had sustained a concussion from high school to the present, based on a diagnosis by a medical professional at the time of the event. To reduce the potential of underreporting, they were informed that that the data were for research only, and that their injury history would not be put in their medical record nor would their coaching staff be informed. These data were then confirmed by chart review by the chief team physician (PHS) for all players. PHS based his confirmation on (a) data from their time playing at PSU when any concussion was diagnosed by him, from clinical signs (as opposed to reported symptoms), or (b) medical records coming to PSU for each player from their previous football history with documented clinical signs of concussion.

Based on these procedures, the total cohort had a mean age of (±standard deviation [SD]) 20.52±1.32 years, 41/87 identified as African-American and 46/87 identified as Caucasian. The total cohort had a mean exposure playing football of (±SD) 11.92±3.53 years. Of all subjects, 46 played "speed" positions, and 34 played "non-speed" positions. The racial background of this cohort is consistent with that reported by the National Collegiate Athletic Association (NCAA) regarding collegiate football players: https://www.ncaa.org/sites/default/files/Final%2B2012%2BCollege%2BRGRC.pdf.

Measures:

Candidate Gene Selection

A candidate gene approach was taken to analyze (1) brain injury, (2) brain development and (3) impulsive behavior. The links between the biological functions of each of these candidate genes and the aforementioned constructs are summarized in Table 5, and described below.

Table 5. Candidate Genes

Gene	***SNP Abbreviation***	***SNP ID***	***Biological Link***	***(A) Brain Injury/Development vs. (B) Impulsive behavior***
catechol-O-methyltransferase	COMT	rs4680	Dopamine breakdown in prefrontal cortex; cell death & cellular dysfunction	(A) TBI Cognitive outcomes[34,35] (B) Impulsivity[25, 34, 35]
atypical chemokine receptor 1 (Duffy blood group)	DARC	rs2814778	Removes inflammatory chemokines	(A) Inflammatory chemokines across BBB[17, 39, 40]
brain derived neurotrophic factor	BDNF	rs6265	Neuron growth & maturation; neuroplasticity	(A) Neuroplasticity[13, 15, 47, 48]
poly(ADP-ribose) polymerase 1	PARP1	rs3219119	Regulation of differentiation, proliferation, tumor transformation; neuronal damage	(A) TBI Neurological outcomes[18]; neuroprotective effect[19]
neuroglobin	NGB	rs3783988	Oxygen transfer across BBB; neuroprotection against oxidative stress	(A) Recovery after TBI[20]; lesion volume[21]
interleukin 6	IL6	rs1800795	Immune response & inflammation	(A) Inflammation & injury[22, 61, 62]
KIAA0319	KIAA0319	rs4504469	Regulates neuronal migration & cell adhesion	(A) neuronal migration and cell adhesion[23, 24, 63]
tryptophan hydroxylase 2	TPH2	rs1386483	Serotonin regulation through axon & dendrite growth	(B) Impulsivity & response inhibition[25, 70, 71]
apolipoprotein E	APOE	rs429358 rs7412	Lipid transportation across tissue	(A) Post-injury cognitive & neuropathological outcomes[11, 75, 76]

(1) *COMT* codes for an enzyme that degrades catecholamines,[28,29] including dopamine,[30] especially in the prefrontal cortex,[31,32] and has been associated with concussion and the subsequent increase in impulsivity.[25] The SNP rs4680 (Val158Met)[33] was selected for its association with cognitive outcomes and TBI, with the A allele (methionine) associated with improved measures of executive function in both children and adults with severe TBI, and for its effect as a non-synonymous variant that alters COMT activity (A allele causes low enzyme activity and higher prefrontal DA concentration).[34,35]

(2) *DARC* is involved with the transport, presentation, and concentration of chemokines,[36,37] which may aid in modulating the inflammatory response.[17,37] In the case of central nervous system inflammation—a common consequence of TBI[38]—DARC has been reported to shuttle inflammatory chemokines across the blood-brain barrier (BBB).[17] In addition, it is expressed on a subset of neurons, suggesting a role for *DARC* in modulation of neuronal activity by chemokines produced by astrocytes.[39,40] The SNP is associated with stroke, rs2814778, and was selected for these analyses.

(3) *BDNF* is involved in neuronal growth[41] and maturation[42] and can facilitate neuroplastic changes leading to adaptive repair,[43] as occurs in response to head trauma.[44–48] The SNP rs6265 (Val66Met)[49] is a non-synonymous variant selected for its effect on altering BDNF activity, and association of the T allele (methionine) in prior studies with increased concussion risk in both pre- and post-deployment military populations.[13,15]

(4) *PARP-1* is involved in the regulation of cell growth and differentiation and in neuronal death/damage.[50,51] It is also involved in TBI neuroprotective effects through reducing the neuroimmune response.[19] The SNP rs3219119 was selected to be consistent with other studies that chose the SNP on the basis of having a minor allele frequency of at least 20% in the

population. This SNP is also an independent predictor in adults of favorable neurologic outcome (AA genotype associated with Glasgow Outcome Scale, GOS>3) after severe TBI (Glasgow Coma Scale, GCS ≤8).[18]

(5) *NGB* is predominately expressed in neurons[52] and is involved with encoding oxygen-binding proteins that increase oxygen availability.[53,54] *NGB* protects against hypoxic and ischemic conditions[55] and influences recovery and functional outcomes after TBI.[21,56] The SNP rs3783988 was selected to represent a major haplotype block, and for its association with functional outcomes after severe TBI (GCS ≤8), measured by GOS, Disability Rating Scale, and Neurobehavioral Rating Scale-Revised.[20]

(6) *IL-6* acts as a pro- and anti-inflammatory cytokine[57–59] and can suppress post-injury neuroinflammation, neuronal injury, and deficits in motor coordination after concussion.[22] The *IL-6* SNP rs1800795[60] was selected for its role in the promoter region of the *IL6* gene, where it affects production of the cytokine (higher levels with the G allele) in many tissues.[61,62]

(7) *KIAA0319* is critical for neuronal migration and cell adhesion, especially in the cerebral cortex.[23,63] The selected SNP, rs4504469,[64] is a non-synonymous mutation which leads to the substitution of amino acid alanine (C) by threonine (T), potentially affecting neuronal migration,[63] and impacting neuropsychological measures.[24,64,65]

(8) *TPH2* is involved in the production of serotonin[66] and, furthermore, it contributes to control of axonal and dendritic growth,[67,68] with excess serotonin reducing growth of both.[69] Further, *TPH2*'s association with post-injury impulsivity and impaired response inhibition in a rat model[25] suggests an association with post-injury impulsivity in humans. The SNP rs1386483 in particular was selected because of association with impulsivity in carriers of the A allele,

especially in the prefrontal cortex,[25] and for its potential involvement with reduced serotonin synthesis.[70,71]

(9) *APOE* encodes a lipoprotein and exists in three isoforms (ε2, ε3, and ε4) that are formed from two non-synonymous SNPs, rs429358 and rs7412.[72,73] Both of these are located in exon 4 of *APOE* and, specifically, the *APOE* ε4 allele, defined by rs429358, has been associated with risk for Alzheimer's Disease[74,75] as well as prolonged post-concussion symptoms and adverse cognitive outcomes.[11,76,77]

Deoxyribonucleic acid (DNA) extraction

DNA extraction was performed using methods described by Freeman and colleagues.[78] Briefly, buccal mucosa cells were collected with cotton swabs (Medical Wire & Equipment, Corsham, Wiltshire, England) and placed in 15 mL centrifuge tubes containing 2.5 mL of lysis buffer containing salts, detergent, and proteinase K. The tubes containing the swabs were identified by a bar code and the identity was anonymous to the Genomics Core Facility. After extraction, the DNA was resuspended in 250 µL of Tris ethylenediaminetetraacetic acid (EDTA) (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) buffer. The DNA was quantified by measuring the absorbance at 260 nm using a Nanodrop spectrophotometer (Nanodrop, Wilmington, DE). After quantification, samples were aliquoted into storage vials and placed in a –80^{o}C locked freezer. The sample was tracked in the freezer location with other purification details using a LIMs system purchased from the Institute of Psychiatry in London.

SNP assays

TaqMan® SNP Genotyping Assays were performed using an allelic discrimination assay protocol (Life Technologies, Carlsbad, CA). One hundred nanograms of DNA were combined in a volume of 5 µL with 2X Universal PCR Mix (Lifetech) and 1/40 the volume of the TaqMan

SNP assay in a 384 well plate. A pre-read was performed and then polymerase chain reaction (PCR) as follows: a 10 min hold at 95ºC, followed by 45 to 50 cycles of 15 sec at 92°C and then 1:30 min at 60°C in a QuantStudio® System 12K FLEX (Life Technologies, Carlsbad, CA). After amplification, fluorescent signal was detected and automatic and manual calls were made based on the segregation of clusters of homozygous and heterozygous subjects using TaqMan Genotyper software.

Statistical Analyses:

Overview

Although the cohorts were collected a year apart, we combined them for Hardy-Weinberg Equilibrium computation and statistical analysis of the relationship of genotype to concussion history. For evaluation of the relationship of genotype to concussion history, we used an MLRA with graphical analysis to assess findings against the existing literature. These analyses incorporated covariates as described below. We further performed an MLRA of any significant effects from the first analyses, using a grouping of genotypes (e.g., T-carriers vs. CC). In parallel, we performed MLRA of grouped genotypes for genes with a low incidence of minor alleles (e.g., *BDNF* and *TPH2*).

An MLRA approach was used given it provides a discriminability analysis in addition to a basic prescription analysis for categorization. If an analysis was significant after correcting for multiple comparisons, we then (1) reported the accuracy of discrimination produced by the MLRA in terms of the accuracy in predicting who would have none, one, two or more concussions (numbers of individuals with 2 or 3 concussions were collapsed into one group given their low incidence), and (2) performed an analysis of none (0) vs. present (1+)

concussions to confirm effects were not driven by potential subject recall bias, or physician error in diagnosis and record keeping.

This type of analysis can be considered quite stringent relative to other approaches used in neuroscience in that it moves past prescription/association to quantify the extent to which the dependent variable is predicted by the independent variable (i.e., what percentage of 0, 1, and 2+ concussion cases were predicted by genotype).

Given demographic factors such as race can impact the analyses, as can years of football played with regard to numbers of concussions players receive historically, these were used as covariates in the MLRA analysis. Accordingly, the MLRA used genotype as the independent variable, race and years of play as covariates, and number of concussions as a dependent variable.

Hardy-Weinberg Equilibrium computation

A test for Hardy-Weinberg Equilibrium was performed for each SNP (Table 6) to assess the possibility of allele dropout, which is a technical artifact of a bias in amplifying the signal from one of the two alleles in a SNP in heterozygous individuals. Allele dropout results in assigning heterozygous individuals as homozygous for the preferentially amplified allele, thus resulting in a significant finding of disequilibrium in the HWE test. Using chi-square, all candidate genes had a non-significant difference between observed number of genotypes and calculated expected genotypes. Because of potential differences in minor allele frequencies based on race, however, the dbSNP database (https://www.ncbi.nlm.nih.gov/projects/SNP/) was consulted and the allele frequencies are presented in Table 6.

Table 6. Hardy-Weinberg calculations and allele frequencies

Gene (SNP)	Observed	Racial Variation in Allele Frequency[a]		Aggregated Allele Frequency[ab]	Gene (SNP)	Observed	Racial Variation in Allele Frequency[a]		Aggregated Allele Frequency[ab]
COMT (rs4680)		Eur	Afr	N = 121358	NGB (rs3783988)		Eur	Afr	N/A
AA	12	0.50	0.28	0.46	TT	57	0.80	0.85	
AG	39				CT	19			
GG	35	0.50	0.72	0.54	CC	9	0.20	0.15	
Total	86*				Total	85*			
DARC (rs2814778)		Eur	Afr	N/A	IL6 (rs1800795)		Eur	Afr	N/A
CC	24	0.01	0.96		GG	53	0.58	0.98	
CT	15				CG	21			
TT	48	0.99	0.04		CC	11	0.42	0.02	
Total	87*				Total	85*			
BDNF (rs6265)		Eur	Afr	N = 121350	KIAA0319 (rs4504469)		Eur	Afr	N = 121396
CC	67	0.80	0.99	0.81	CC	33	0.58	0.96	0.67
CT	18				CT	34			
TT	1	0.20	0.01	0.19	TT	18	0.42	0.04	0.33
Total	86*				Total	86*			
PARP1 (rs3219119)		Eur	Afr	N/A	TPH2 (rs1386483)		Eur	Afr	N/A
AA	23	0.33	0.71		CC	52	0.62	0.70	
AT	33				CT	29			
TT	30	0.67	0.29		TT	5	0.38	0.30	
Total	86*				Total	86*			
APOE 158 (rs7412)		Eur	Afr	N = 13534	APOE 112 (rs429358)		Eur	Afr	N = 28926
CC	72	0.94	0.90	0.93	CC	2	0.16	0.27	0.18
CT	12				CT	31			
TT	2	0.06	0.10	0.07	TT	53	0.85	0.73	0.82
Total	86*				Total	86*			

*All nonsignificant by Chi square using calculated expected values
[a]From the dbSNP database (https://www.ncbi.nlm.nih.gov/projects/SNP/)
[b]Aggretated Population data used if available; sample size 2n
Eur = European descent
Afr = African descent

MLRA of Genotype and Graphical Analysis (N=87):

We used the total sample in an MLRA predicting number of previous diagnosed concussions (three categories: none, one, two or more) by genetic allele (e.g., CT, CC, or TT). We collapsed the numbers of subjects with two (*n*=4) or three *(n*=3) previous concussions into one group given their low incidence. For SNPs for which the minor allele count was five or

fewer (i.e., *BDNF* and *TPH2*), the homozygotes for the minor allele (i.e., TT) were grouped with the heterozygotes (i.e., CT), resulting in two rather than three genotypes. As is customary in the literature, the two SNPs in APOE were combined into one analysis of the three genotypes (ε2, ε3, and ε4).[72,73] We used race and number of years participating in football as covariates.

Significant effects were followed by a graphical analysis for interpretation of the results. The diagrams displayed (Figure 5) show the genotype effects on the estimated marginal mean number of previous concussions in a standard mean±confidence interval plot, and a relative incidence plot.

Post hoc testing of Concussion History vs. KIAA0319 (N=87)

Independent of the MLRA evaluation of the predictive power of the *KIAA0319* allele on history of concussion, a multinomial test on prior concussion categories (none, one, two or more) was applied to test whether concussion history differed significantly across genotypes when variants were combined to lessen disparity in group sizes being statistically tested.

Correction for Multiple Comparisons

For MLRA analysis with combined cohorts, any result with $p < 0.05/10 = 0.005$ was considered significant, where the correction reflects the number of potential comparisons performed across 10 SNPs.

Results

Hardy-Weinberg Equilibrium: There were no significant differences between any candidate genotype frequencies observed suggesting that these SNPs were not affected by allele drop-out (Table 6).

MLRA and Graph Analysis: For the combined cohorts, MLRA revealed a significant model (p=0.005) after correction for multiple comparisons, predicting number of diagnosed concussions (three categories: none, one, two or more) by *KIAA0319* genotype (i.e., CT, CC, or TT), while covarying race and number of years participating in football (Table 7). As noted in Table 7, five other genes showed trend effects (p<0.1) with this analysis: *COMT, DARC, BDNF, TPH2*, and *APOE* (ε4 carriers).

Table 7. Total cohort analysis using multinomial logistic regression predicting number of concussions by genotype, covarying race, and number of years played

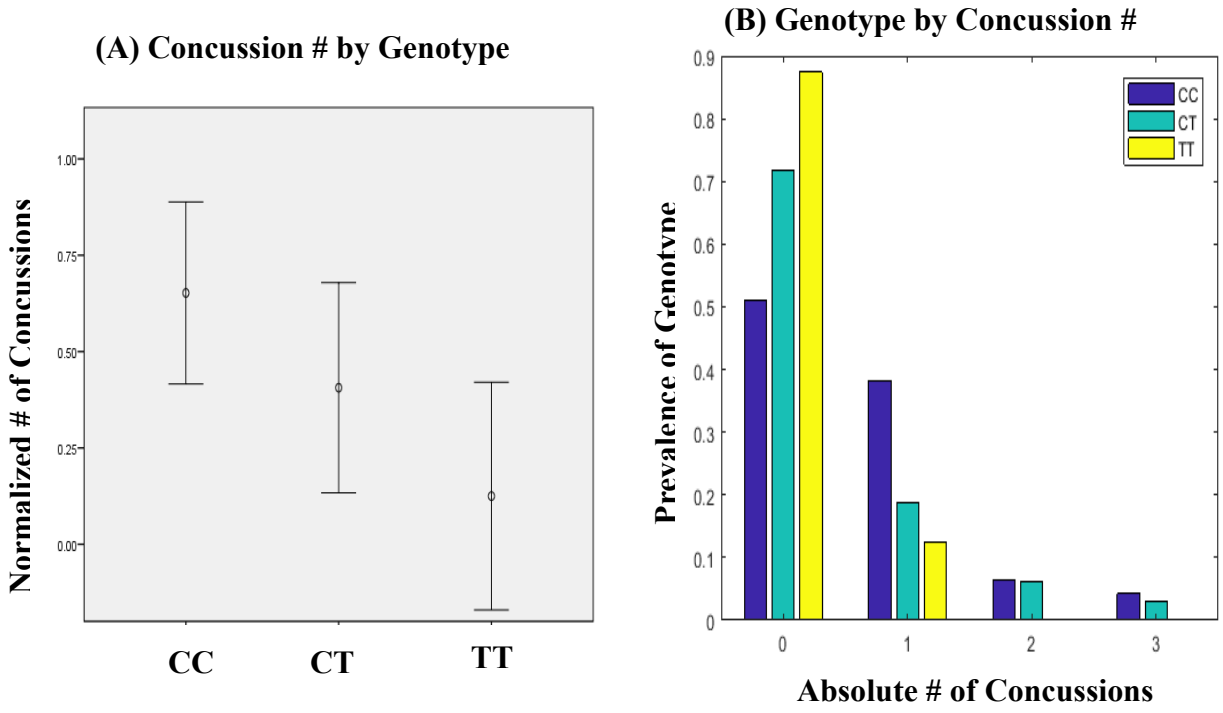
	Combined cohorts		
Genotype	χ^2	R^2	p
COMT	14.651	0.158	0.066**
DARC	16.058	0.170	0.042**
BDNF	12.256	0.134	0.056**
PARP1	9.708	0.107	0.286
NGB	11.897	0.129	0.292
IL6	12.275	0.136	0.139
KIAA0319	22.204	0.228	**0.005**
TPH2	14.927	0.159	0.061**
APOE e4 carriers	13.335	0.150	0.038**

Analysis of genotype effects on concussion number for nine genes/10 single nucleotide polymorphisms (SNPs) is listed with resultant chi-square, the Cox and Snell R^2, and associated p value. These results reflect the combined set of subjects from Cohorts 1 and 2 collected a year apart. Any result with $p < 0.05/10 = 0.005$ is listed in bold type, where the correction reflects the number of comparisons performed (10 tests for 10 SNPs). Any effect with $0.1 < p < 0.005$ is listed as a trend effect with double asterisks (**).

For this combined cohort, the prediction success of *KIAA0319* genotype was overall 68.6% (92.5% for 0, 36.0% for 1, and 12.5% for 2 or more previous concussions).

Graphs of genotype by normalized concussion number (Figure 5) indicated that the *KIAA0319* genotype TT had the lowest number of concussions. A similar analysis for *BDNF* indicated that the genotype (TT) had an increased likelihood of past concussion in this cohort of Division I football players.

Figure 5. KIAA0319 genotype significantly predicts number of previously reported concussions. (A) There is a linear relationship between KIAA0319 genotype and number of concussions. Football players with the TT genotype are the least likely to have a previously reported concussion. Error bars denote 95% confidence interval. (B) Histogram of number of concussions per KIAA0319 further illustrating that football players with the TT genotype had fewer concussions.



Post hoc* testing of Concussion History vs. Grouped Genotype of *KIAA0319, BDNF, TPH2: A follow-up multinomial logistic regression was conducted predicting history of concussion by *KIAA0319* genetic allele to examine the effect of the presence of the "T" allele such that individuals with CT or TT alleles (n=40) were grouped together versus individuals with CC alleles (n=46). Race and number of years played were added as covariates. The overall model was significant (χ^2=19.653, Cox and Snell R^2 =.204, p<0.003) when correcting for the total number of SNPs that were tested (N=10, for a correction of p<0.05/10=0.005) and suggests individuals with the CC allele are more likely to have experienced previous concussion in comparison to those with the CT or TT alleles.

Similar analyses were done for *BDNF* and *TPH2* given a lower incidence of minor alleles, producing results that remained at the trend level. Specifically, for *BDNF*, the overall model was not significant (χ^2=12.256, Cox and Snell R^2 =.134, p<0.056). It was also not significant for *TPH2* (χ^2=11.845, Cox and Snell R^2 =.129, p<0.066).

***Post hoc* testing of grouped concussion history and cohort subsamples:** A follow-up multinomial logistic regression was further conducted predicting history of concussion by *KIAA0319* genetic allele to examine the effect of grouping number of concussions as absent (0) or present (1), regardless of potential number, and using race and number of years played as covariates. The overall model was significant (χ^2=16.610, Cox and Snell R^2 =.176, p<0.002; zero prior concussions predicted to be 53 and 1+ concussions as 33) when correcting for multiple comparisons, supporting the generalizability of the results in the context of uncertainty about concussion events.

Although race was used as a consistent covariate in the MLRA analyses, we also performed *post hoc* MLRAs of the two primary races in the cohort independently of each other, and continuing to use years of play as a covariate. As there was no TT genotype in the African-American cohort, this *post hoc* analysis grouped genotypes (CT or TT alleles were grouped together versus individuals with CC alleles). The overall model remained significant for African-Americans (χ^2=10.782, Cox and Snell R^2 =.231, p<0.029) and Caucasian subsamples (χ^2=9.666, Cox and Snell R^2 =.193, p<0.046).

Discussion

In this candidate gene study of athletes in a Division I football team, multinomial logistic regression analysis (MLRA) of the relationship of genotype on the history of past concussions showed that *KIAA0319* was the only genotype to significantly predict number of diagnosed concussions of the 10 loci tested, while allowing for control of race and numbers of years of football played. The overall predictive accuracy of *KIAA0319* genotype on diagnosed history of concussion for the combined cohorts was about 68%. Graphical analysis indicated that the TT genotype, reported by others to have an increased risk for dyslexia,[23] had the lowest risk for prior diagnosed concussion. A significant multinomial test of T-carriers further suggested a testable hypothesis for fruit fly and animal studies. It should be noted that the trend effects were also observed for five of the nine genes studied. Together, these findings in a small cohort of subjects (N=87) point to the likelihood that genetic effects may play a role in susceptibility to past concussion in athletes competing at the highest levels of contact sports. These points are discussed in the paragraphs that follow.

Abnormal expression of *KIAA0319* may connect several observations from this work and the historical concussion literature, which could potentially be tested in animal models of brain

trauma. Paracchini and colleagues[63] reported that reduced expression of *KIAA0319* (in the animal analog of the TT genotype that in humans is associated with dyslexia) adversely affects adhesion of neurons to the glial skeleton, thereby impairing neuronal migration during development and regrowth. The differences in white matter structure and volume corresponding to the *KIAA0319* genetic polymorphism may result in differences in energy transfer from the whole skull to individual neurons.

From an engineering perspective, healthy, aligned, axon bundles are stiffer in the fiber direction by orders of magnitude, so one might hypothesize that individuals with reduced *KIAA0319* expression (potentially both CT and TT genotypes) would have less regularly oriented axonal fibers, making their white matter slightly more resilient to pressure waves from trauma. Further reduction in the cellular level strain could arise from each fiber being longer than is essential (to cover the path between any two points in the brain), meaning that trauma energy would initially lead to straightening of the axons without additional stress on the membranes. In addition, less adhesion of the neurons to the glial skeleton may introduce another source of compliance that could absorb some of the trauma energy. Hypothetical differences of this sort could reduce the potential for diffuse axonal injury, especially in the TT genotype, which could, in turn, reduce the likelihood of a diagnosed concussion.[79]

Together, these potential effects of reduced *KIAA0319* expression suggest a testable reduction in risk for diffuse axonal injury under head impact conditions.[80,81] Porcine models of head rotational acceleration have been shown to have high face and ecological validity in support of the traumatic axonal injury (TAI) model of head trauma (for review see Cullen and coworkers, 2016).[82] The TAI model of head trauma provides a compelling basis for the long-term neuropathological processes leading to subsequent neurodegeneration and CTE, along with

multiple functional brain disorders such as depression, general anxiety, and suicidality (for review see Johnson and associates, 2017).[83] The effects of *KIAA0319* on the TAI model could readily be tested both in translational porcine and rodent models of head trauma, and with fruit fly work.[84,85] An integrated systems research approach might also allow one to study if impaired migration would be expected to slow the process of recovery from a brain injury, which might contribute to an explanation for the longer recovery times reported for concussed athletes with dyslexia.[86–88] Such a study could also follow up on the trend effects observed for the other candidate genes in this project (i.e., *BDNF, APOE, COMT, DARC*, and *TPH2*).

Of the five genes showing trend effects, two had *p* values reaching a nominal threshold of $p<0.05$. One such gene, *APOE,* has been widely studied with respect recovery and outcome following TBI,[8–10] or headache and other symptoms in concussed collegiate athletes,[11] and our findings support these studies in contrast to those arguing against associations between *APOE* and TBI or TBI-related outcomes.[12–14] The other gene reaching a nominal threshold, *DARC,* has been implicated in the transport, presentation, and concentration of chemokines,[36,37] potentially modulating inflammatory responses[17,37] and modulating neuronal activity by astrocytes.[39,40] The nominal findings of *DARC* in this study are consistent with multi-modal imaging studies pre- and postseason in Division I football players,[26] which suggest the hypothesis of mild TBI as a neuroimmune problem. Last, the trend findings with *BDNF* support the published findings of Dretsch and colleagues,[13] even though they studied a different risk group for concussion, namely, active duty soldiers.

It is important that the results from this study be interpreted in light of a number of limitations or caveats. Our sample included only one team at the Division I level and only had a total sample size of 87 subjects, which suggests these results be considered pilot findings in the

context of calls for large-scale genetic studies that can adequately deal with population variance.[89–91] This sample also had little diversity in race and no diversity in gender. This limits the generalizability of these results to a general population, although most other studies of Division I athletes are going to face significant challenges given the relatively small number of Division I athletes in the United States and the close supervision of their time, which limits participation in research. Given the likelihood of some Division I athletes to continue on to play professional sports (e.g., particularly from teams such as PSU that has been the consensus national champion multiple times), which extends the duration of exposure to potential head trauma, we believe that examining genetic risk factors for concussion in this sample was appropriate.

It should be noted that these data do not suggest a binary screening protocol for participation in contact or collision sports. They do suggest that much of the observed variability may have a genetic basis, and this may allow clinicians to advocate for more careful monitoring of athletes with a particular genotype. Before such a recommendation can be made, additional work is required. The dramatic under-reporting of concussions[92,93] and the deleterious effects of sub-concussive head impacts,[26,94–100] strongly indicate that future work should incorporate the measurement of structural, functional, and biochemical alterations as assessed by MRI-based analysis as a function of number and magnitude of head accelerations,[95,101] along with integrating such measures with prevention research in contact sports, such as appropriate tackling technique. Integrating genotype with head impact exposure and quantitative biomedical imaging will make it possible to determine the effective risk potential for each athlete, and connect it via model-based functional MRI and genetic imaging to its functional implications (e.g.,[102,103]). Future

directions could include larger sample sizes to better study associations meeting nominal significance and other trends in the data.

Future work should also explore the interaction between sex and genotype. Several studies have documented differences in white matter volume,[104] gray matter volume,[105] myelination,[106–108] and axonal structure and response to injury[83] between males and females. Given that the *KIAA0319* polymorphism also induces a change in axonal structure, the interaction between sex and genotype merits investigation.

A further caveat was that the number of concussions reported was self-reported as part of a research staff interview, followed up by thorough chart review of all 87 players by the lead team physician (PHS), and confirmation of 0, 1, or 2+ concussion based on documented clinical signs (as opposed to self-reported symptoms). Although this type of reporting might lead to overestimation or underestimation of the true number of concussions, self-report was confirmed by clinical staff based on documented clinical signs. Lastly, because *KIAA0319* has not been linked with concussion before, only one SNP of *KIAA0319* was assayed in this study. Future work should explore other SNPs in *KIAA0319* (e.g., rs6935076).

This pilot study provides evidence for a potential link between the number of previous concussions and T-carriers of the rs4504469 SNP within *KIAA0319*, using an analysis framework that combined discriminability analysis with prescription. These results directly suggest hypotheses for further human and preclinical studies.

Acknowledgements:

Funding: Data collection and initial genetic analysis for this project were entirely funded by Semyon Slobounov's lab. Funding for data analysis and manuscript preparation was funded by Hans Breiter's lab. We would like to thank PSU football players for their time and efforts

participating in this study. We also would like to thank Katie Finelli for her assistance with this project and Ashley Price, Kerry Hair, Deborah Grove, and Craig Praul at the PSU Genomics Core Facility – University Park, PA for performing the initial genetic analysis. Lastly, we would like to thank Doug Smith for his supportive guidance and key revisions to the manuscript.

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Chapter 4: The Effect of Head Acceleration Events on Functional Outcomes: A Two Football Season Comparison Study

Abstract

Context: In collision sports, particularly football, athletes can accumulate thousands of subconcussive impacts or head acceleration events (HAEs) across a single season; however, the short-term consequences of these impacts are not well understood.

Objective: To investigate the effects of the accumulation of impacts on cognitive functions over a single season of football and to compare two separate seasons of data (2015 and 2017) for reproducibility and how coaching and medical decisions and governing body rules can indirectly influence outcomes.

Design: Prospective observational cohort study

Setting: Athletic training room and University laboratory

Participants: Twenty-three NCAA Football Bowl Subdivision players in 2015 and 34 players in 2017.

Main Outcome Measures: Helmet accelerometers during practices and virtual reality testing (VR; balance, reaction time, spatial memory) before, during, and after the season.

Results: In the 2015 season, preseason had the majority of ≥80G impacts while during the season had the majority of ≥25G to <80G impacts and positional differences showed that linemen had the majority of both types. Virtual reality analysis revealed that scores significantly decreased after the season for spatial navigation ($p<0.05$) but not for balance or reaction time. Significant correlations ($p<0.05$) were found between accelerometer and cognitive measures, as well as, player demographic variables. The 2017 season revealed that practices during the season had the majority of both categories of impacts. Virtual reality analysis revealed changes in score across the season with the balance module changing significantly across time ($p<0.05$).

Comparisons in data between the seasons revealed significant decreases in the incidence rates of impacts at both ≥80G and ≥25G to <80G from 2015 to 2017 (p<0.05) but not for raw number of impacts per player (p>0.05). Additionally, there were differences in virtual reality scores at postseason testing timepoint with players from the 2017 season having significantly worse scores on balance and reaction time and significantly better scores on spatial memory (p<0.05) than players from the 2015 season.

Conclusions: Even in the absence of clinical symptoms and concussion diagnosis, repetitive impacts may cause cognitive or functional alterations. Documenting the distribution of impact quantity and intensity as a function of time and position may be considered by coaches and clinicians to reduce the accumulation of impacts in athletes exposed in contact sports. However, coaching technique, medical decisions, and governing body rule changes indirectly could have beneficial or protective outcomes for individuals in contact sports. Monitoring athletes over the course of a season could provide useful information for athlete safety and provide more information on athletes' exposure to impacts.

Introduction

Repetitive subconcussive impacts, or head acceleration events (HAEs), those impacts that are less severe than a full-blown concussive injury, have become a major concern due to their possible accumulative effect and link to neurological impairments. These impacts involve the transfer of mechanical energy to the brain with sufficient force to injure axonal or neuronal integrity but do not result in clinical symptoms.[1–3] However, these repetitive impacts are often unmanaged or undiagnosed leading to a larger number of impacts over the course of a season or career.[4,5] Post-mortem studies, done in both humans and animals, have shown that repeated impacts may have an accumulative effect[6] and may affect cognitive processes.[7] The link between

repetitive impacts and cognitive function,[8–11] white matter changes on diffusion tensor imaging (DTI;[12,13]), alterations of cerebral blood flow,[14] functional brain alterations,[8,11,14,15] and cerebral spinal fluid changes[16] have been previously shown. However, the intensity and frequency of the impacts necessary to cause damage is still unknown.

Players of collision sports, such as football, are at a high risk for concussion and the accumulation of repetitive head impacts. Previous studies have shown that high school football players average 600 impacts over a season and collegiate players average 1000 impacts.[1] However, these exposures can also vary based on player position and previous concussive history. Research has begun to link these exposure metrics to cognitive and neurobehavioral disturbances, including increased risk for Alzheimer's disease, chronic traumatic encephalopathy (CTE), or amyotrophic lateral sclerosis (ALS).[5] However, there is still much debate regarding the progression of these disturbances and their link to repetitive impacts.

Accelerometers are now commonly used in research to track the number of head acceleration events in football. College football players (compared to high school football and other college sports) experience the most impacts across a season, the highest average peak linear and rotational acceleration per impact, and the highest cumulative linear and rotational acceleration per impact.[17] The type of practice is also a contributing factor to the number of occurring impacts. Full-pad practices have been shown to have the highest number of impacts per session and these numbers increase in game settings.[17–19]

Position played is a major confounding factor and current literature suggests that offensive and defensive linemen sustain the greatest number of impacts, at a lower magnitude however, than other positions.[18–20] Statistically significant differences in impacts based on position played have been found.[18] Offensive linemen were shown to have more accelerative

head impacts as they are normally on the line of scrimmage and are involved in short distance, low magnitude impacts on almost all plays.[19,20] Athletes in the skill or speed positions while in the open field, experience fewer, but higher magnitude impacts.[19] These positions include quarterback, running back, fullback, tight end, corner back, safety, and linebacker; players that often build up momentum prior to tackling or being tackled.[21] Playing position also was a significant factor in the athletes' symptom reporting of more non-clinically observable post-impact symptoms (headache, concentration difficulties, dizziness, etc.), with offensive linemen in particular reporting more-frequent symptoms after repetitive impacts, suggesting that these linemen experience more subconcussive injury.[22]

Commonly, the processes affected after concussion are memory, attention, concentration, processing speed, and reaction time.[23–25] Additionally, cognitive changes have also been associated with repetitive HAEs. Visual working memory impairments, with altered activation in the dorsolateral prefrontal cortex,[11] and abnormal regional cortical activation patterns[12] have been demonstrated in football players after a single season, even in the absence of clinical symptoms of concussion.

This two-part study examined the effect of multiple high intensity impacts on general brain function after participation in a single season of football using behavioral and neuropsychological tools. By monitoring cognitive changes after a season, we can examine how exposure to repetitive impacts affects these processes in a way that is both time and cost effective and could help inform clinical protocols. Over two separate seasons, the 2015 season and the 2017 season, players were monitored as it was expected that the number of HAEs accumulated throughout the season (as an additive effect) would influence functional properties critical in athletes (balance, reaction time, spatial memory). Additionally, the number of impacts would be

a confounding factor along with player position, and/or previous history of concussive injury. More broadly, these separate seasons would also allow us to examine how best to design protocols and how, indirectly, changes made by medical and coaching personnel at a single institution and governing bodies of sport could influence outcomes.

Methods

Design for 2015 Season: Before the season started, all participants underwent a baseline test on virtual reality and the accelerometer BodiTrak system was installed in their helmets. Once preseason began, participants wore their accelerometers during 53 practice sessions. Participants were tested on a virtual reality system during the season (including preseason) if they met specific, predetermined thresholds during a single practice session: 1 hit ≥80G and/or ≥28 impacts ≥25G and ≤80G. These thresholds were set based upon a combination of previous literature[18] and pilot work done, however this data will not be presented here. After the season ended, all participants were tested again on the virtual reality system *(see Procedures below for details on the specific modalities).*

Design for 2017 Season: Before the season started, all participants underwent a baseline test on virtual reality and the accelerometer BodiTrak system was installed in their helmets. Once preseason began, participants wore their accelerometers during 69 practice sessions. All participants were testing using virtual reality at the end of preseason (weeks 1 – 3) and after the first half of the season (weeks 4 – 8). After the season ended, all participants were tested again on the virtual reality system *(see Procedures below for details on the specific modalities).*

Participants: All participants were Penn State University NCAA Football Bowl Subdivision football players. Background information is contained in Table 8 for both the 2015 and 2017 seasons. All participants provided informed written consent, as approved by the Penn State University Institutional Review Board. These participants from 2015 were part of a larger study that included imaging data that has been previously published.[14]

Table 8. Participant demographic information for both the 2015 and 2017 season

Variables	***2015 Season (n=23)***	***2017 Season (n=34)***
Demographics, *range (M ± SD)*		
Age	19 – 23 (20.78 ± 1.28)	18 – 23 (20.48 ± 1.60)
Number of Years Playing	6 – 19 (10.89 ± 3.34)	8 – 17 (10.88 ± 2.83)
Number of Previous Concussions, *n (%)*		
0	14 (60.9)	18 (52.9)
1	7 (30.4)	13 (38.2)
2	2 (8.7)	2 (5.9)
3	0 (0)	1 (2.9)
ADHD Diagnosis, *n (%)*	2 (8.7)	11 (32.4)
Psychiatric Diagnoses, *n (%)*	0 (0)	1 (2.9)
Position Breakdown, *n (%)*		
Offensive Line	8 (34.8)	16 (47.1)
Defensive Line	8 (34.8)	18 (52.9)
Tight End	2 (8.7)	0 (0)
Linebacker	2 (8.7)	0 (0)
Safety	2 (8.7)	0 (0)
Running Back	1 (4.3)	0 (0)

Procedures:

BodiTrak System: Impacts to the head for all participants were monitored using helmet sensors from the Head Health Network BodiTrak system. These sensors, created by Vista Medical, were adapted to be individually placed inside each individual participants' helmet using a 3M VHB

adhesive and are comprised of elastic fabric with pressure monitors and impact sensors. The sensors provide estimates on linear acceleration (in units of G) and location of impact. The sensors were in place throughout the season, but impacts were only recorded during practices (not games) for a total of **53** possible sessions in 2015 and **69** possible sessions in 2017.

Virtual Reality System: A 3D TV system (HeadRehab.com) with a head mounted accelerometer was used. Data were collected before season (baseline) and after the season. Three different modules were used: balance, reaction time, and spatial memory (see [26–28] for reliability and validity of all modules). In the balance task, participants are instructed to hold tandem Romberg position for all trials. In the first trial, the virtual room is completely still (for a baseline measure) and in the subsequent 6 trials, the virtual room moves in various directions. The system measures the position and orientation in yaw, pitch, and roll directions. In the reaction time module, participants are instructed to stand feet shoulder width apart, hands on their hips. The virtual room moves and they are instructed to move their body in the same direction as the virtual room. The system measures both reaction time (in ms) and errors of anticipation (wrong direction of response). In the spatial memory module, participants are shown a randomized virtual pathway with multiple turns to a door and then the return trip. Afterwards, they are instructed to repeat the exact pathway using a joystick. The system measures how many errors it takes to get to the door and the total time to complete the task. Raw data were analyzed using SideLine v10.1 test reporting module which uses mathematical algorithms to produce a score on a scale of 0 (fail) to 10 (perfect) and this score was used for analysis.

Statistical Analysis: All statistical analyses were performed using SPSS V25. For all analyses, statistical significance was considered reached at $p<0.05$.

2015 Season Data: Due to non-normality, a Wilcoxon Signed Rank test was used to assess differences in virtual reality scores before the season versus after the season for each modality. Additionally, participants were grouped into categories of concussion history (i.e., yes or no) or position categories (i.e, speed or non-speed based off of Lehman's classifications[21]) and then differences in virtual reality scores before the season versus after the season were also examined. Point-Biserial correlations were used, given the ability to measure the association between continuous and dichotomous variables, to assess the relationship between accelerometer data, virtual reality data, and participant demographic information. Impacts were categorized into categories of ≥80G and ≥25G to <80G based on work done by Mihalik[18] and on pilot work. Correction for multiple comparisons was not done as each correlation was considered an independent hypothesis.

2017 Season Data: Due to non-normality, virtual reality scores across time were examined for all participants using a Friedman's test with post hoc analysis done using Wilcoxon Signed Rank tests with a Bonferroni correction. Participants were again grouped into categories of concussion history (i.e., yes or no) or position categories (i.e offensive line versus defensive line) and differences in virtual reality scores across the season for each subgroup were examined using the Friedman's test with post hoc analysis done using Wilcoxon Signed Rank tests with a Bonferroni correction, while differences between groups were examined using the Kruskal-Wallis test.

Comparison Between Seasons: Accelerometer data was compared between seasons using independent t-tests for all players under study, as well as, just the non-speed players under study.

Comparisons were made both on the raw accelerometer data and the incidence rate of impacts. Virtual reality data from the two seasons was compared using a Kruskal-Wallis test for each module.

Results

***2015 Accelerometer Data*:** A total of 129 impacts at ≥80G were recorded across the season (53 sessions) with 78.3% (101 impacts) occurring during the preseason (13 sessions) and 21.7% (28 impacts) occurring during the regular season (40 sessions). A total of 3557 impacts at ≥25G to <80G were recorded across the season with 32.6% (1158 impacts) occurring during the preseason and 67.4% (2399 impacts) occurring during the regular season. The overall number of individual impacts at ≥80G and ≥25G to <80G obtained over the season are presented in Table 9. The average incidence rate for impacts at ≥80G was 0.0047±0.0038 while the average incidence rate for impacts ≥25G to <80G was 0.1228±0.0878.

By practice type, full pads had the highest cumulative number of ≥80G (78) impacts followed by scrimmages (30), upper pads only (19) and then helmets only (2). For the ≥25G to <80G impacts by practice type, full pads have the highest raw cumulative number (1934) followed by upper pads only (1225), scrimmages (300), and then helmets only (98). However, practice type rate showed that the scrimmage practices had the highest average number of impacts per practice followed by full pads, uppers, and lastly, helmets.

Table 9. Accelerometer data for all participants (n=23) from the 2015 season including position information.

Participant Number	Position	Monitored Sessions (53 total)	Total ≥80G	Incidence Rate of ≥80G[a]	Total ≥25G and ≤80G	Incidence Rate of ≥25G and ≤80G[a]
1*	Tight End	27	0	0.0000	17	0.0139
2	Offensive Line	42	3	0.0025	95	0.0779
4	Defensive Line	37	5	0.0041	116	0.0952
5	Defensive Line	38	7	0.0057	180	0.1477
6	Safety	45	5	0.0041	101	0.0829
7	Defensive Line	47	11	0.0090	148	0.1214
8	Offensive Line	47	10	0.0082	455	0.3733
9*	Offensive Line	26	0	0.0000	28	0.0230
10	Offensive Line	49	15	0.0123	344	0.2822
11	Offensive Line	30	5	0.0041	221	0.1813
12	Running Back	43	13	0.0107	110	0.0902
13	Safety	51	2	0.0016	124	0.1017
14	Defensive End	37	4	0.0033	155	0.1272
15	Defensive End	32	7	0.0057	95	0.0779
16**	Defensive Line	2	2	0.0016	9	0.0074
17	Defensive Line	36	2	0.0016	99	0.0812
18	Defensive Line	47	2	0.0016	74	0.0607
19	Offensive Line	38	1	0.0008	183	0.1501
20	Linebacker	45	1	0.0008	151	0.1239
21	Offensive Line	48	7	0.0057	301	0.2469
22	Tight End	37	13	0.0107	205	0.1682
23**	Linebacker	17	12	0.0098	83	0.0681

Players who missed part of the season due to a non-neurological injury are indicated by an asterisk (*). Players whose season ended early due to non-neurological injury are indicated by a double asterisk (**).

[a]*Incidence rate calculated as: (number of impacts)/(population size*time of study). For example, for participant 1: 17/(23*53).*

***2015 Virtual Reality Data*:** Virtual reality results were produced on a scale of 0 (fail) to 10 (perfect) for each module: spatial memory, balance, and reaction time. Additionally, a comprehensive score is calculated using the scores from the three modules. Average scores for the before season and after season were calculated for all 23 participants and Wilcoxon Signed Rank Test were run (significance, $p<0.05$). In the spatial memory module, a significant change

(Z= -2.160, *p*=0.03) in score from before season (M=8.79, SD=1.90) to after season (M=7.74, SD=2.85) was observed. In the balance module, there was a non-significant change (Z= -1.02, *p*=0.31) in scores from before season (M=7.89, SD=2.49) to after season (M=8.97, SD=0.74). The reaction time module also showed a non-significant change (Z= -1.40, *p*=0.16) in score from before season (M=4.78, SD=3.30) to after season (M=5.98, SD=2.18).

Additionally, when data was examined based on previous concussion history (yes or no) there were significant differences found. Players with a previous history of concussion (n=9) had a significant decrease (Z= -2.016, *p*=0.04) in the spatial memory module from before the season (M=9.03, SD=1.12) to after the season (M=6.44, SD=3.19). No other significant differences were found for either group in the other VR modules.

2015 Correlation Data: Point-Biserial Correlations were run between predictors of interest (position category, years played football, and number of previous concussions) and main outcomes (before and after VR scores and impacts) and are presented in Table 10. Years played was significantly positively correlated with how many previous concussions the athlete had. Additionally, there was a statistically significant negative association between how many previous concussions and Total 80G impacts, while Total 80G impacts across the season was significantly positively associated with Total 25G to <80G impacts across the season. Lastly, non-speed versus speed position category was significantly negatively correlated with before the season spatial memory VR score.

Table 10. Point-biserial correlations

	Non Speed vs Speed	Years Played	How Many Cx	Total 80G	Total 25G	Before Spatial	Before Balance	Before Rxn Time	Post Spatial	Post Balance	Post Rxn Time
Non Speed vs Speed	1	-0.181	-0.051	0.015	-0.185	-.537**	0.123	0.111	-0.367	0.122	0.107
Years Played		1	.486*	-0.075	0.275	-0.019	-0.046	0.283	-0.283	-0.211	-0.107
How Many Cx			1	-.452*	-0.330	0.008	0.072	0.083	-0.282	-0.175	0.043
Total 80G				1	.435*	-0.095	0.068	-0.096	0.061	-0.143	-0.159
Total 25G					1	-0.123	0.089	0.230	0.197	-0.013	-0.311
Before Spatial						1	-0.115	-0.036	0.375	-0.219	-0.014
Before Balance							1	0.256	-0.174	-0.129	-0.117
Before Rxn Time								1	0.002	0.348	0.194
Post Spatial									1	0.026	0.029
Post Balance										1	-0.023
Post Rxn Time											1

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).
cx = concussion; Total 80G = impacts ≥80G; Total 25G = impacts ≥25G to <80G; Before = before season; rxn time = reaction time; Post = after season

2017 Accelerometer Data: A total of 147 impacts at ≥80G were recorded across the season (69 sessions) with 29.3% (43 impacts) occurring during the preseason (13 sessions) and 70.7% (104 impacts) occurring during the regular season (56 sessions). A total of 5733 impacts at ≥25G to <80G were recorded across the season (69 sessions) with 27.8% (1595 impacts) occurring during the preseason (13 sessions) and 72.2% (4138 impacts) occurring during the regular season (56 sessions). The raw number of individual impacts at ≥80G and ≥25G to <80G obtained over the season are presented in Table 11. The average incidence rate for impacts at ≥80G was 0.0018±0.0018 while the average incidence rate for impacts ≥25G to <80G was 0.0719±0.0375.

Table 11. Accelerometer data for all participants (n=34) from the 2017 season including position information.

Participant Number	Position	Total ≥80G	Incidence Rate of ≥80G[a]	Total ≥25G and ≤80G	Incidence Rate of ≥25G and ≤80G[a]
1	Defensive Line	2	0.0009	43	0.0183
2	Defensive Line	8	0.0034	107	0.0456
3**	Defensive Line	17	0.0072	107	0.0456
4	Offensive Line	3	0.0013	210	0.0895
5	Offensive Line	6	0.0026	147	0.0627
6	Offensive Line	3	0.0013	355	0.1513
7	Defensive Line	1	0.0004	119	0.0507
8	Defensive Line	3	0.0013	74	0.0315
9	Offensive Line	0	0.0000	0	0.0000
10	Offensive Line	4	0.0017	172	0.0733
11	Defensive Line	1	0.0004	194	0.0827
12	Defensive Line	1	0.0004	157	0.0669
13*	Offensive Line	0	0.0000	119	0.0507
14	Offensive Line	5	0.0021	370	0.1577
15	Offensive Line	0	0.0000	124	0.0529
16	Defensive Line	3	0.0013	79	0.0337
17	Offensive Line	0	0.0000	169	0.0720
18	Offensive Line	1	0.0004	126	0.0537
19	**Offensive Line**	**5**	**0.0021**	**160**	**0.0682**
20	Defensive Line	4	0.0017	107	0.0456
21	Defensive Line	4	0.0017	210	0.0895
22	Offensive Line	5	0.0021	289	0.1232
23	Defensive Line	6	0.0026	201	0.0857
24	Defensive Line	16	0.0068	257	0.1095
25	Defensive Line	4	0.0017	137	0.0584
26	Offensive Line	1	0.0004	71	0.0303
27	Defensive Line	0	0.0000	276	0.1176
28	Defensive Line	8	0.0034	138	0.0588
29	Offensive Line	6	0.0026	235	0.1002
30	**Offensive Line**	**3**	**0.0013**	**84**	**0.0358**
32	Offensive Line	3	0.0013	201	0.0857
33	Defensive Line	3	0.0013	341	0.1454
34	Defensive Line	13	0.0055	231	0.0985
35	Defensive Line	8	0.0034	123	0.0524

Players who missed part of the season due to a non-neurological injury are indicated by an asterisk (*). Players whose season ended early due to non-neurological injury are indicated by a double asterisk (**). Players who were diagnosed with a concussion during the season are ***bolded****.*

[a]*Incidence rate calculated as: (number of impacts)/(population size*time of study). For example, for participant 1: 43/(34*69).*

For impacts ≥80G (n=147), the average GForce reading (in G) for linear acceleration was 102.07 ± 18.21 and the average gyro (in rad/sec^2) for rotational acceleration was 922.83 ± 734.03. For impacts ≥25G to <80G (n=5733), the average GForce reading (in G) was 36.84 ± 11.07 and the average gyro (in rad/sec^2) was 821.84 ± 462.23. Impacts ≥80G were primarily located to the front right of the helmet (n=25; 17%) as were impacts ≥25G to <80G (n=1481; 25.8%).

2017 Virtual Reality Data: A Friedman's test was run for each modality of virtual reality (comprehensive, balance, spatial memory, reaction time) using the four testing timepoints and the descriptive data for each modality is presented in Table 12.

Table 12. Descriptive statistics for all virtual reality modalities across the season

	Baseline			**Preseason**			**Midseason**			**End of Season**		
	Mean	Std Dev.	N	Mean	Std Dev.	N	Mean	Std Dev.	N	Mean	Std Dev.	N
Comprehensive	6.56	1.60	33	7.52	1.39	33	6.80	1.79	33	6.83	1.56	33
Balance	7.40	3.06	33	8.72	1.62	33	7.23	3.07	33	7.24	2.61	33
Spatial Memory	8.31	2.38	33	8.42	2.28	33	8.53	2.18	33	8.71	1.85	33
Reaction Time	3.98	3.00	33	5.41	3.01	33	4.63	2.69	33	4.54	2.41	33

The Friedman's test revealed a nonsignificant difference in comprehensive score across the season $\chi^2(3)=4.382$, $p=0.228$. Wilcoxon signed-rank test with Bonferroni correction applied resulted in a significance level of $p<0.008$. There was a significant Wilcoxon signed-rank tests for the comprehensive module with scores from baseline and after preseason being significantly different ($Z=-2.984$, $p=0.003$).

There was a significant difference in balance score across the season $\chi^2(3)=8.131$, $p=0.042$. Wilcoxon signed-rank test with Bonferroni correction applied resulted in a significance

level of $p<0.008$. There was a significant Wilcoxon signed-rank tests for the balance module with scores from after preseason and at the end of the season being significantly different (Z=-2.877, $p=0.004$).

The spatial memory module showed a statistically significant change across the season $\chi^2(3)=9.821$, $p=0.019$. Wilcoxon signed-rank test with Bonferroni correction applied resulted in a significance level of $p<0.008$. However, there were no significant Wilcoxon signed-rank tests for the spatial memory module.

Additionally, there was a nonsignificant difference in reaction time score across the season $\chi^2(3)=6.358$, $p=0.094$. Wilcoxon signed-rank test with Bonferroni correction applied resulted in a significance level of $p<0.008$. However, there were no significant Wilcoxon signed-rank tests for the reaction time module.

Comparisons of VR data were made using a Kruskal-Wallis test based on position (offensive versus defensive line) and previous concussion history (yes versus no). Based on position, there were no significant differences between scores for comprehensive, balance, spatial memory, or reaction time modules between offensive and defensive linemen ($p>0.05$). When examining previous concussion history, there were no significant differences between groups on comprehensive, spatial memory, or reaction time ($p>0.05$), however there was a significant difference between groups on the balance module (mean rank: yes (12.19) and mean rank: no (21.53)) only at the postseason timepoint (H=7.695, $p=0.006$).

Across time, using the Friedman Test, there were no significant changes across time in any module for defensive line players ($p>0.05$). Offensive line players had no significant changes across time in comprehensive, spatial memory or reaction time modules ($p>0.05$). There were statistically significant differences in balance scores ($\chi^2(3)=9.755$, $p=0.021$). Post hoc

testing using Wilcoxon signed-rank test with Bonferroni correction applied resulted in a significance level of $p<0.008$. However, after correction for multiple comparisons, there were no significant Wilcoxon signed-rank tests for the balance module.

Comparison Data for the 2015 and 2017 Seasons: Accelerometer Data for each season for all players, as well as for just the non-speed players from the 2015 season, was compared and is presented in Table 13.

Table 13. Comparison accelerometer data between the 2015 and 2017 seasons

	2017 (n = 34)	2015 (n = 23)	2015 non-speed only (n = 16)
# Sessions	69	53	53
Total Impacts ≥80G	147	129	83
Average 80 per session	2.13	2.44	1.57
Average 80 per player	4.32	5.61	5.19
Average 80 per session per player	0.063	0.106	0.098
Total Impacts ≥25G & <80G	5733	3557	2818
Average 25 per session	83.09	67.11	53.17
Average 25 per player	168.62	156.91	176.125
Average 25 per session per player	2.444	2.918	3.323
Preseason			
Sessions	13	13	13
Total Impacts ≥80G	43	101	62
Total Impacts ≥25G & <80G	1595	1158	873

Independent t-tests were run to compare accelerometer data between seasons for both raw values and incidence rates (raw data found in Tables 9 and 11). Table 14 presents the differences in raw number of impacts per player between seasons for a) all players under study and b) just linemen under study. Table 15 presents the differences in incidence rates of impacts between seasons.

Table 14. Independent t-test for players in 2015 vs. 2017

	Season	N	Mean	Std. Dev.
Total ≥80G Impacts	2017	34	4.32	4.23
	2015[a]	23	5.61	4.64
	2015 Linemen only[c]	16	5.19	4.12
Total ≥25G & <80G Impacts	2017	34	168.62	88.08
	2015[b]	23	156.91	110.14
	2015 Linemen only[d]	16	176.13	123.06

[a]*non-significant when compared with Total 80G 2017 data (p=0.284)*
[b]*non-significant when compared with Total 25G 2017 data (p=0.658)*
[c]*non-significant when compared with Total 80G 2017 data (p=0.500)*
[d]*non-significant when compared with Total 25G 2017 data (p=0.806)*

Table 15. Independent t-test for Incidence Rates of Impacts between seasons

	Season	Mean	Std Dev	t	p
Incidence Rate of Impacts ≥80G	2015	0.0047	0.0038	3.291	0.003*
	2017	0.0018	0.0018		
Incidence Rate of Impacts ≥25G & <80G	2015	0.1228	0.0878	2.573	0.016*
	2017	0.0719	0.0375		

**significant (p<0.05)*

Comparison of VR data between seasons on all players under study from 2015 and 2017 was assessed using a Kruskal-Wallis test. There were no significant differences between scores at the before the season timepoint on comprehensive, balance, spatial memory, or reaction time modules (p>0.05).

At postseason, there were statistically significant differences in balance scores (H=5.555, p=0.018), with a mean rank score of 34.65 for 2015 and 24.21 for 2017. Additionally, there was a statistically significant difference in spatial memory scores (H=4.302, p=0.038), with a mean rank score of 23.09 for 2015 and 32.37 for 2017. Lastly, there was a statistically significant difference in reaction scores (H=5.097, p=0.024), with a mean rank score of 34.39 for 2015 and 24.39 for 2017. There were no significant differences in score for the comprehensive module (H=3.480, p=0.062).

Discussion

To our knowledge, this study is the first to explore neurocognitive changes, via VR measures, over a single season of football. By implementing testing throughout the season, we were able to begin to track players' neurocognitive abilities and how it relates to the accumulation of impacts. Additionally, over the course of two seasons, this paper allowed for a preliminary examination of reproducibility and how, indirectly, changes made at coaching, medical, or governing body levels can influence outcomes. This study revealed several findings of interest that will be discussed in the following text.

In terms of raw accelerometer data, the 2015 preseason data showed the vast majority of high threshold impacts (≥80G). Second, practice type influenced the cumulative number of impacts received. Full pads had the highest raw number of impacts both at ≥80G and at ≥25G to <80G, which has been previously demonstrated.[15] Third, speed players (tight end, linebacker, defensive back, running back) experienced more cumulative impacts at ≥80G and non-speed players (linemen) had more cumulative impacts at ≥25G to <80G, which has been previously demonstrated.[19,20]

Virtual reality data from the 2015 season revealed a significant decrease in spatial memory scores from before the season to after the season ($p<0.05$) as well as non-significant increases ($p>0.05$) in balance and reaction time scores. Furthermore, when players were separated into groups based on previous concussion diagnosis (yes or no), only players with previous history of concussion had significant decreases in spatial memory scores after the season ($p<0.05$). Cognitive impairments have been previously demonstrated, mostly in MRI studies, showing visual working memory impairments being associated with altered activation in the dorsolateral prefrontal cortex (DLPFC)[11] and decreases in cortical thickness.[29] However, this study shows impairments in working memory without the use of advanced MRI and highlights

the importance of spatial memory as a beneficial and low cost (both time and money) test for detecting neurocognitive changes as a function of cumulative impacts that players experience over a single football season in the absence of diagnosed concussion.

The correlation analyses on the 2015 season data also revealed several findings of interest. Years played was significantly positively correlated with total number of concussions. Additionally, how many previous concussions was significant negatively associated with Total 80G impacts while Total 80G impacts across the season was significantly positively associated with Total $\geq$25G to <80G impacts across the season. These significant correlations begin to highlight the importance of understanding a player's individual background and how those factors could be used in a clinical setting to better help identify those who may be at greater risk for impact accumulation or cognitive changes.

Lastly, non-speed versus speed position category was significantly negatively correlated with before-season spatial memory VR score, with non-speed players having higher spatial memory score before the season. These correlations suggest that in the future, spatial memory assessment could be a critical clinical tool in determining which players may be at risk for cognitive deficits throughout the season and that monitoring players' neurocognitive function during the entire season, even in the absence of any clinical symptoms, may be an important injury prevention approach. The information from this study could potentially help identify players at risk for high number of repetitive head impacts and cognitive deficits, as previous literature has shown links between concussion history and cognitive functioning.[30] Retired professional football players, diagnosed with three or more concussions, were found to report more cognitive symptoms, specifically memory impairment and cognitive impairment, than retired players with no history of concussion.[31] Our finding of a cognitive decline from before

the season to after the season are in line with previous reports that a history of concussions in college football players may result in lower cognitive functions including memory changes.[32] It is important to acknowledge that simply examining players before and after the season for deficits could potentially be misleading. These pre to postseason changes provide support for the notion that clinical symptoms are not necessarily observed in the presence of deficits in brain functioning. However, it is still unclear if these changes could be compensatory adaptation to cumulative impacts or more lasting alteration of brain functional integrity.

To that point, the 2017 season of data collection was adapted slightly to gain a better understanding of these issues discussed above. The protocol was adapted to include more timepoint testing, adding sessions at the end of preseason and halfway through the season. Additionally, when using college athletes as participants in research, certain factors are out of researchers control including medical and coaching decisions made by the individual team and rule changes made by governing bodies, like the National Collegiate Athletic Association (NCAA). In between the 2015 and 2017 seasons, there were important changes that need to be discussed and considered when interpreting this data. The NCAA implemented two rules changes that could influence this data: instant replay for targeting and the elimination of multiple contact practices a day during preseason. Additionally, decisions made at the Penn State level that could influence the way this data is interpreted were changes to practice format (the number of hitting practices a week), changes to coaching staff (new defensive coaches), and new equipment (helmets specifically chosen for each individual based on fit and the use of protective equipment including the Protech cover). While these factors will most likely directly influence data, there is no way to quantity their effects on this data so they will be considered indirect effects on data and be examined as part of a larger scope discussion.

In the 2017 season data alone, the majority of impacts at both thresholds occurred during the regular season. This partly contradicts the 2015 data in that the majority of ≥80G impacts moved from the preseason to during the regular season in 2017. Interesting to note is the high rotational acceleration of these impacts. On average, impacts in 2017 had rotational accelerations of 922.83 at ≥80G and 821.84 at ≥25G to <80G. While the computation of rotational acceleration has been debated, these numbers are lower than rotational accelerations reported in other studies on football players.[8,12,19,33–35]

In terms of virtual reality data, the 2017 data showed no significant changes from pre to postseason directly, contradicting the 2015 data. When examining changes in score across time, however there were mixed findings. The reaction time and comprehensive modules had no changes in score over time while the spatial memory and balance modules did have changes over time. When further probing these changes however, no direct comparisons of spatial memory scores remained significant. Balance, however, did change from preseason to end of season showing a decrease in score at the end of the season. The fluctuation of virtual reality scores over time highlights the importance of consistent monitoring of athletes even in the absence of clinical symptoms. Many factors – like fatigue, stress, effort, etc., – have the ability to affect testing results so more frequent monitoring could be beneficial. Balance, in particular, is considered a more functional task, and while likely affected by physical fatigue, has also been shown to be negatively affected by a season of exposure to repetitive HAEs.[36–38]

These findings highlights a few potential limitations that will be discussed. The absence of significant changes pre to postseason only in the spatial memory data could be due to the participants selected in this study. In particular, the cohort of players in 2017 had a much higher rate of diagnosed ADHD and many were on medication. This has great potential to influence

outcomes, particularly cognitive outcomes like a spatial memory task. Furthermore, the 2015 season contained both speed and non-speed players while 2017 contained only non-speed players. This could continue to highlight how different the exposure, and risk, are by position as well as necessary skills that needed to be successful in each. Additionally, since many protective measures were set in place between the seasons it can be potentially inferred that these contributed to the lack of changes seen. However, this is purely speculation at this time as there is no way to definitively quantify if these changes contributed. Lastly, the Friedman's test revealed significant changes only in the balance modality over time. This highlights the potential utility of a more wide-range and inclusive battery of tests when examining athlete functioning. The use of cognitive and functional components when assessing athletes may have the ability to identify more nuanced changes that are occurring compared to the use of one single clinical test.

When directly comparing the data from the 2015 and 2017 seasons, some interesting findings emerge. First, although nonsignificant, there were decreases in the raw number of impacts per player at both ≥80G and ≥25G to <80G in 2017. However, since the use of speed players in 2015 has the potential to confound this, another comparison was made just using the linemen (non-speed players) from each season. This decrease in impacts, although still nonsignificant, remained and has the potential to be clinically meaningful. The use of incidence rates to examine exposure to impacts, however, revealed significant decreases in impacts in 2017 at both the both ≥80G and ≥25G to <80G threshold. Incidence rates allow for consideration of both different sample sizes and different practice schedules between seasons and highlights the influence that medical, coaching, and rule changes can have on outcome. These indirect measures in this study could provide utility when considering what changes to implement for player safety.

Comparisons in the virtual reality data between seasons showed no differences at the preseason timepoint but differences at the postseason timepoints. Players from the 2017 had significantly better spatial memory scores, but significantly worse reaction time scores and balance scores compared with players from the 2015 season. These differences could be multifactorial ranging from player selection and sample size differences to indirect influences like practice schedules, strength and conditioning load, and coaching personnel changes. However, this suggests another interesting line of research to continue as balance and reaction time reflect more functional outcomes as opposed to purely cognitive outcomes and as mentioned, multifactorial testing with both components may prove more beneficial.

This study is not without limitations. The present studies were based on fairly small cohorts of football players so future work at continuing to replicate these findings as well as examining a greater variety of players (including females) would prove beneficial. Furthermore, only practice accelerometer data, and not game data, was used collected which could confound the results. Future studies should examine these sessions as well as there is potential for higher overall number of impacts, especially the impacts at higher intensities. Lastly, there is concern over the use of accelerometers in contact sports due to the accuracy of the systems used. Forces measured by the helmet systems may not accurately represent the forces experienced by the brain itself. The system used in this study likely has inconsistencies in forces measured and margins of error like many other systems used in research. Lastly, incorporating new technologies along with virtual reality could lead to the development of more beneficial assessment tools than currently used in clinical practice.

Conclusions and Clinical Implications:

Presently, the medical community does not have proven quantitative objective measures to diagnose concussions, nor any proven measures to predict an individual's susceptibility to concussive and subconcussive injury. The quantitative data regarding impacts obtained via helmet sensors throughout the football season allowed us to assign an objective value to individual events that had previously only been assessed subjectively. This information, combined with the results from the virtual reality testing, may allow coaches and policymakers to more confidently embrace the idea that minimizing the number and intensity of head impacts will benefit the participant and prevent decreased cognitive testing scores after participating in one football season.

No exact diagnostic testing for concussion exists as of yet and the accelerometer technology should not be used as a diagnostic measure. However, medical professionals may be able to use technologies such as virtual reality and helmet sensors to assist in making decisions about the health and well-being of patients. The importance of monitoring athletes throughout a season, not just before and after, is also something that should be considered to furthermore increase athlete safety. As comparisons between seasons made here indirectly reveal, these changes at both an individual school, as well as a governing body, have the potential to greatly influence outcomes for players. More work will be required in this area as coaches adapt new techniques and rules continue to change. Understanding the effects of these changes, as well as other factors that influence the likelihood a player receives repetitive impacts, will continue to be critical in providing optimal medical care for athletes.

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Chapter 5: Changes in White Matter Tracts of the Cervical Spinal Cord After a Single Season of Collegiate Football

Abstract

Importance: Examine the involvement of the central nervous system, outside of the brain, in sports-related concussion and exposure to repetitive head acceleration events.

Objective: To investigate the effects of a single season of collegiate football on the integrity of white matter tracts in the cervical spinal cord using diffusion tensor imaging.

Design: Prospective observational study.

Setting: Athletic training room and University laboratory.

Participants: Fifteen NCAA Division I Football players.

Exposure: Repetitive Head Acceleration Events.

Main Outcome and Measure: Diffusion tensor imaging preseason and postseason, and helmet accelerometer data throughout the season.

Results: From preseason to postseason, a significant decrease ($p<0.05$) of axial diffusivity was seen within the right spino-olivary tract. In addition, a significant decrease ($p<0.05$) in global white matter fractional anisotropy along with increases ($p<0.05$) in global white matter mean diffusivity and radial diffusivity were found. These changes from preseason to postseason fractional anisotropy were significantly moderated by previous concussion history ($p<0.05$) and number of head acceleration events over 80G ($p<0.05$).

Conclusions and Relevance: Despite the absence of sports-related concussion, we present measurable changes in the white matter integrity of the cervical spinal cord suggesting injury from repetitive head acceleration events, or sports-related concussion, may include the entirety of the central nervous system, not just the brain.

Introduction

Sports-related concussion (SRC) is a traumatic brain injury originating from the transmission of biomechanical forces to the head following exposure to impacts anywhere on the body.[1] The mechanism of injury in SRC shares many similarities with whiplash-associated disorders (WAD)[2] that leave the cervical spine vulnerable to injury.[3,4] Consequently, there is a large overlap in SRC and WAD symptomology. SRC signs and clinical symptoms include: headache, neck pain, impaired memory and concentration, dizziness, irritability, sleep disturbance, and fatigue which also can be attributed to a concurrent cervical injury.[5] For most SRC, clinical symptoms and neurocognitive deficits resolve in 10-14 days,[1] with a minority (10-30%)[6] of athletes reporting symptoms months to years post-injury.[7] This is in stark comparison to WAD where upwards of 50% of individuals report symptoms one year post-injury.[8] Furthermore, it is clinically accepted that there is comorbid cervical injury from SRC[3] and that any damage to the cervical spinal cord may have a direct influence on vestibular, visual and/or somatosensory functioning.[2] Despite the anatomical proximity and function of the spinal cord in the central nervous system (CNS), there is a paucity of research regarding the involvement of the cervical spinal cord in SRC,[9] while the interaction of the head and soft-tissue neck in SRC has been greatly debated and studied.[10]

Subconcussive impacts have the potential to transfer a high degree of linear and rotational acceleration forces, but do not elicit any SRC clinical symptoms.[11] However, controversy remains over the appropriate name and threshold of forces needed to be considered a subconcussive impact[12] therefore, in the following text, repetitive head acceleration events (HAEs) will be used. HAEs involve the transfer of mechanical energy at enough force to injure axonal or neuronal integrity[13] and therefore are also capable of producing cervical injuries.[3,14] In addition, contact sports have an inherent risk of exposure to repetitive impacts and it has been

shown that athletes involved in contact sports can be exposed to upwards of 1000 HAEs during the course of a season.[13,15]

Diffusion tensor imaging (DTI) has become a valuable and recommended technique to objectively quantify white matter microstructure by assessing changes in diffusivity (mean, axial, and radial) and fractional anisotropy (FA) from SRC[16,17] and exposure to HAEs. HAEs studies utilizing DTI have reported reductions in white matter integrity in the form of decreased FA and increased diffusivity.[18–21] Focusing on the brainstem, specifically the midbrain, Hirad and colleagues[22] demonstrated reductions in FA from exposure to HAEs during the course of a single collegiate football season. Furthermore, this reduction in white matter integrity was correlated to the amount of rotational acceleration forces from HAEs experienced by the players. However, to date, all of the HAEs literature has concentrated exclusively on the brain excluding the spinal cord, despite it being a vital component of the CNS for relaying sensory and motor information.[2]

The objective of this study was to examine the influence of repetitive HAEs on the white matter tracts of the cervical spinal cord (C1 – C4) over the course of a single collegiate football season using DTI. Helmet accelerometer data were also recorded to evaluate the relationship between HAEs and its effects on spinal cord integrity. To our knowledge, this is the first study to examine the role of the cervical spinal cord in relation to repetitive HAEs. We hypothesized that there would be subtle changes in DTI metrics in the form of decreased FA and axial diffusivity (AD) along with increased mean diffusivity (MD) and radial diffusivity (RD) when comparing preseason to postseason scans indicating injured white matter tracts.[23,24] Moreover, the extent of these changes would be influenced by the volume of HAEs sustained over the course of a season. Given the diffuse nature of SRC injury in the brain, we also speculated that both afferent and efferent white matter tracts would be affected from HAEs exposure and would be modulated by

demographic factors known to affect outcome (position played, number of years played, and previous concussion history).

Methods

Design, Setting, and Participants: This was a prospective, observational study conducted from July to December 2015. Power calculations were done *a priori* using G*Power, with effect size ($\delta = 0.8$), significance ($\alpha = 0.05$), and power ($\beta = 0.95$) recommending a total sample size of 23 participants. Twenty-three Pennsylvania State University Football Bowl Subdivision players were enrolled in this study for a single season. All participants provided written informed consent, as approved by the Pennsylvania State University Institutional Review Board. Three players were lost to follow-up so a total of 20 players completed both MRI scans – the first before any contact practice began for the season (preseason) and the second within one week of the last regular season game (postseason). MR quality issues (i.e. motion) allowed only 15 scans to be analyzed at the spinal cord level.

Head Acceleration Event Assessment: Impacts to the head were monitored using HeadHealth Network's BodiTrak system. The sensors are individually placed in each athlete's helmet and are comprised of elastic fiber with pressure monitors and impact sensors. Output includes linear acceleration (in units of G) and data was collected over the course of the season.

MRI acquisition: MRI scans were performed on a Siemens 3T Prisma MR scanner (Siemens, Erlangen, Germany) with a 20-channel head/neck coil. Identical imaging protocols were obtained at both preseason and postseason and included the following sequences (Figure 6): 2D sagittal T2-weighted fat saturated turbo spin echo, 3D axial T2*-weighted multi-echo gradient

echo, and 2D axial diffusion weighted imaging. Sagittal T2-weigthed images were acquired (TE=92ms, TR=3520ms, resolution=0.7x0.7x3.0mm, slices=15, fat saturation = SPAIR, TA= 1:58) to evaluate the cervical spine for any surrounding soft tissue or ligamentous injury. Axial multi-echo gradient echo images (TE=17ms, ms, TR=530ms, resolution=0.5x0.5x4.0mm, slices=20, magnetization transfer= yes, TA= 7:14) were acquired to provide superior gray white matter contrast for segmentation and registration. Diffusion tensor (DTI) metrics were computed from diffusion-weighted images acquired with a multi-segmented readout spin-echo echo-planar imaging sequence (TE=63ms, TR=3000, resolution= 1.0x1.0x4.0mm, slices= 20, b-values= 0 & 600 s/mm^2, directions= 20, TA= 10:41) to assess white matter integrity.

Images were reviewed to rule out disc herniation and soft-tissue/ligamentous injury. Image analysis was performed with Spinal Cord Toolbox[25] and consisted of: spinal cord segmentation, affine and nonlinear registration to the MNI-Poly-AMU template,[26] and warping of white matter atlas to template (Figure 7). White matter atlas consists of 30 white matter tracts (Table 16). DTI images were motion corrected,[27] co-registered, and fractional anisotropy (FA: a simplistic measure of structural integrity of white matter), mean diffusivity (MD: a simplistic measure of molecular diffusion rates or membrane density), axial diffusivity (AD: a simplistic measure of axonal degeneration), and radial diffusivity (RD: a simplistic measure of myelination) metrics were computed.[28]

Figure 6: Acquired scans, A) 2D sagittal T2-weighted fat saturated turbo spin echo, B) 3D axial T2*-weighted multi-echo gradient echo, and C) 2D axial diffusion tensor imaging.

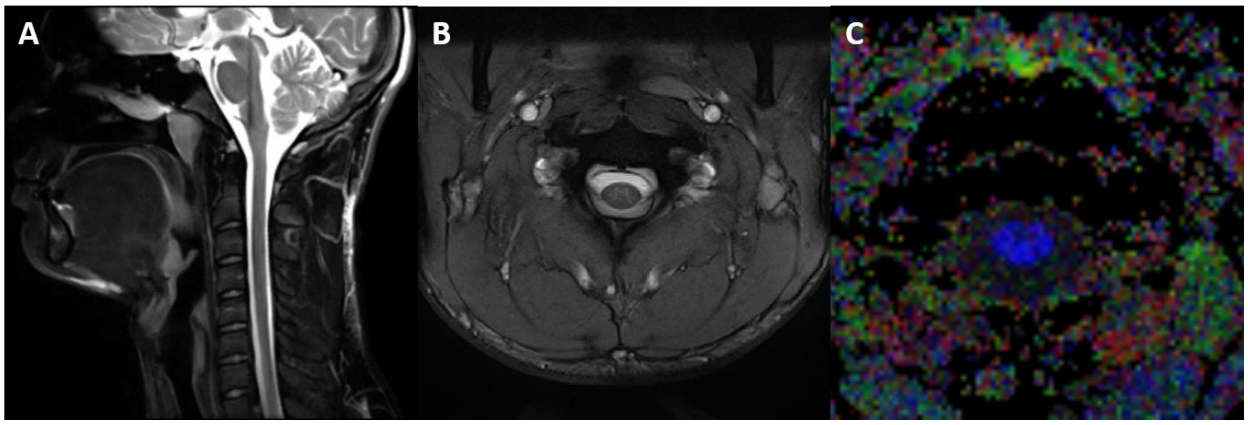


Figure 7: Example of post-processing A) white matter segmentation, B) registration, and C) warping of white matter atlas with 30 spinal tract regions of interest.

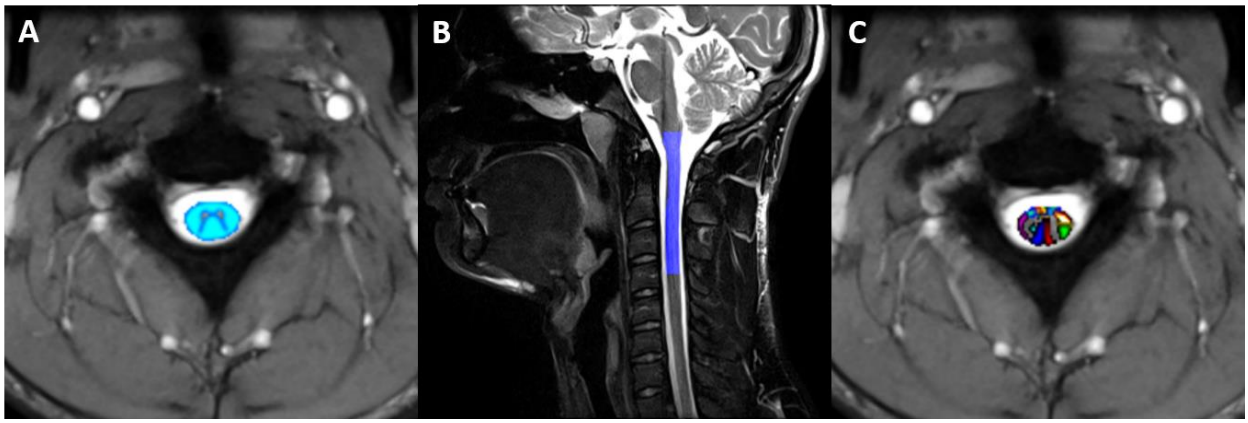


Table 16. Spinal cord white matter (WM) tracts

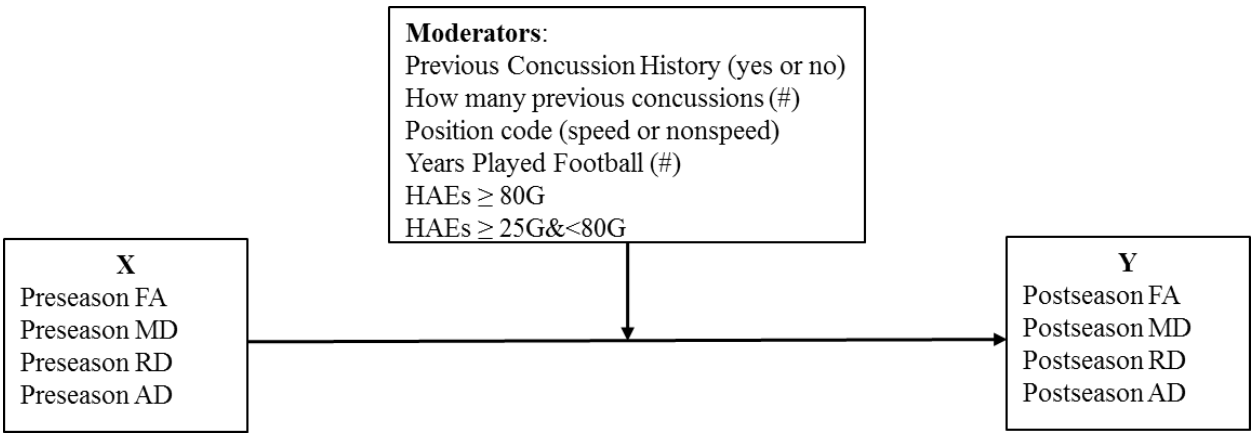
ROI	ROI Label Name	Efferent or Afferent
1	WM left fasciculus gracilis	Afferent
2	WM right fasciculus gracilis	Afferent
3	WM left fasciculus cuneatus	Afferent
4	WM right fasciculus cuneatus	Afferent
5	WM left lateral corticospinal tract	Efferent
6	WM right lateral corticospinal tract	Efferent
7	WM left ventral spinocerebellar tract	Afferent
8	WM right ventral spinocerebellar tract	Afferent
9	WM left rubrospinal tract	Efferent

10	WM right rubrospinal tract	Efferent
11	WM left lateral reticulospinal tract	Efferent
12	WM right lateral reticulospinal tract	Efferent
13	WM left spinal lemniscus (spinothalamic and spinoreticular tracts)	Afferent
14	WM right spinal lemniscus (spinothalamic and spinoreticular tracts)	Afferent
15	WM left spino-olivary tract	Afferent
16	WM right spino-olivary tract	Afferent
17	WM left ventrolateral reticulospinal tract	Efferent
18	WM right ventrolateral reticulospinal tract	Efferent
19	WM left lateral vestibulospinal tract	Efferent
20	WM right lateral vestibulospinal tract	Efferent
21	WM left ventral reticulospinal tract	Efferent
22	WM right ventral reticulospinal tract	Efferent
23	WM left ventral corticospinal tract	Efferent
24	WM right ventral corticospinal tract	Efferent
25	WM left tectospinal tract	Efferent
26	WM right tectospinal tract	Efferent
27	WM left medial reticulospinal tract	Efferent
28	WM right medial reticulospinal tract	Efferent
29	WM left medial longitudinal fasciculus	Both
30	WM right medial longitudinal fasciculus	Both

Statistical Analysis: All statistical analysis was performed using SPSS V25 (IBM Corp., Somers, NY). Average FA, AD, MD, and RD values were calculated for each of the 30 segmented ROIs for each individual at both preseason and postseason time points. Paired t-tests were run to evaluate within person changes from preseason to postseason for each ROI. Additional paired t-tests were run on all white matter ROIs (1-30) collectively, as well as, efferent and afferent pathway tracts. Independent t-tests were run to examine differences in two primary grouping variables: previous concussion history (yes or no) and position category (speed or nonspeed). These were also run from preseason to postseason using individual ROIs and the grouping of white matter ROIs (data presented in Supplemental File 1). Lastly, moderation regression analyses were run by calculating 95% CIs and using bootstrapping with 5,000 resamples via the

Process procedure in SPSS (Figure 8).[29] For moderation analyses, the interaction term of X by M was used to determine significance (p<0.05).

Figure 8. Moderation Model for White Matter ROIs Preseason to Postseason.



Results

Image quality measures or motion artifacts resulted in 8 participants being removed for a final pool of 15 participants used for analysis. These 15 participants were 20.4 ± 1.45 years old, all right handed, and played football for an average of 11.25 ± 3.92 years. Additionally, n=9 players had no history of previous concussion while n=6 had a previously medically diagnosed concussion (1, n=4; 2, n=2) and, based on the criteria by Lehman and colleagues,[30] n=9 were considered nonspeed (offensive and defensive lineman) and n=6 were considered speed (wide receivers, running backs, linebackers, cornerbacks, etc.). No athletes under study were diagnosed with a concussion during the season.

Head acceleration event assessment data was collected over the course of the season (Table 17). Impact events were collected during practices and assessed using thresholds of ≥25G&<80G and ≥80G to quantify impacts likely relevant to brain health.[31,32]

Table 17. Accelerometer data for all participants (n=15).

Participant	Position	Monitored Sessions (53 total)	Total ≥80G	Total ≥25G&<80G
1*	Speed	27	0	17
2	Nonspeed	42	3	95
3	Nonspeed	37	5	116
4	Nonspeed	38	7	180
5	Speed	45	5	101
6	Nonspeed	30	5	221
7	Speed	43	13	110
8	Speed	51	2	124
9	Nonspeed	37	4	155
10**	Nonspeed	2	2	9
11	Nonspeed	36	2	99
12	Speed	45	1	151
13	Nonspeed	48	7	301
14	Speed	37	13	205
15	Nonspeed	39	2	315

Players who missed part of the season due to a non-neurological injury are indicated by an asterisk (*). Players whose season ended early due to non-neurological injury are indicated by a double asterisk (**).

Overall individual ROI analysis showed no significant change in FA, AD, MD, and RD from preseason to postseason with only a significant decrease in AD of the right spino-olivary tract (p=0.026) (see Supplemental File 2 for each individual ROI paired t-test). However, characterization of white matter tracts (ROIs 1-30), as a whole, revealed significant changes from preseason to postseason (see Table 18) in the form of decreased FA (p=0.005) with increased MD (p=0.05) and RD (p=0.004). Moreover, significant decreases in FA (Table 18) were seen in both the composite subdivision of afferent neurons (p=0.012) and efferent neurons (p=0.041) with significant increase seen in RD for efferent neurons only (p=0.034).

Table 18. Paired t-test pre to post season for all white matter tracks and all afferent and efferent tracts

		Mean	Std. Dev.	t	p-value
White Matter	Pre FA	0.72961	0.07124	2.851	0.005*
	Post FA	0.71868	0.08393		
	Pre MD	0.00099	0.00020	-1.969	0.050*
	Post MD	0.00100	0.00023		
	Pre RD	0.00043	0.00023	-2.869	0.004*
	Post RD	0.00046	0.00025		
	Pre AD	0.00209	0.00024	0.373	0.709
	Post AD	0.00209	0.00028		
Afferent	Pre FA	0.74807	0.04725	2.545	0.012*
	Post FA	0.73471	0.06625		
	Pre MD	0.00095	0.00017	-1.048	0.296
	Post MD	0.00096	0.00021		
	Pre RD	0.00036	0.00020	-1.699	0.091
	Post RD	0.00039	0.00024		
	Pre AD	0.00212	0.00016	0.405	0.686
	Post AD	0.00211	0.00022		
Efferent	Pre FA	0.72257	0.07890	2.052	0.041*
	Post FA	0.71138	0.09212		
	Pre MD	0.00100	0.00023	-1.219	0.224
	Post MD	0.00102	0.00025		
	Pre RD	0.00046	0.00024	-2.133	0.034*
	Post RD	0.00049	0.00026		
	Pre AD	0.00209	0.00027	0.809	0.419
	Post AD	0.00208	0.00031		

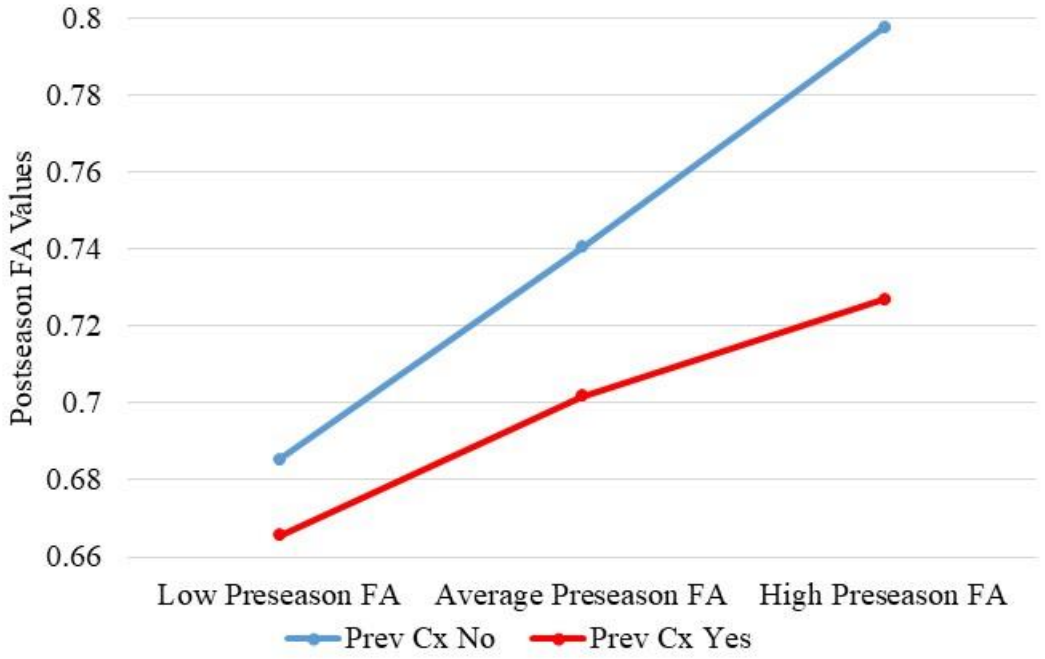
**significant ($p<0.05$)*
Pre= preseason; Post= postseason; FA= fractional anisotropy; MD= mean diffusivity; RD= radial diffusivity; AD= axial diffusivity

Previous concussion history (both yes versus no and number of previous concussions), years playing football, position code, and number of HAEs ≥80G and ≥25G&<80G were examined as independent moderators of the relation between preseason to postseason imaging white matter values.

Preseason white matter FA and previous concussion were entered into the regression analysis and the interaction term explained a significant increase in the variance of postseason FA values ($R^2 = 0.2683$, $F(3, 446) = 54.5045$, $p<0.001$). Thus, previous concussion history

(*b*=.1286, *t*(446)= 1.7239, p=0.08) was a significant moderator (*b*=-.2265, *t*(446)= -2.2393, *p*=0.0256) of the relationship between preseason FA (*b*=.2559, *t*(446)=5.9197, *p*<0.001) and postseason FA values. For both those players with (*b*=0.4293 t=5.2528, *p*<0.001) and without history of concussion (*b*=0.6558 t=11.0095, *p*<0.001), there is a positive relationship between history and postseason FA levels (Figure 9).

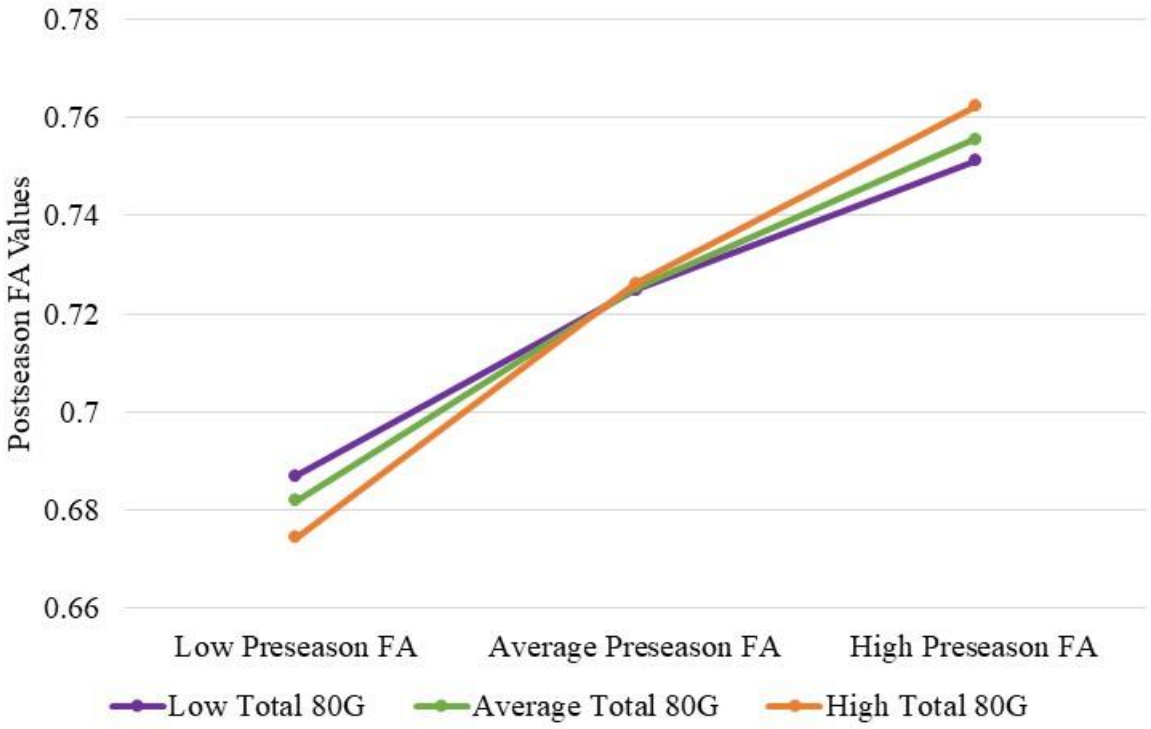
Figure 9. Moderation Results of Preseason and Postseason FA Values with Previous Concussion History



Preseason white matter FA and HAEs ≥80G throughout the season were entered into the regression analysis and the interaction term explained a significant increase in the variance of postseason FA values (R^2 = 0.2230, F(3, 446) = 42.6737, *p*<0.001). Thus, HAEs ≥80G (*b*=-.0241, *t*(446)= -2.4661, *p*=0.014) was a significant moderator (*b*=.0330, *t*(446)= 2.4287, *p*=0.0155) of the relationship between preseason FA (*b*=.3833, *t*(446)=7.3674, *p*<0.001) and

postseason FA values. For both those players with low (b=.4493 t=7.2470, p<0.001), average (b=.5154 t=10.0839, p<0.001), and high numbers of 80G HAEs (b=.6144 t=10.3325, p<0.001), there is a positive relationship between impacts and postseason FA levels (Figure 10).

Figure 10. Moderation Results of Preseason and Postseason FA Values with Total HAEs ≥80G



Although not reaching statistical significance (p>0.05), FA, AD, RD, and MD showed a moderate relationship for number of prior concussions (FA: p=0.065; AD: p=0.052; RD: p=0.051; MD: p=0.063) and FA and AD showed a moderate relationship with HAEs over 25G (FA: p=0.098; AD: p=0.047).

Discussion

In this study, we examined the effects of exposure to repetitive HAEs on spinal cord integrity over the course of a single season of NCAA Football. Specifically, athletes underwent preseason and postseason DTI scans to evaluate white matter integrity of the cervical spinal cord. There are several findings of interest that will be discussed. First, we documented a significant reduction in AD in the right spino-olivary tract sustained during the course of a competitive football season. Second, looking at the global white matter integrity of the cervical spinal cord, we observed a substantial decrease in FA and increase in MD and RD, which was not specifically confined to either afferent or efferent pathways. Third, these changes from preseason to postseason were moderated by previous concussion history and exposure to impacts. It is important to note that no participants under study received a clinically diagnosed SRC during the course of this study. Yet, despite not suffering SRC, we observe measurable changes in the white matter integrity of the cervical spinal cord implicating injury from repetitive HAEs may extend outside the brain to include the entirety of the CNS.

Previous research using DTI in SRC has revealed widespread white matter abnormalities in the brain as reported by changes in FA, MD, AD, and RD. However, these studies have yielded mixed findings highlighting potential confounds including timing of DTI acquisition.[17] Consistent with our observed reduction in FA and increased diffusivity measures, studies employing a comparable experimental setup, with DTI being performed at preseason and postseason intervals, have reported decreases in FA[33,34] along with increases in MD and RD indices.[21,33,35–38] Nonetheless, all of these studies have focused exclusively on the brain, omitting evaluation of spinal cord white matter. DTI has also been used to assess white matter changes in spinal cord injuries (SCI) and has also demonstrated significant decreases in FA and increased RD.[39–42] In addition, DTI of chronic SCI has also revealed significant reductions in FA and

increased diffusivity indices.[42,43] Following neck flexion and extension there is potential for injury to occur from disc herniation and ligamentous injury that may influence clinical symptoms, recovery, and molecular diffusion.[42] However, anatomical MRIs of these participants revealed no radiological findings for disc herniation or suggested concomitant soft-tissue injury.

Demyelination, remyelination, axonal loss, and atrophy are all associated with changes in molecular diffusion in chronic SCI which may also be a result of exposure to HAEs over the course of a season or career.[44–46] DTI analysis revealed a widespread distribution of affected spinal cord tracts including afferent and efferent pathways which suggests that the hallmark diffuse axonal injury (DAI) seen in concussive injuries[47] may not be localized to just the brain. Given the parallel between the mechanism of injury in both SRC and WAD, injury to the spinal cord is expected; however, we cannot rule out transneuronal degeneration of the spinal cord as an evolution of injury from repetitive HAEs.[48] Moreover, injury to the spinal cord from HAEs may reflect a similar relationship between subconcussive brain injury and SRC, but under the threshold to produce a spinal cord concussion.[49]

The potential for HAEs to infer injury to the spinal cord has major implications on the diagnosis, management, and monitoring of symptoms resolution in SRC. Missed diagnoses of concussive injuries in WAD and SCI is a concern and may occur in upwards of 74% of cases.[50] Consequently, it is likely that injury to the spinal cord is overlooked in SRC. Here we observed a significant change in the right spino-olivary tract, which can have anterograde and retrograde effects on the afferent transmission of important balance and proprioception information from tendons and muscles to the cerebellum.[51] Additionally, trending effects were seen in the right ventral corticospinal (decrease in FA, increase in RD) and right medial reticulospinal tracts (increase in RD). The ventral corticospinal tract, an efferent pathway, is crucial for control of

axial and proximal limb muscles involved in posture and balance while the medial reticulospinal tract, an efferent pathway, is crucial for regulation of extensor, or proximal, muscle function to maintain posture and balance.[52] In combination, the involvement of these three pathways suggests a potential role of balance and postural stability being one of the processes most negatively affected after exposure to HAEs. Given the recommended evaluation of neurocognitive functions, such as balance and reaction time,[1] spinal cord involvement may have a direct effect on outcomes, performance, and success rate on these tests[5] and this should be explored further. Moreover, injury to the spinal cord may shed light into post-concussive syndrome (PCS) and long-term disabilities[53] associated with SRC and symptoms resolution.

Interestingly, moderation analyses revealed that years playing football and position code, both known to increase exposure to HAEs and risk for long-term deficits,[54–57] did not moderate the changes on any DTI metric. However, concussion history and exposure to impacts ≥80G did significantly moderate these changes over the season, but only for FA values. These findings suggest that using exposure to high intensity impacts and previous concussion history, as well as FA values, could be potentially useful clinical factors to consider both in terms of an individual's risk and outcome after exposure. Furthermore, given that those individuals with a previous history of concussion had worse imaging outcomes at postseason, this could suggest an accumulative effect of damage that continues to be compounded with exposure to HAEs after diagnosis.

The etiology of PCS remains unclear with most researchers adopting the view it is a compilation of many factors that span the spectrum from biological to psychological.[58] PCS is one of the most controversial syndromes in sports medicine today with a constellation of symptoms[59] that may also mimic traumatic cervical injuries resulting in chronic pain, headache,

and neck pain.[60] This presents many challenges for the clinician and the need for a more systematic approach in the evaluation of PCS to better handle the possible contributing differential diagnoses, co-morbidities, and psychological factors is important.[60] The neural mechanisms, pathophysiology, and other contributing factors to the symptomology of concussion are poorly understood and could be caused by abnormalities anywhere in the CNS.[61]

Limitations

It should be noted that this study has limitations. It involved a small sample size of only male, football athletes from one collegiate team. This limits generalizability and future work should be done to replicate these findings, as well as, expand on this work at different ages and sexes. Additionally, it was done over a short time frame so more studies involving longitudinal data are warranted to better identify if the compromised cervical spinal cord seen here worsens over time with consistent exposure or the extent of recovery in the absence of HAEs.

Conclusions

In a study of collegiate football players, it was found that there are significant changes in white matter integrity of the spinal cord with exposure to repetitive HAEs. Despite not suffering a SRC, exposure to these repetitive impacts suggests damage to the cervical spinal cord; however, the extent and permanence of this damage is still unknown. Future work should be taken to replicate these findings and relate it to functional outcomes, especially its potential link to postural instability. These findings suggest that injury from repetitive HAEs or SRC may extend outside of the brain to include the entire CNS and clinical consideration of these other areas, especially the cervical spine cord, should be considered. The localization of the source of

injury in SRC is still unknown and this could confound and complicate symptom presentation, outcome, and recovery.

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Supplemental Material

Supplemental File 1:

Exploratory independent t-tests were run to examine differences in two primary grouping variables: previous concussion history (yes or no) and position category (speed or non-speed). For each individual ROI, preseason to postseason changes were evaluated and significant differences existed for those with and without a previous concussion history. Those players with no previous concussion history only had significant changes from preseason to postseason in FA13 (left spinal lemniscus; up; $p=0.032$), MD18 (right ventrolateral reticulospinal; down; $p=0.037$), RD23 (left ventral corticospinal; up; $p=0.018$), and AD16 (right spino-olivary; down; $p=0.041$). Those players with a history of previous concussive injury only had significant changes from preseason to postseason in RD5 (left lateral corticospinal; up; $p=0.033$) and RD24 (right ventral corticospinal; up; $p=0.002$). Globally, in all white matter tracts, there were no significant changes pre to post season for those individuals with no history of concussion ($p>0.05$). In those players with a history of concussion, there were global significant decreases in FA ($p<0.001$), increases in MD ($p<0.001$), and increases in RD ($p<0.001$).

Additionally, for each individual ROI, preseason to postseason changes were evaluated and significant differences existed for those playing non-speed versus speed positions. Non-speed players only had significant changes from preseason to postseason in AD12 (right lateral reticulospinal; up; $p=0.010$). Speed players however, had significant changes from preseason to postseason in FA16 (right spino-olivary; down; $p=0.011$), AD7 (left ventral spinocerebellar; down; $p=0.002$), and AD13 (left spinal lemniscus; down; $p=0.002$). Globally, in all white matter tracts, there were significant changes pre to post season for those speed players having a significant decrease in AD ($p<0.001$) and for non-speed players showing significant global decreases in FA ($p=0.010$), increases in MD ($p=0.002$), increases in RD ($p=0.007$), and decreases

in AD (p=0.011). Differences between groups at each time point for all white matter ROIs are presented for previous concussion history (SupTable1) and position (SupTable2).

SupTable1. Group Statistics for White Matter based on Previous History of Concussion (Yes or No)

			N	Mean	Std. Dev	t	Sig. (2-tailed)
White Matter	Pre FA	no	270	0.7219	0.0737	-2.836	0.005*
		yes	180	0.7412	0.0659		
	Pre MD	no	270	0.0010	0.0002	1.146	0.252
		yes	180	0.0010	0.0002		
	Pre RD	no	270	0.0004	0.0002	1.69	0.092
		yes	180	0.0004	0.0002		
	Pre AD	no	270	0.0021	0.0002	-0.257	0.797
		yes	180	0.0021	0.0002		
	Post FA	no	270	0.7293	0.0880	3.329	0.001*
		yes	180	0.7027	0.0748		
	Post MD	no	270	0.0010	0.0002	-1.685	0.093
		yes	180	0.0010	0.0003		
	Post RD	no	270	0.0004	0.0002	-2.207	0.028*
		yes	180	0.0005	0.0003		
	Post AD	no	270	0.0021	0.0003	-0.237	0.813
		yes	180	0.0021	0.0003		

significant (p<0.05)

SupTable2. Group Statistics for White Matter based on Position Code (Non-speed vs Speed)

			N	Mean	Std. Deviation	t	Sig. (2-tailed)
White Matter	Pre FA	non-speed	270	0.7259	0.0692	-1.364	0.173
		speed	180	0.7352	0.0740		
	Pre MD	non-speed	270	0.0010	0.0002	0.435	0.664
		speed	180	0.0010	0.0002		
	Pre RD	non-speed	270	0.0004	0.0002	0.956	0.340
		speed	180	0.0004	0.0002		
	Pre AD	non-speed	270	0.0021	0.0002	-0.702	0.483
		speed	180	0.0021	0.0002		
	Post FA	non-speed	270	0.7133	0.0807	-1.659	0.098
		speed	180	0.7267	0.0881		
	Post MD	non-speed	270	0.0010	0.0002	2.319	0.021*
		speed	180	0.0010	0.0002		
	Post RD	non-speed	270	0.0005	0.0003	1.481	0.139
		speed	180	0.0004	0.0003		
	Post AD	non-speed	270	0.0021	0.0003	3.186	0.002*
		speed	180	0.0020	0.0003		

significant (p<0.05)

Supplemental File 2:

SupTable 3A. Fractional Anisotropy (FA) values at preseason (FA.#) and postseason (PostFA.#) and paired t-test results for each individual ROI.

	Mean	Std. Deviation	t	Sig. (2-tailed)		Mean	Std. Deviation	t	Sig. (2-tailed)
FA.1	**0.7731**	**0.0436**	**1.411**	**0.180**	**FA.16**	**0.7329**	**0.0402**	**1.456**	**0.167**
PostFA.1	**0.7044**	**0.2017**			**PostFA.16**	**0.6596**	**0.2014**		
FA.2	**0.7670**	**0.0455**	**1.355**	**0.197**	**FA.17**	**0.7637**	**0.0617**	**1.008**	**0.331**
PostFA.2	**0.7023**	**0.2024**			**PostFA.17**	**0.7105**	**0.2056**		
FA.3	**0.7671**	**0.0379**	**1.284**	**0.220**	**FA.18**	**0.7622**	**0.0462**	**0.985**	**0.341**
PostFA.3	**0.7052**	**0.1988**			**PostFA.18**	**0.7093**	**0.2035**		
FA.4	**0.7662**	**0.0367**	**1.348**	**0.199**	**FA.19**	**0.6956**	**0.0859**	**0.968**	**0.349**
PostFA.4	**0.7022**	**0.1990**			**PostFA.19**	**0.6350**	**0.1889**		
FA.5	**0.7312**	**0.0359**	**1.191**	**0.254**	**FA.20**	**0.7033**	**0.0653**	**1.164**	**0.264**
PostFA.5	**0.6723**	**0.1944**			**PostFA.20**	**0.6473**	**0.1870**		
FA.6	**0.7213**	**0.0438**	**1.243**	**0.234**	**FA.21**	**0.6877**	**0.0927**	**1.328**	**0.205**
PostFA.6	**0.6666**	**0.1914**			**PostFA.21**	**0.6094**	**0.2176**		
FA.7	**0.7274**	**0.0649**	**1.019**	**0.326**	**FA.22**	**0.7394**	**0.0801**	**1.290**	**0.218**
PostFA.7	**0.6698**	**0.2003**			**PostFA.22**	**0.6561**	**0.2192**		
FA.8	**0.7558**	**0.0477**	**1.350**	**0.198**	**FA.23**	**0.6205**	**0.0388**	**0.934**	**0.366**
PostFA.8	**0.6819**	**0.2015**			**PostFA.23**	**0.5816**	**0.1783**		
FA.9	**0.7832**	**0.0607**	**1.232**	**0.238**	**FA.24**	**0.6467**	**0.0845**	**1.945**	**0.072**
PostFA.9	**0.7217**	**0.2055**			**PostFA.24**	**0.5603**	**0.1670**		
FA.10	**0.7878**	**0.0543**	**1.473**	**0.163**	**FA.25**	**0.6695**	**0.0622**	**0.811**	**0.431**
PostFA.10	**0.7149**	**0.2058**			**PostFA.25**	**0.6301**	**0.1939**		
FA.11	**0.7511**	**0.0402**	**0.929**	**0.369**	**FA.26**	**0.7258**	**0.0572**	**0.602**	**0.557**
PostFA.11	**0.7048**	**0.2044**			**PostFA.26**	**0.6921**	**0.2027**		
FA.12	**0.7748**	**0.0525**	**0.981**	**0.343**	**FA.27**	**0.7112**	**0.0820**	**1.092**	**0.293**
PostFA.12	**0.7279**	**0.2089**			**PostFA.27**	**0.6482**	**0.2069**		
FA.13	**0.7548**	**0.0227**	**1.120**	**0.281**	**FA.28**	**0.7393**	**0.0985**	**1.580**	**0.137**
PostFA.13	**0.6991**	**0.1966**			**PostFA.28**	**0.6583**	**0.2025**		
FA.14	**0.7604**	**0.0338**	**1.350**	**0.199**	**FA.29**	**0.6826**	**0.0793**	**0.561**	**0.583**
PostFA.14	**0.6979**	**0.1976**			**PostFA.29**	**0.6557**	**0.1982**		
FA.15	**0.6931**	**0.0460**	**1.218**	**0.243**	**FA.30**	**0.7075**	**0.0764**	**1.101**	**0.289**
PostFA.15	**0.6316**	**0.1879**			**PostFA.30**	**0.6593**	**0.1951**		

SupTable 3B. Mean Diffusivity (MD) values at preseason (MD.#) and postseason (PostMD.#) and paired t-test results for each individual ROI.

	Mean	Std. Deviation	t	Sig. (2-tailed)		Mean	Std. Deviation	t	Sig. (2-tailed)
MD.1	0.0010	0.0001	-0.875	0.396	MD.16	0.0009	0.0001	0.649	0.527
PostMD.1	0.0010	0.0001			PostMD.16	0.0009	0.0002		
MD.2	0.0010	0.0001	-1.259	0.229	MD.17	0.0009	0.0001	1.427	0.176
PostMD.2	0.0011	0.0001			PostMD.17	0.0009	0.0001		
MD.3	0.0010	0.0001	-0.959	0.354	MD.18	0.0009	0.0001	-0.337	0.741
PostMD.3	0.0011	0.0001			PostMD.18	0.0010	0.0001		
MD.4	0.0010	0.0001	-0.905	0.381	MD.19	0.0013	0.0001	0.335	0.742
PostMD.4	0.0010	0.0001			PostMD.19	0.0013	0.0002		
MD.5	0.0011	0.0001	-1.203	0.249	MD.20	0.0012	0.0001	0.077	0.939
PostMD.5	0.0011	0.0001			PostMD.20	0.0012	0.0002		
MD.6	0.0011	0.0000	-1.481	0.161	MD.21	0.0006	0.0003	-0.759	0.461
PostMD.6	0.0011	0.0001			PostMD.21	0.0007	0.0004		
MD.7	0.0007	0.0001	-0.183	0.857	MD.22	0.0005	0.0003	-0.006	0.995
PostMD.7	0.0007	0.0002			PostMD.22	0.0005	0.0003		
MD.8	0.0006	0.0002	-0.450	0.660	MD.23	0.0013	0.0001	-0.508	0.620
PostMD.8	0.0007	0.0002			PostMD.23	0.0013	0.0002		
MD.9	0.0010	0.0001	-0.407	0.690	MD.24	0.0011	0.0002	-1.257	0.229
PostMD.9	0.0010	0.0001			PostMD.24	0.0012	0.0002		
MD.10	0.0010	0.0001	-1.410	0.180	MD.25	0.0012	0.0001	-0.405	0.692
PostMD.10	0.0011	0.0001			PostMD.25	0.0012	0.0001		
MD.11	0.0009	0.0001	-0.364	0.721	MD.26	0.0011	0.0001	1.103	0.289
PostMD.11	0.0009	0.0001			PostMD.26	0.0010	0.0001		
MD.12	0.0009	0.0001	-0.043	0.966	MD.27	0.0010	0.0001	-0.293	0.774
PostMD.12	0.0009	0.0001			PostMD.27	0.0010	0.0001		
MD.13	0.0010	0.0001	-0.494	0.629	MD.28	0.0010	0.0001	-1.470	0.164
PostMD.13	0.0011	0.0001			PostMD.28	0.0010	0.0001		
MD.14	0.0010	0.0001	-1.422	0.177	MD.29	0.0010	0.0001	-1.151	0.269
PostMD.14	0.0011	0.0001			PostMD.29	0.0010	0.0001		
MD.15	0.0010	0.0001	0.511	0.617	MD.30	0.0010	0.0001	-1.443	0.171
PostMD.15	0.0010	0.0002			PostMD.30	0.0011	0.0001		

SupTable 3C. Radial Diffusivity (RD) values at preseason (RD.#) and postseason (PostRD.#) and paired t-test results for each individual ROI.

	Mean	Std. Deviation	t	Sig. (2-tailed)		Mean	Std. Deviation	t	Sig. (2-tailed)
RD.1	0.0004	0.0001	-1.368	0.193	RD.16	0.0004	0.0001	-0.188	0.853
PostRD.1	0.0005	0.0001			PostRD.16	0.0004	0.0002		
RD.2	0.0004	0.0001	-1.558	0.142	RD.17	0.0004	0.0001	0.509	0.619
PostRD.2	0.0005	0.0001			PostRD.17	0.0004	0.0001		
RD.3	0.0004	0.0001	-0.968	0.350	RD.18	0.0004	0.0001	-0.466	0.648
PostRD.3	0.0005	0.0001			PostRD.18	0.0005	0.0001		
RD.4	0.0004	0.0001	-1.063	0.306	RD.19	0.0007	0.0002	-0.397	0.697
PostRD.4	0.0005	0.0001			PostRD.19	0.0007	0.0002		
RD.5	0.0005	0.0001	-1.344	0.200	RD.20	0.0007	0.0001	-0.618	0.547
PostRD.5	0.0006	0.0001			PostRD.20	0.0007	0.0002		
RD.6	0.0005	0.0001	-1.190	0.254	RD.21	0.0001	0.0003	-0.996	0.336
PostRD.6	0.0006	0.0001			PostRD.21	0.0002	0.0005		
RD.7	0.0001	0.0002	-0.384	0.706	RD.22	-0.0001	0.0003	-0.474	0.643
PostRD.7	0.0001	0.0003			PostRD.22	0.0000	0.0003		
RD.8	0.0000	0.0002	-0.825	0.423	RD.23	0.0007	0.0001	-0.671	0.513
PostRD.8	0.0000	0.0002			PostRD.23	0.0008	0.0002		
RD.9	0.0004	0.0001	-0.894	0.386	RD.24	0.0006	0.0002	-2.065	0.058
PostRD.9	0.0004	0.0001			PostRD.24	0.0007	0.0002		
RD.10	0.0004	0.0001	-1.638	0.124	RD.25	0.0007	0.0001	0.020	0.984
PostRD.10	0.0005	0.0001			PostRD.25	0.0007	0.0002		
RD.11	0.0004	0.0001	-0.088	0.931	RD.26	0.0005	0.0001	0.657	0.522
PostRD.11	0.0004	0.0001			PostRD.26	0.0005	0.0002		
RD.12	0.0004	0.0000	0.190	0.852	RD.27	0.0005	0.0001	-0.683	0.506
PostRD.12	0.0004	0.0001			PostRD.27	0.0005	0.0002		
RD.13	0.0005	0.0000	-0.779	0.449	RD.28	0.0005	0.0001	-1.926	0.075
PostRD.13	0.0005	0.0001			PostRD.28	0.0005	0.0001		
RD.14	0.0005	0.0001	-1.656	0.120	RD.29	0.0005	0.0001	-0.283	0.781
PostRD.14	0.0005	0.0001			PostRD.29	0.0005	0.0001		
RD.15	0.0005	0.0001	0.123	0.904	RD.30	0.0005	0.0001	-1.144	0.272
PostRD.15	0.0005	0.0002			PostRD.30	0.0005	0.0001		

SupTable 3D. Axial Diffusivity (AD) values at preseason (AD.#) and postseason (PostAD.#) and paired t-test results for each individual ROI.

	Mean	Std. Deviation	t	Sig. (2-tailed)		Mean	Std. Deviation	t	Sig. (2-tailed)
AD.1	**0.0022**	**0.0001**	**-0.031**	**0.976**	**AD.16**	**0.0021**	**0.0002**	**2.496**	**0.026**
PostAD.1	**0.0022**	**0.0002**			**PostAD.16**	**0.0020**	**0.0002**		
AD.2	**0.0022**	**0.0001**	**-0.537**	**0.600**	**AD.17**	**0.0020**	**0.0002**	**0.749**	**0.466**
PostAD.2	**0.0022**	**0.0002**			**PostAD.17**	**0.0019**	**0.0002**		
AD.3	**0.0022**	**0.0001**	**-0.725**	**0.481**	**AD.18**	**0.0020**	**0.0002**	**-0.101**	**0.921**
PostAD.3	**0.0022**	**0.0002**			**PostAD.18**	**0.0020**	**0.0002**		
AD.4	**0.0022**	**0.0001**	**-0.462**	**0.651**	**AD.19**	**0.0024**	**0.0001**	**1.236**	**0.237**
PostAD.4	**0.0022**	**0.0002**			**PostAD.19**	**0.0023**	**0.0002**		
AD.5	**0.0021**	**0.0001**	**-0.730**	**0.477**	**AD.20**	**0.0023**	**0.0002**	**0.821**	**0.425**
PostAD.5	**0.0022**	**0.0002**			**PostAD.20**	**0.0023**	**0.0002**		
AD.6	**0.0022**	**0.0001**	**-1.506**	**0.154**	**AD.21**	**0.0017**	**0.0003**	**0.210**	**0.836**
PostAD.6	**0.0022**	**0.0002**			**PostAD.21**	**0.0017**	**0.0004**		
AD.7	**0.0019**	**0.0001**	**0.232**	**0.820**	**AD.22**	**0.0017**	**0.0003**	**0.641**	**0.532**
PostAD.7	**0.0019**	**0.0003**			**PostAD.22**	**0.0016**	**0.0003**		
AD.8	**0.0019**	**0.0002**	**0.293**	**0.774**	**AD.23**	**0.0023**	**0.0002**	**-0.111**	**0.913**
PostAD.8	**0.0019**	**0.0002**			**PostAD.23**	**0.0024**	**0.0002**		
AD.9	**0.0022**	**0.0001**	**0.384**	**0.707**	**AD.24**	**0.0022**	**0.0002**	**0.719**	**0.484**
PostAD.9	**0.0022**	**0.0001**			**PostAD.24**	**0.0022**	**0.0002**		
AD.10	**0.0022**	**0.0001**	**-0.658**	**0.521**	**AD.25**	**0.0022**	**0.0002**	**-0.742**	**0.471**
PostAD.10	**0.0022**	**0.0001**			**PostAD.25**	**0.0022**	**0.0002**		
AD.11	**0.0018**	**0.0001**	**-0.596**	**0.560**	**AD.26**	**0.0021**	**0.0002**	**1.072**	**0.302**
PostAD.11	**0.0018**	**0.0003**			**PostAD.26**	**0.0021**	**0.0002**		
AD.12	**0.0019**	**0.0002**	**-0.233**	**0.819**	**AD.27**	**0.0020**	**0.0002**	**0.479**	**0.639**
PostAD.12	**0.0019**	**0.0002**			**PostAD.27**	**0.0020**	**0.0002**		
AD.13	**0.0021**	**0.0001**	**-0.058**	**0.955**	**AD.28**	**0.0020**	**0.0002**	**-0.396**	**0.698**
PostAD.13	**0.0021**	**0.0002**			**PostAD.28**	**0.0020**	**0.0002**		
AD.14	**0.0021**	**0.0001**	**-0.928**	**0.369**	**AD.29**	**0.0020**	**0.0002**	**-1.531**	**0.148**
PostAD.14	**0.0022**	**0.0001**			**PostAD.29**	**0.0020**	**0.0003**		
AD.15	**0.0021**	**0.0001**	**0.864**	**0.402**	**AD.30**	**0.0020**	**0.0001**	**-1.623**	**0.127**
PostAD.15	**0.0021**	**0.0002**			**PostAD.30**	**0.0021**	**0.0002**		

Chapter 6: Overall Conclusions

This dissertation aimed to examine the concept of susceptibility versus resiliency to sports-related concussion and exposure to repetitive head acceleration events. While this idea is relatively novel in its concept, it has the potential to shed light on why certain individuals may develop long-term problems after participation in contact sports. While we have a great understanding of the numerous benefits of participation in sport on many types of development, the concern surrounding contact sport's relationship with brain health cannot be ignored. Given the link to neurodegenerative diseases, including CTE,[23] that have been discovered, a better understanding of the after-effects of exposure to SRC and the accumulation of HAEs is necessary.

Each chapter included in the dissertation highlights various factors that could contribute to the concept of resiliency versus susceptibility. Chapter 2 was a systematic review highlighting clinical/cognitive, imaging, and biomarker changes that can occur after a single season of participation in contact sports. The timeframe was chosen as it matches the study designs of most of the individual studies in this dissertation. Additionally, this systematic review focused on college-aged and younger athletes. During these ages, there is substantial development, especially in regards to the brain,[26–28] that needs to be considered when participating in contact sports. Overall, Chapter 2 highlights the inconsistencies in study design employed in the field of study of HAEs and how these inconsistencies could contribute to varying results. At this time, it is difficult to make concrete conclusions about a single season of participation in contact sports on the overall health of the athlete and his or her brain. Interestingly, the cognitive/clinical papers revealed little change over the course of the season, perhaps suggesting that more sensitive measures, or a combination of measures, are needed to adequately study the effects of

HAEs. On the other hand, biomarker and imaging studies tended to reveal more negative findings after the season, yet there were discrepancies among those studies. These imaging and biomarker studies demonstrate the need for reproducibility and the use of highly-specific measures in studies. This review highlights the need for more standardization in the field and more prospective chronological studies to better understand the true effects of exposure to repetitive HAEs.

Chapter 3 used genetics to predict an individual's previous history of concussion. The use of individuals' genetics for health purposes has been a growing area of research and it can be assumed that there is a link between concussion and genetics. Using a candidate gene study, with specific genes selected based on their relationship to brain injury or impulsive behavior, we identified one SNP, *KIAA0319*, that was a significant predictor of the number of previous concussions. Reduced expression of *KIAA0319* can negatively affect adhesion of neurons to the glial skeleton, thereby impairing neuronal migration during development and possibly after injury.[29] This suggests both a potential theoretical framework to consider, in addition to the role of genetics in susceptibility to concussion in contact sports.

Chapter 4 used various tools to examine cognitive and functional outcomes over the course of a single season of football. The use of accelerometers in contact sport has been growing both as a research tool and a clinical one. The data, as reported in this study, has the potential to identify individuals at risk for a greater accumulation of HAEs, as well as to highlight how individual differences, such as position or premorbid status, can influence functional outcome. Additionally, although an indirect and observational measure, the comparison of data between the two seasons highlights how individual teams' coaching and medical decisions and governing body rule changes have the potential to greatly influence

outcomes after a season. This could have great implications for clinical practice moving forward. Lastly, this chapter highlights the variability that can occur among seasons so, as Chapter 2 identified, study design and participant selection is crucial for reproducibility.

Chapter 5 identified significant changes in white matter integrity of the cervical spinal cord with exposure to repetitive HAEs. Despite no diagnosis of SRC, there was damage to the white matter of the spinal cord; however, the extent and permanence of that damage is still unknown. This study highlights the importance, both scientifically and clinically, of considering the entire central nervous system when studying SRC and HAEs. Localization of damage during injury is still unknown and could be contributing to or confounding symptom presentation, outcome, and recovery.

While the individual chapters employed different experimental techniques and modalities, in combination they reveal some core trends and findings that will be discussed. As identified in the Chapter 2 systematic review, changes in function over the course of a season matched general findings from the review. Chapter 4 with its clinical/cognitive focus matched trends of the systematic review reporting mixed findings in cognitive changes after the season. However, the imaging findings in Chapter 5 aligned with most papers identified in the systematic review regarding negative changes after a season of play. Additionally, individual chapters highlight other areas of injury to consider that may be commonly overlooked. Chapter 3 highlights the beneficial use of genetics as a growing field to consider when understanding injury susceptibility while Chapter 5 highlights the importance of considering the cervical spinal cord, or more broadly the entirety of the central nervous system, when studying these injuries.

Each study is not without its limitations which were discussed individually in detail in the above text. However, from a broader view, major limitations in this field are also identified in

this dissertation. Primarily these studies are done on college-aged male, football players and are typically smaller sample sizes. Additionally, as well as little diversity in sex, there is often little diversity in race. This limits generalizability of results to other sports teams as well as the general population. The time frame of study may also be considered a limitation. The studies were done over the course of a single sports season so the long-term effects, especially their potential link to future neurological problems, is unknown. Future work, therefore, should be undertaken to obtain longitudinal data on contact sport athletes. Furthermore, the use of an individual's premorbid status as a binary screening tool for participation is currently cautionary. Each of these factors, at this time, should not be used in isolation, but instead should be evaluated to shed light on the observed variability seen and to allow for more careful monitoring of athletes with specific factors.

Overall, this dissertation employed various techniques, ranging from clinically focused to more physiologically based approaches, to study the athletes involved in contact sports. The continued overlap between basic science and clinical science is needed for the field to move forward and this dissertation highlights possible paths. The concept of susceptibility versus resiliency will hopefully continue to develop and more work is needed to better understand these complex factors. When physiology of the brain after different levels of injury is better defined, we will have better understanding of the true definition of injury, its diagnosis, risk, and the threshold needed for injury. This, in turn, will lead to improved understanding of the resultant cognitive, imaging, and biomarker changes. With increased knowledge of the complex physiology of the brain after exposure to SRC or repetitive HAEs, both scientific and clinical questions will be easier to answer. In its entirety, this dissertation highlights the concept of susceptibility versus resiliency and offers ways to study it, both physiologically and clinically,

with the overall goal of better protecting the neurologic health of athletes involved in contact sports.

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EDUCATION

Penn State University, University Park, PA
Ph.D. in Kinesiology **2017 – current**
Advisor: Semyon Slobounov, Ph.D.

Penn State University, University Park, PA
M.S. in Kinesiology **2015 – 2017**
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Thesis: Neurobiological Effect of Selective Brain Cooling After Concussive Injury

Penn State University, University Park, PA
B.S. in Biology: Neuroscience **2011 – 2015**
Minor: Spanish
Honors Thesis: The Effects of Subconcussive Impacts on Virtual Reality Test Performance and Academics

TEACHING EXPERIENCE

Co-Instructor for Kinesiology 360: Neurobiology of Motor Control and Development **Fall 2019 – Spring 2020**

SELECTED PUBLICATIONS & PRESENTATIONS

1. **Walter**, A.E., and Slobounov, S.M. (2018). Psychological Aspects in Sport Concussions. *Handbook of Sport Psychology, 4th Ed*., ch58 [in press].
2. **Walter**, A., Herrold, A.A., Gallagher, V.T., Lee, R., Scaramuzzo, M., Bream, T., Seidenberg, P.H., Vandenbergh, D., O'Connor, K., Talavage, T.M., Nauman, E.A., Slobounov, S.M., Breiter, H.C. (2018). KIAA0319 Genotype Predicts the Number of Past Concussions in a Division I Football Team: A Pilot Study. *J Neurotrauma*, 36(&): 1115-24. doi: 10.1089/neu.2017.5622.
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4. **Walter**, A.E., Slobounov, S.M. (2018). Application of Virtual Reality in Assessment and Treatment of Concussion. In *Neuropsychology of Sports-Related Concussion*, ch12.
5. Slobounov, S.M., **Walter**, A, Breiter, H.C., Zhu, D.C., Bai, X., Bream, T., Seidenberg, P., Mao, X., Johnson, B., Talavage, T.M., (2017). The effect of repetitive subconcussive collisions on brain integrity in collegiate football players over a single football season: a multi-modal neuroimaging study. *Neuroimage Clin*, 14, 708-718. doi: 10.1016/j.nicl.2017.03.006.
6. **Walter**, A., Finelli, K., Bai, X., Johnson, B., Neuberger, T., Seidenberg, P., Bream, T., Hallett, M., Slobounov, S. (2017). Neurobiological Effect of Selective Brain Cooling After Concussive Injury. *Brain Imaging Behav*, 12(3), 891-900. doi: 10.1007/s11682-017-9755-2.
7. **Walter**, A., Finelli, K., Bai, X., Arnett, P., Bream, T., Seidenberg, P., Lynch, S., Johnson, B., Slobounov, S. (2017). Effect of Enzogenol® Supplementation on Cognitive, Executive, and Vestibular/Balance Functioning in Chronic Phase of Concussion. *Dev Neuropsychol*, 42(2), 93-103. doi: 10.1080/87565641.2016.1256404.
8. Invited Speaker: "*KIAA0319 Genotype Predicts the Number of Past Concussions in a Division I Football Team"* Genotyping and Gene Expression: Thermo Fisher Scientific Applications Supported by the PSU Genomics Core Facility, September 2018
9. Oral Presentation: *"Baseline Virtual Reality Scores in a Large Cohort of Division I Athletes: Differences Based on Gender, Sport, and Previous Concussion History"* The 13th World Congress on Brain Injury: Toronto, CA March 2019
10. Oral Presentation: *"The Accumulation of Subconcussive Impacts Across a Single Season of Division I Football"* The 12th World Congress on Brain Injury: New Orleans, LA March 2017