INVESTIGATING THE EFFECT OF SUBCONCUSSIVE IMPACT EXPOSURE ON WHITE MATTER INTEGRITY IN COLLEIGATE FOOTBALL PLAYERS

by

Blaire Magee

A thesis submitted to the Centre for Neuroscience Studies Graduate Program

In conformity with the requirements for

the degree of Master of Science

Queen's University

Kingston, Ontario, Canada

(July, 2021)

Abstract

Background: Recently, there has been growing concern around subconcussive impacts (SCI) in football and the potential implications on neurologic health. Of particular interest are athletes who undergo repeated exposure to SCI over a single football season. This form of neurotrauma is often overlooked due to lack of observable symptomatology, however previous work has shown evidence of microstructural damage to white matter (WM).

Methods: 22 Canadian Collegiate varsity football players were studied over a single competitive season using accelerometers and diffusion tensor imaging (DTI). Accelerometers were used to assess impacts received by each player throughout the season. Imaging was conducted at 3 timepoints (PRE-season; PRE, post-training camp; PTC, POST-season; POST) and probabilistic tractography was used to delineate WM tracts of interest. Changes in WM microstructure were assessed at each time point and between exposure groups (high exposure; HE, low exposure; LE, based on impacts over the season) using the diffusion metrics fractional anisotropy (FA) and mean diffusivity (MD).

Results: The HE group had significantly lower FA in the left and right corticospinal tracts (CST), left anterior thalamic radiation (ATR), and left superior thalamic radiation (STR), and higher MD in the left and right CST, left ATR, and left inferior fronto-occipital fasciculus (IFO) compared to the LE group. FA was found to decrease in the LE group from PRE and PTC to POST timepoints in the right posterior thalamic radiation (PTR) and right CST respectively. The HE group had decreased FA from PTC to POST in forceps minor (FMI). MD was found to be significantly decreased from PRE to PTC in the right superior longitudinal fasciculus (SLF) in the HE group.

Conclusions: Our findings indicate changes to WM microstructure occurs silently due to repetitive SCI exposure which may persist beyond a single football season.

Co-Authorship

Research was conducted under the guidance of the principal investigator Dr. D.J. Cook. MRI data was collected by fellow lab members Drs. Allen Champagne and Nicole Coverdale. Accelerometer data was collected with the help of Emile Peponoulas, Boris Baker, and Kristen Lacelle.

Acknowledgements

Firstly, thank you to my supervisor, Dr. D.J. Cook, for the mentorship, guidance, and support I have received throughout my research experience in your lab. Thank you for all the opportunities you have provided me with and ongoing encouragement to pursue my goals. Thank you to Dr. Nicole Coverdale, your input and guidance has been instrumental to the completion of this degree and my research career. Thank you for the countless edits and helping to make sure I was able to finish this degree on time. I would not have been able to do this without you. To Dr. Allen Champagne, thank you for introducing me to the field of concussion research and always bringing such enthusiasm and passion to everything you do. Thank you for taking the time to teach me and all your words of encouragement along the way. To my fellow colleagues, Kristen Lacelle and Kaden Shearer; thank you both for making the late nights at the football field and at the lab more enjoyable. Working with both of you has been a highlight of this degree. Thank you to Emile and Boris – my project wouldn't have been possible without the data you collected.

I would also like to thank my friends and family that have supported me throughout this degree. To my friend and roommate Alexandria DeCarlo, thank you for your constant support and unconditional friendship throughout and beyond both of our degrees. Thank you to my oldest friend Mae-Lin DeLange for your graphic design support and always being there for me. Thank you for always believing in me and helping me to growing to the woman I am today. Thank you for always being there no matter what and teaching me the importance of perseverance. Mum, I'm so lucky to have had you as an amazing role model to look up to. I will never be able to express what an impact that has made on my life and how thankful I am to have you as my mother. Dad, you've always been there for me no matter what the circumstance, always picked me up when I was down, and encouraged me to pursue my dreams. I'll never be able to thank either of you enough for everything you've done for me the past 24 years. Cameron and Jonathan, you two are the best little brothers I could have asked for. Thank you for always standing up for me, putting a smile on my face, and providing our family with constant entertainment.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

3D..Three Dimensional
AD..Axial diffusivity
ADHD...Attention Deficit Hyperactivity Disorder
ATP...Adenosine triphosphate
ATR..Anterior thalamic radiation
b0...b-zero
BBB...Blood brain barrier
BET...Brain extraction tool
BEDPOSTX................Bayesian estimation of diffusion parameters obtained using sampling techniques
BOLD...Blood-oxygenation level dependent
BSI...Brief Symptom Inventory
CBF..Cerebral blood flow
CBT..Cognitive Behavioral Therapy
CGC...Cingulate gyrus of cingulum
CGH...Parahippocampal part of cingulum
CNS..Central Nervous System
CSF..Cerebrospinal fluid
CST..Corticospinal tract
CTE..Chronic Traumatic Encephalopathy
DB...Defensive back
DL...Defensive line
DLPFC...Dorsolateral prefrontal cortex
DTI..Diffusion tensor imaging
DW..Diffusion weighted
DWI...Diffusion weighted imaging
DW-MR...Diffusion weighted magnetic resonance
EPI...Echo-planar imaging
FA..Fractional anisotropy
FLIRT...FMRIB's linear image registration tool
FNIRT..FMRIB's non-linear image registration tool
FMA...Forceps major

FMI....Forceps minor
fMRI....Functional magnetic resonance imaging
FMRIB....Functional MRI of the brain
FSL....FMRIB's software library
FOV....Field of view
GCS....Glasgow coma scale
GFT....gForce Tracker
GM....Grey matter
HIII....Hybrid III
HE....High exposure
HIC_{15}....Head Impact Criteria
HIT....Head Impact Telemetry
IFO....Inferior fronto-occipital fasciculus
ILF....Inferior longitudinal fasciculus
ImPACT....Immediate Post-Concussion Assessment and Cognitive Testing
K....Kicker
LB....Linebacker
LE....Low exposure
LOC....Loss of consciousness
MD....Mean diffusivity
MDD....Major Depressive Disorder
MNI....Montreal Neurological Institute
MP-RAGE....Magnetization prepared – RApid gradient echo
MR....Magnetic resonance
MRI....Magnetic resonance imaging
mTBI....Mild traumatic brain injury
MVA....Motor vehicle accident
NFL....National Football League
NFT....Neurofibrillary Tangle
NMDA....N-methyl D-aspartate
OL....Offensive line
PCS....Post-concussion Syndrome
PGSE....Pulsed gradient spin echo

PTA..Post-traumatic Amnesia
PTR..Posterior thalamic radiation
PTSD..Post-traumatic Stress Disorder
RB..Running back
RD..Radial diffusivity
RF..Radiofrequency
ROI..Region of interest
RPQ..Rivermead Post-Concussion Symptom Questionnaire
SCAT..Sport Concussion Assessment Tool
SCI..Subconcussive Impact
SIS..Second Impact Syndrome
SLF..Superior longitudinal fasciculus
SNR..Signal-to-noise ratio
SRC..Sport Related Concussion
STR..Superior thalamic radiation
TBI..Traumatic Brain Injury
TC..Training camp
TE..Echo time
TI..Inversion time
TR..Repetition time
UNC..Uncinate fasciculus
WM..White matter
WR..Wide receiver

Chapter 1

Introduction

1.1 Types of Neurotrauma

1.1.1 Concussion

Concussion is a form of mild traumatic brain injury (mTBI). Both are subsets of traumatic brain injury (TBI) and these terms are typically used interchangeably (Steenerson & Starling, 2017). There is currently no universally accepted definition of concussion but it can be considered a “complex pathophysiological process affecting the brain, induced by traumatic biomechanical forces” (McCrory et al., 2017). When concussion occurs as a direct result of participation in sport, the injury is termed sport-related concussion (SRC). SRC is a common injury among contact sport athletes, with an estimated 1.6-3.8 million cases per year in the United States (Langlois, Rutland-Brown, & Wald, 2006). This number likely an underestimation as many athletes do not seek medical attention after head injury, which may elevate their risk for subsequent injury and prolonged recovery (Asken et al., 2016; Elbin et al., 2016).

Generally, concussion results from exposure of the brain to acceleration-deceleration forces. The cerebrospinal fluid (CSF), one of the brain’s natural protective mechanisms, does not protect against these types of blunt injuries (Blennow, Hardy, & Zetterberg, 2012). Application of these forces can cause the brain to ricochet off the skull in a mechanism called “contre coup” (Martin, 2016). There are two main types of head impacts; straight impacts that generate linear acceleration of the head and impacts to the side of the head or face that result in rotational acceleration of the head. The brain tends to tolerate impacts resulting in linear acceleration better than those causing rotational acceleration (Cantu, 1996). This is thought to be attributed to increased axonal shearing and damage leading to a cascade of harmful neurochemical events that are associated with rotational impacts (Blennow et al., 2012).

Concussion can arise from a variety of biomechanical mechanisms and as such, manifests with a wide range of symptoms. Commonly reported concussion symptoms include, but are not limited to; headache, sensitivity to light or noise, dizziness, nausea, changes in mood, post-traumatic amnesia, and loss of consciousness (McCrory et al., 2017). It is important to note that post-traumatic amnesia, and loss of consciousness (LOC) do not need to be present to satisfy the diagnostic criteria of concussion as previously believed (McCrory et al., 2017). Recovery from concussion or SRC is highly variable, and patients report differing rates of symptom resolution and return to normal routine. As well, symptom resolution may not be an accurate indicator of recovery as abnormalities in brain metabolism, cerebral blood flow (CBF) and microstructural integrity may persist beyond symptomatic injury (Koerte et al., 2015). This suggests that the complex neuropathophysiology of concussion is not well understood and requires further investigation.

1.1.2 Subconcussive Impacts

A subconcussive impact (SCI) can be described as a head impact that does not produce symptoms or meet the clinical definition of concussion (Bailes, Petraglia, Omalu, Nauman, & Talavage, 2013; Mainwaring, Ferdinand Pennock, Mylabathula, & Alavie, 2018). Although there is a lack of observable symptomatology typically present in SRC, repetitive SCI exposure is suspected to produce pathophysiological sequalae similar to that of concussion and frequently encountered in contact sports (Bailes et al., 2013). These changes likely arise due to the same acceleration-deceleration forces that occur with concussion, resulting in axonal shearing and stretching, and subsequent disruption of neuronal homeostasis (MacFarlane & Glenn, 2015). Despite an increasing number of studies, the long-term consequences, frequency and magnitude of subconcussive impacts remain poorly understood.

Currently, the neuropathophysiological changes that occur with SCI are not well established. Neuroimaging studies have found widespread, heterogenous changes associated with repetitive SCI exposure, such as changes in brain metabolism, cerebral blood flow, cortical thinning, and structural and functional integrity (Abbas et al., 2015; Bailes et al., 2013; Bazarian et al., 2014; Bernick et al., 2015; Champagne, Peponoulas, et al., 2019; McAllister et al., 2014; Montenigro et al., 2017; Slobounov et al., 2017; Svaldi et al., 2020; Talavage et al., 2014). In one such study, Talavage and colleagues (2014) conducted a longitudinal assessment of a group of football players using functional magnetic resonance imaging (fMRI) and found significantly decreased fMRI activation in the dorsolateral prefrontal cortex (DLPFC) and cerebellum over a season of football in the absence of clinically diagnosed concussion. These findings were associated with poorer performance on tests of verbal and visual working memory (Talavage et al., 2014). The authors concluded that neurocognitive impairment could occur in the absence of overt concussion symptomatology and may persist over time (Talavage et al., 2014).

These findings were substantiated by Slobounov and colleagues (2017), who conducted a multi-modal imaging study investigating the effect of repetitive SCI in collegiate football players over a single season. The authors reported abnormalities in functional connectivity within the left and right cingulate cortices and the left hippocampus, and globally increased CBF at the post season timepoint (Slobounov et al., 2017). There is conflicting evidence regarding whether abnormalities such as these persist or resolve with time, and as such, further investigation is required to better understand the long-term implications of this type of neurotrauma (Mainwaring et al., 2018).

Further, it remains poorly established whether the magnitude or frequency of subconcussive impacts is more harmful. Slobounov and colleagues (2017) found greater neuroimaging abnormalities in football athletes with a history of larger magnitude impact exposure, while Montenigro and colleagues (2017) found a dose-response relationship between cumulative head impacts and risk for cognitive impairment later in life (Montenigro et al., 2017; Slobounov et al., 2017). Additionally, there has been some evidence that suggests the period of time between neurological insults may play a role in the cumulative effect (McAllister et al., 2014). When taken together these findings suggest that the neuropathophysiology of SCI is multi-faceted and long-term outcomes are likely dependent on many different critical factors.

The high rates of SCI in contact sports, particularly American football (Crisco et al., 2011), have given rise to escalating concerns in the past years, surrounding the implications of long-term exposure to repetitive head impacts, concussive or otherwise. SCI is a much more common occurrence than concussion, as demonstrated by previous studies using helmet-based accelerometers to investigate the frequency of head impacts over the course of a single season of football. For example, high school athletes sustain an average of 600 impacts and collegiate athletes up to 1400 over the course of a football season (Bailes et al., 2013; Broglio et al., 2009; Stern et al., 2011). Moreover, the number and magnitude of impacts an athlete sustains is expected to vary significantly by position played (Crisco et al., 2011; Crisco et al., 2012; Slobounov et al., 2017).

1.1.3 Long-Term Effects

1.1.3.1 Chronic Traumatic Encephalopathy

Chronic Traumatic Encephalopathy (CTE) is a progressive neurodegenerative disease only diagnosed post-mortem, first described in boxing athletes as “Punch-drunk syndrome”(Martland, 1928) or *dementia pugilistica* (Millspaugh, 1937). Similar to concussion, CTE remains poorly characterized without a globally accepted definition. CTE is thought to arise from repetitive head trauma, either in the form of concussive or subconcussive impacts, manifesting as a variety of impairments (McKee, Alosco, & Huber, 2016; McKee, Cairns, et al., 2016; McKee et al., 2013; Solomon & Zuckerman, 2015). CTE tends to present as an inconsistent combination of mood disorders, neuropsychiatric, behavioral and cognitive impairments of variable and progressive severity. Additionally, CTE presents clinically after a prolonged period of latency of relatively unpredictable duration (McKee, Alosco, et al., 2016; McKee, Cairns, et al., 2016; McKee et al., 2013; Omalu et al., 2011). The lack of consensus on cause and symptomology reflects the poor understanding of the specific pathology, etiology, presentation, incidence and prevalence of CTE.

Despite years of research, the pathology of CTE remains poorly characterized. Post-mortem neuropathological analysis has yielded one finding which is currently the only diagnostic standard: tauopathy, distinct from other neurodegenerative diseases (McKee, Cairns, et al., 2016). Histopathological findings must include the presence of perivascular p-tau aggregations in neurons, astrocytes and cell processes in an irregular pattern within the depths of the cortical sulci (McKee, Cairns, et al., 2016), but often includes additional findings of microglial activation, parenchymal histiocytes and amyloidopathy (Omalu et al., 2011). The cause of this unique tauopathy in CTE is unknown, however two possible theories are (i) a reduction of phosphate activity leading to increased production of aberrantly phosphorylated proteins or (ii) axonal injury inducing dissociation of tau from microtubules, resulting in the abnormal phosphorylation of tau.

CTE has predominantly been reported in professional and amateur athletes as young as 17 (McKee et al., 2013). The majority of cases reported have been linked to multiple mTBI, TBI or repetitive exposure to SCI, such as those frequently observed in collision sports. Specifically, the acceleration and deceleration forces applied to the brain are thought to produce axonal damage resulting in concussive symptomatology or, with respect to SCI, damage that accumulates asymptomatically over time. Forces causing rotational acceleration of the head are thought to be particularly harmful as they produce shearing injuries to axons (Choe, 2016; McKee et al., 2009; Steenerson & Starling, 2017). Furthermore, the irregular, patchy deposition of CTE tauopathy suggests that distribution may be related to the variable mechanism of injury (i.e., blows/impacts to the side or top of the head) observed in contact sports such as football and boxing (McKee et al., 2009). There has been increasing evidence supporting the possibility of long-term exposure to SCI playing a direct role in the development of CTE (McKee et al., 2013). This adds to concerns surrounding prolonged SCI exposure in football players, due to limited understanding of characteristics, neuropathophysiological changes, and associated risks of this form of neurotrauma.

1.2 The Neurobiology and Neuropathology of Concussion

Head impacts can initiate a complex pathophysiological process termed "The Neurochemical Cascade of Concussion" (Giza & Hovda, 2001, 2014). This process characterizes the pathophysiological changes that begin at the time of impact and are thought to continue throughout the course of recovery (Blennow et al., 2012; MacFarlane & Glenn, 2015). While this process has been largely studied in concussion and TBI, the acceleration deceleration forces that result in shearing and tearing of axons inducing the pathophysiological changes in the brain can still occur following SCI, likely producing more subtle damage that accumulates over time (Choe, 2016; McKee & Daneshvar, 2015; McKee, Daneshvar, Alvarez, & Stein, 2014).

The initial head trauma causes disruption of neuronal homeostasis, typically due to the shearing and stretching of axons from the biomechanical forces of the impact. This disrupts the neuronal membranes through a process called mechanoporation resulting in aberrant efflux of intracellular K^{+}, consequently triggering non-discriminatory release of excitatory neurotransmitters. In particular, glutamate causes neuronal excitation, further K^{+} efflux, forming a feed-back loop and influx of extracellular Ca^{2+}, which will be sequestered in the mitochondria (Giza & Hovda, 2014; MacFarlane & Glenn, 2015). Additionally, excess glutamate can bind to N-methyl D-aspartate (NMDA) receptors causing additional cortical depolarization, intracellular Ca^{2+} accumulation, and hyperexcitability (Blennow et al., 2012; MacFarlane & Glenn, 2015; Steenerson & Starling, 2017).

After the events described above, the brain is in a hyperexcitable state causing both the ATP-dependent Na^{+}-K^{+} pumps and glial cells capable of up taking extra K^{+} go into overdrive in an attempt to restore homeostasis. This results in a massive consumption of and demand for ATP. Furthermore, the brain enters a hypometabolic state due to decreased CBF, resulting in a decreased supply of glucose. This, in combination with the increased energy demand, can deplete ATP stores resulting in an energy crisis. In an attempt to mitigate the energy crisis and compensate for impaired cerebral oxidative metabolism, glycolysis is often upregulated to satisfy ATP demands. However, this increases the production of lactate, which accumulates as it is unable to be properly metabolized through the Krebs cycle (MacFarlane & Glenn, 2015). Accumulation of lactate has paradoxical effects that are not fully understood. Increased lactate can be very harmful to the brain resulting in acidosis which can subsequently cause cell membrane damage, increased permeability, breakdown of the blood brain barrier (BBB) and cerebral edema (MacFarlane & Glenn, 2015). Conversely, there have been some studies suggesting that increased lactate could be used as an additional energy source by the brain, helping to restore homeostasis (Glenn et al., 2003). While these contradictory effects require further investigation to fully understand their roles in the pathophysiology of concussion, the effect of lactate is suspected to be linked to the degree of both injury and impairment of oxidative metabolism (Glenn et al., 2003; MacFarlane & Glenn, 2015).

As previously described, the increased Ca^{2+} sequestered in the mitochondria can impair oxidative metabolism exacerbating the energy crisis, resulting in widespread neuronal depression (Blennow et al., 2012; Giza & Hovda, 2014; MacFarlane & Glenn, 2015; Steenerson & Starling, 2017). Despite the harmful neurochemical cascade that occurs following concussion and throughout recovery, it typically does not, if ever, result in cell death. However, these neurological sequalae can have long-term detrimental effects on neuronal function and the specific mechanism involved requires further investigation (MacFarlane & Glenn, 2015; Steenerson & Starling, 2017).

1.3 Accelerometers

Due to growing concerns surrounding SCI there has been increasing demand to track and quantify these types of head impacts. This has resulted in the development and application of accelerometers to characterize and quantify head impacts in contact sports. There have been several attempts to quantify head impacts in the past, however, more recent development of minimally intrusive accelerometers, such as the Head Impact Telemetry (HIT) and gForce Tracker

(GFT) systems, have made this goal much more feasible as previous models have been large and bulky and impaired movement (Moon, Beedle, & Kovacic, 1971; Reid, Tarkington, Epstein, & O'Dea, 1971).

Development of these types of sensors has allowed for quantification of several different metrics of head impacts such as impact frequency, linear acceleration, rotational acceleration and impact location to be better characterized in contact sports (Campbell et al., 2016). These devices can be affixed inside a player's helmet, or alternatively can be attached directly to the head with adhesive, or worn using a cap, band or mouthguard for non-helmeted athletes (Cortes et al., 2017). Sensors can be activated remotely and record and wirelessly transmit impact data to a computer in real-time.

The gForce Tracker system (Artaflex Inc., Markham, Ontario, Canada) measures 6 degrees of freedom kinematics of head impacts to compute linear acceleration, rotational velocity – which can be differentiated to obtain rotational acceleration – and measures of impact severity such as Head Impact Criteria (HIC_{15}) (Campbell et al., 2016). The GFT sensors are composed of a triaxial accelerometer and gyroscope which quantify these measures. The accuracy of the GFT system was validated in a study comparing GFT outputs to the Hybrid III (HIII) headform test device, which is regarded as the gold-standard and often used as reference values for studies that use accelerometers (Campbell et al., 2016). The authors determined that corrected GFT data produced accurate values for linear acceleration, rotational velocity and acceleration, and HIC_{15} when compared to reference values from the HIII headform device. Furthermore, the GFT values were comparable to other similar devices used in head impact studies (Campbell et al., 2016). The authors did note that GFT outputs may vary based on location of the sensor within the helmet and the type of helmet used (Campbell et al., 2016).

Despite advancements in the understanding of head impact kinematics, the point or threshold at which asymptomatic head impacts become harmful and elevate an athlete's risk of long-term neurocognitive deficits remains elusive. Furthermore, it remains poorly established which elements of head impact kinematics are the most predictive of subsequent injury risk or long-term impairment. Previous studies using accelerometers to examine SCI in football players have demonstrated that characteristics of subconcussive head impacts, such as frequency and linear acceleration, can vary significantly based on factors like position played (Crisco et al., 2011; Crisco et al., 2012), suggesting an athlete's individualized risk may be heavily dependent on their role on the field.

A more recent study attempted to characterize the threshold dose-response relationship between SCI and long-term neurocognitive impairment in former high-school and collegiate football players (Montenigro et al., 2017). Based on previous accelerometer studies, the authors suggested risk for long-term impairment is relatively stable (referred to as "baseline risk") until a certain threshold of cumulative head impacts is reached, at which point the risk of impairment increases with every 1000 impacts. The baseline was found to be dependent on the specific outcome measures in question (e.g., behavior dysregulation, cognitive impairment, apathy) but fell between 2216-7251 lifetime subconcussive impacts for all measures of impairment (Montenigro et al., 2017). Although the authors note limitations to their findings like small sample size, as high-school and collegiate football players sustain on average anywhere between 400 and 1400 subconcussive impacts per season (Bailes et al., 2013; Broglio et al., 2009; Stern et al., 2011), these findings highlight the potential detrimental effect of cumulative SCI exposure on risk for long-term and impairment.

1.4 Assessing White Matter in the Brain

1.4.1 Diffusion Weighted Imaging

In diffusion weighted imaging (DWI), the magnetic resonance (MR) signal is sensitized to the diffusion of water. Diffusion can be either isotropic (unrestricted) or anisotropic (restricted)(Hecke, Emsell, & Sunaert, 2016). Isotropic diffusion occurs in areas such as the CSF and GM, while anisotropic diffusion occurs in structures with a preferential direction of diffusion such as that observed in white matter (WM). The properties of and difference between isotropic and anisotropic diffusion can be used to infer the microstructural integrity of the white matter within the brain and is the basis for the contrast in DWI (Hecke et al., 2016). Diffusion-encoding gradients are applied such that the subtle displacement of water molecules over time can be captured. The signal produced from the gradients is attenuated as a function of the displacement of the water molecules in a given voxel and the amount of diffusion weighting applied (Hecke et al., 2016). The amount of diffusion weighting used for an imaging sequence is determined by the *b*-value parameter. A higher *b*-value will result in greater signal attenuation and increased image contrast compared to a lower *b*-value (Hecke et al., 2016; Tournier, Mori, & Leemans, 2011). A *b*-value of zero will result in no diffusion weighting and as such DWI acquisitions typically require one or more *b*-zero (b0) scans for normalization (Hecke et al., 2016). Diffusion occurs in multiple directions, however diffusion-encoding gradients only capture the diffusion along the direction which they are applied. Maximum signal is achieved when the direction of diffusion is parallel to the direction of the gradient and no signal is detected when the diffusion is perpendicular to the gradient. Therefore, multiple acquisitions are required to capture diffusion over a 3D space (Hecke et al., 2016).

1.4.2 Diffusion Tensor Imaging

Diffusion tensor imaging (DTI) can be used to investigate WM properties including but not limited to, microstructural integrity, tract volume and structural connectivity of tracts within the brain (Alexander, Lee, Lazar, & Field, 2007; Soares, Marques, Alves, & Sousa, 2013; Tournier et al., 2011). DTI makes use of the contrast provided by the diffusion weighted images and fits a tensor at each brain voxel to model the diffusion of water molecules. The tensor is a 3x3 symmetric matrix that allows for modelling of the ellipsoidal diffusion process (Basser, Mattiello, & LeBihan, 1994). According to the Eigen Decomposition Theorem, the tensor matrix can be represented by eigenvalues (λ) and eigenvectors (ε). At each voxel the eigenvalues are used to represent the size and the shape of the tensor, and the eigenvectors describe the orientation of the tensor. Anisotropic diffusion is described by an ellipsoid tensor and as such, regions like WM are represented well by DTI (Hecke et al., 2016; Soares et al., 2013). However, in regions where diffusion is approximately isotropic, such as the CSF and GM, the principal eigenvector becomes poorly defined due to increasingly random orientations. This results in a noisy appearance of the resulting DTI image and renders the eigenvectors and eigenvalues essentially meaningless for such areas (Hecke et al., 2016; Soares et al., 2013).

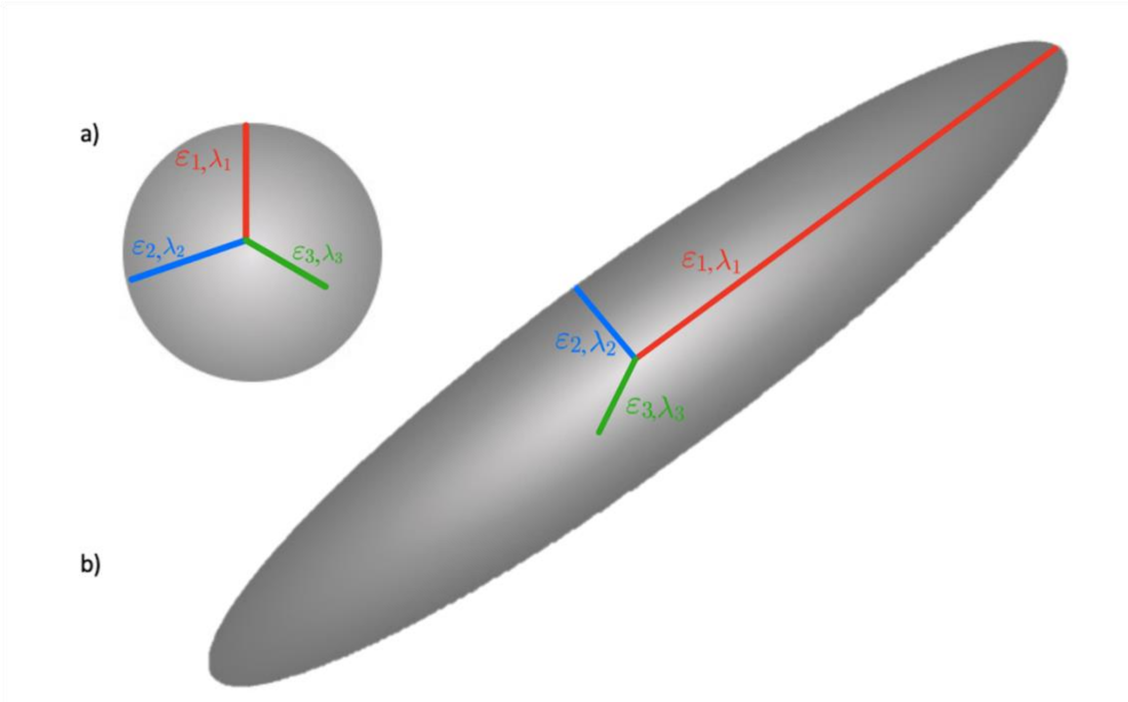

Figure 1. Isotropic and anisotropic tensor models. Depiction of **A)** isotropic and **B)** anisotropic tensor models and their corresponding eigenvalues (λ_1, λ_2, λ_3) and eigenvectors (ε_1, ε_2, ε_3).

From the diffusion tensor matrix, scalar diffusion metrics can be calculated for each voxel and provide information about the size and shape of the tensor, independent of its orientation (Hecke et al., 2016). Mean diffusivity (MD) is the average magnitude of molecular displacement from diffusion or the overall size of the tensor, independent of its shape, and is calculated as an average of the eigenvalues (Eq. 1.). Fractional anisotropy (FA) is a normalized measure of how much the eigenvalues differ from one another and describes the shape of the tensor in terms of its relative length to its width. Simply put, FA represents the directional preference of the diffusion with values ranging from zero to one (Eq. 2.). Axial diffusivity (AD) represents the diffusion along the principal eigenvector or the main axis of diffusion and is equal to the value of the principal eigenvalue (λ_1). Radial diffusivity (RD) is calculated as the average of the two smaller eigenvalues (Eq. 3.) and represents the diffusion perpendicular to the main axis of diffusion (Hecke et al., 2016).

$$MD = \frac{\lambda_1+\lambda_2+\lambda_3}{3} \quad \text{Eq. 1.}$$

$$FA = \sqrt{\frac{3}{2}}\frac{\sqrt{(\lambda_1-MD)^2+(\lambda_2-MD)^2+(\lambda_3-MD)^2}}{\sqrt{\lambda_1^2+\lambda_2^2+\lambda_3^2}} \quad \text{Eq. 2.}$$

$$RD = \frac{\lambda_2+\lambda_3}{2} \quad \text{Eq. 3.}$$

WM integrity and its microstructure can be affected by many different processes such as axotomy and demyelination (Mayer et al., 2010; Messe et al., 2012; Steenerson & Starling, 2017). Injury to WM will be reflected in DTI findings by variations in the metrics previously discussed, with the most commonly used being FA and MD. FA is particularly sensitive to changes in WM microstructure, specifically changes in axon density, axon size, degree of myelination and axonal injury (Hecke et al., 2016; Soares et al., 2013). Changes in MD can reflect deviations from the normal level of myelination and disruption of tissue integrity (Alexander et al., 2007). AD and RD are less frequently used to assess changes in WM integrity as their findings can be difficult to interpret in areas with high amounts of crossing fibers (Tournier et al., 2011). However, they are thought to reflect compromised axonal integrity and demyelination, respectively (Alexander et al., 2007; Tournier et al., 2011).

1.4.3 Probabilistic Tractography

Tractography or fiber tracking can be used to delineate and visualize major white matter tracts in the brain. Tractography works by using DTI data to assess fiber connections between adjacent WM voxels, with the goal of determining which fibers belong to the same tract (Tournier et al., 2011). This allows for 3D reconstruction of tract structure which can then be used with diffusion metrics to infer properties of WM tracts such as integrity.

There are two main methods of tractography. Deterministic tractography uses a “best fit” estimation of tract structure and boundaries while probabilistic tractography attempts to reduce errors that may occur from estimating fiber orientation by outputting results in the form of probability distribution maps (Tournier et al., 2011). For the probabilistic approach, with every step the tractography algorithm takes, a number of fiber orientations are estimated. Once the pre-defined stop or termination point is reached, the algorithm selects the orientation estimations with a higher density of connections to the pre-set start or seed point and produces probability distributions for these. Probabilistic tractography is considered unique as it is currently the only MRI modality that can provide this information. Despite its advantages, probabilistic tractography can still be prone to error and may produce findings with streamlines or fiber connections that do not belong to the tract in question. This tends to occur as connectivity at the microstructural level is not particularly well defined due to the unpredictable nature of axonal branching which can complicate defining tract boundaries (Alexander et al., 2007; Hecke et al., 2016; Tournier et al., 2011). Understanding these limitations of tractography is crucial to ensure findings are interpreted correctly and ideally should be combined with prior neuroanatomical knowledge of the tract of interest to minimize error.

1.5 DTI in Head Impacts

1.5.1 DTI Findings

As previously mentioned, FA can be considered a scalar measure, ranging from 0-1, describing both the shape of the ellipsoid tensor and the degree to which there is a preferred direction of diffusion (Van Hecke, Emsell, & Sunaert, 2016). Disruptions in FA are thought to reflect changes in WM microstructure with decreased FA most frequently associated with compromised WM, loss of structural integrity and microscopic tissue damage (Assaf & Pasternak, 2008; Horsfield & Jones, 2002). Decreased FA can also occur due to processes such as demyelination, a decreased number of WM fibers, or a higher proportion of crossing fibers (Tournier et al., 2011). With respect to subconcussion and concussion, decreased FA is thought to occur as a result of axonal injury or damage and compromised WM microstructural integrity (Alexander et al., 2007; Gajawelli et al., 2013; Murugavel et al., 2014; Shenton et al., 2012). Increases in FA can occur when there is increased directionality of diffusion and is most commonly associated with processes such as myelination, a higher axon density, axonal swelling or edema (Huppi & Dubois, 2006; Niogi & Mukherjee, 2010; Shultz, MacFabe, Foley, Taylor, & Cain, 2012).

Neurochemical changes and subsequent damage to axons can also be reflected by changes in MD. MD can be considered to be a representation of the size of the tensor, independent of its orientation and describes the diffusion in an individual voxel (Van Hecke et al., 2016). An increase in MD occurs due to greater diffusion in a given voxel and is thought to indicate axonal degeneration or injury, or other processes that increase the extracellular space, such as demyelination or aging (Deary et al., 2019; Metwalli et al., 2010; Vinciguerra et al., 2020).

Decreased MD is commonly associated with higher levels or increased myelination, dense axonal packing, and white matter maturation (Huppi & Dubois, 2006; Lebel, Walker, Leemans, Phillips, & Beaulieu, 2008). However, decreased MD can also be representative of processes that constrict the extracellular space, such as cellular edema, and axonal injury events occurring outside the axon, resulting in axonal swelling and decreased space between axons (Povlishock & Katz, 2005). This is thought to be the cause of decreased MD in neurotrauma while increased MD is thought to be due to axonal degeneration (Bazarian, Zhu, Blyth, Borrino, & Zhong, 2012; DiCesare et al., 2020; Tayebi et al., 2021).

1.5.2 DTI in Concussion

Diffusion tensor imaging is a valuable methodology for *in vivo* study of WM pathologies, namely changes in WM structure and integrity with alterations in diffusion of water molecules used as a biomarker. The use of DTI to examine WM integrity abnormalities following SRC in the past has yielded somewhat inconsistent findings, with studies reporting DTI metric changes of opposite directionality (Henry et al., 2011; Mustafi et al., 2018; Niogi et al., 2019; Wu et al., 2020). This has been largely attributed to a variety of factors such as a lack of consensus surrounding image acquisition protocols and subsequent analysis. Additionally, factors related to pathology itself such as the time course of recovery may play a role in the discrepancies observed in the literature (Bazarian et al., 2007). Inconsistencies in identified brain areas and WM tracts found to be damaged in SRC are thought to be due to the heterogenous nature of SRC, and the susceptibility of the structures themselves to the shearing forces of concussion (Crisco et al., 2012; Hellewell, Nguyen, Jayasena, Welton, & Grieve, 2020; Stojanovski et al., 2019).

Studies investigating DTI irregularities in the acute phase of SRC have produced conflicting results. Many studies have found widespread areas of decreased FA and/or increased MD following concussion (Arfanakis et al., 2002; Inglese et al., 2005; Miles et al., 2008; Mustafi et al., 2018; Niogi et al., 2019; Wu et al., 2020). For example, Niogi et al. (2019) examined DTI changes in active NFL athletes and found several areas with markedly reduced FA within 5 days following SRC compared to pre-morbid baseline imaging (Niogi et al., 2019). Moreover, a study investigating collegiate football players found diffusely increased MD in players that had sustained SRC within 48 hours of testing (Mustafi et al., 2018). These findings of reduced FA and increased MD are thought to reflect loss of WM integrity and compromised microstructure. Reductions in FA may occur due to loss of diffusion directionality following axonal injury and tissue damage, while increased MD arises due to greater diffusion in a given brain voxel as a result of axonal degeneration. Additionally, there is some evidence supporting the association of diffusion metric changes with clinical measures of symptoms (i.e., Sport Concussion Assessment Tool; SCAT, Brief Symptom Inventory; BSI) and/or poorer performance on neuropsychological tests (Miles et al., 2008; Mustafi et al., 2018; Wu et al., 2020), indicating that there may be a relationship between DTI changes and symptomatology.

Conversely, a number of studies have also reported increased FA and decreased MD following concussion. For instance, such changes in diffusion metrics were found within 6 days of injury in brain regions such as the corpus callosum, corticospinal tracts, cingulum and superior longitudinal fasciculus (Chu et al., 2010; Henry et al., 2011; Lancaster et al., 2016). Furthermore, Lancaster and colleagues (2016) found WM integrity changes were associated with worse performance on measures of cognitive functioning in concussed subjects (Lancaster et al., 2016). These changes in FA and MD are thought to represent inflammation, axonal swelling and edema.

The discrepancies between these findings with those above could be attributed to studies examining participants at differing time points after concussion, with diffusion metric findings reflecting distinct pathophysiology. Alternatively, analysis methods (i.e., voxel-based analysis, tract-based spatial statistics, tractography) often vary between DTI studies, as there is currently no standardized approach to this neuroimaging method. Therefore, analysis methodologies may also account for the inconsistencies in the literature.

1.5.3 DTI in SCI

While DTI has been used more extensively to study SRC, less research has been done with this imaging modality to investigate the effect of repetitive SCI on WM integrity. As recent evidence has consistently suggested cumulative SCI exposure may have harmful effects on long-term cognition and brain health (McKee, Alosco, et al., 2016; Montenigro et al., 2017; Stern et al., 2011), there is a growing need to better understand the potential effects of these kinds of impacts, including the impact on WM microstructure. As SCI is a form of neurotrauma that is thought to have an additive effect, the majority of studies have been longitudinal in design. With respect to football, studies have often examined changes in diffusion metrics throughout the course of a competitive season, reporting changes in FA and MD over this time frame, suggesting cumulative SCI may affect WM integrity and microstructure (Asselin et al., 2020; Bazarian et al., 2014; Chun et al., 2015; McAllister et al., 2014). However, reported changes in diffusivity measures have been inconsistent in both directionality and anatomical location.

Asselin et al. (2020) found widespread FA decreases throughout the brain (93.3% of affected voxels) following a single season of football in non-concussed collegiate athletes. The authors additionally found a correlation between the magnitude of FA change and impact exposure, with a greater change in FA being associated with higher cumulative impact exposure, suggesting a dose-response relationship between SCI and WM changes (Asselin et al., 2020). Another study found similar changes in a cohort of collegiate ice hockey and football players, citing increases in MD within the corpus callosum from pre- to post-season. Furthermore, increased MD was associated with greater impact exposure and poorer performance on verbal learning and memory assessments, indicating that such changes may play a role in functional impairment (McAllister et al., 2014).

Conversely, Chun et al. (2015) examined alterations in diffusivity measures in high-school football players and reported increased FA in the left superior corona radiata and right superior fronto-occipital fasciculus from the pre- to post-season timepoints. This increase in FA showed a significant positive relationship with cumulative head impacts (Chun et al., 2015). As these findings are in contrast to the directionality reported above in collegiate athletes, it is possible that this could be attributed to different impact exposure profiles in less competitive athletes. Lastly, a study conducted by Bazarian and Colleagues (2014) investigated collegiate football players without a clinical diagnosis of concussion using DTI and helmet accelerometers over the pre- and post-season timepoints, with an additional assessment after 6-months of rest. The authors found significant alterations in both FA and MD that persisted beyond and up to 6 months following the conclusion of the football season. The brain regions with altered diffusion metrics showed areas with changes in FA and MD of either directionality (i.e., regions with increased and decreased FA and MD) at both the post-season and 6-month timepoints compared to controls (Bazarian et al., 2014). The presence of both increased and decreased diffusion metrics could be attributed to brain areas at differing phases of axonal injury and subsequent repair.

The inconsistent findings from previous research suggests that the underlying neuropathophysiology and effect of SCI on white matter in contact sport athletes, particularly football players, is still poorly understood. Inconsistencies in both directionality and anatomic location of diffusion metric alterations in SCI highlight this lack of understanding both in terms of phases of WM injury and susceptibility of different WM structures. As different changes in diffusion metrics (i.e., increased versus decreased FA) are thought to reflect differing neuropathophysiological processes there is a clear need for better characterization of the effects of SCI on WM integrity. Additionally, these discrepancies could be attributed in part to variable data collection and different analysis methods, sport played, or the heterogenous nature of SCI itself, but regardless merit additional investigation to further understand of this type of neurotrauma.

1.6 Objectives, Rationale, and Hypothesis

DTI has been used previously to investigate changes in WM integrity following SRC, however less work studying SCI has been done using this imaging modality. DTI and probabilistic tractography are considered powerful methods to delineate and assess diffusion changes in WM tracts *in vivo*. While it has been clearly established that SRC can result in damage to WM tracts, compromising structural integrity, the effects of repetitive SCI exposure and the frequency at which these head impacts pose long-term risks are not well understood. Furthermore, the pathophysiological timeline of these changes is not well characterized.

There is evidence to suggest that repeated SCI, even over a short period of time, may result in measurable brain changes (Bailes et al., 2013; Davenport et al., 2014). However, with respect to WM integrity, previously published studies have produced conflicting results, reporting variable changes in diffusion metrics such as increases and decreases in FA and MD (Asselin et al., 2020; Bazarian et al., 2014; Mayinger et al., 2018), which are thought to signify different pathophysiological processes. These inconsistences may be attributed to factors such as varying time periods between injury and imaging, and variable mechanism of injury, but regardless, have made it challenging to elucidate these features of SCI. Factors such as duration and frequency of exposure necessary to compromise WM integrity and the timeline of recovery for such changes remain difficult to establish. The discrepancy between the directionality of these findings and the limited research regarding the cumulative effects and frequency of SCI highlights a need to further characterize the implications on WM tracts.

The goal of this study is to assess changes in DTI metrics, specifically FA and MD, associated with SCI to better characterize alterations in WM integrity that may occur over and persist beyond a single season of collegiate football. Specifically, the present study aims to (i) examine the effect of differing SCI exposure frequencies, and (ii) the cumulative effects of SCI throughout and beyond the football season on diffusion metrics. We hypothesize that repetitive SCI exposure compromises WM integrity resulting in decreased FA and increased MD, in the absence of clinically diagnosed SRC. Furthermore, we hypothesize that these changes will accumulate over the season and will be more pronounced in athletes with greater exposure to SCI.

These results will provide insight into diffusion abnormalities that occur with SCI. Specifically, these findings will allow for a better understanding of the directionality of these changes to gain insight into the pathophysiological processes which may underlie these types of injuries. Furthermore, this study may help to characterize the frequency at which SCI alters WM integrity by examining groups with distinct amounts of SCI exposure which is not common in the existing literature. Additionally, these findings could help further understanding of the timeline in which SCI compromises WM microstructure as very few studies have investigated multiple timepoints prior, during, and after a season of football. These features of SCI remain elusive, therefore, examining the potential implications of factors described above will allow us to better understand the risk posed to contact sport athletes and build a foundation for studying the long-term implications of SCI on neurocognitive functioning.

Chapter 2

Materials and Methods

2.1 Subject Recruitment

Subjects were recruited from the Queen's University Varsity Men's Football Team in Kingston, Ontario. The cohort consisted of 22 athletes aged 18-22 (mean 20 ± 1) years. Each athlete completed a neuroimaging protocol and SCAT3 neuropsychological testing based on the criteria set at the International Consensus Agreement on Concussion in Sport (McCrory et al., 2013) at three timepoints over a single season of football. Timepoints included: pre-season (PRE-), after training camp but prior to the first game of the season (post-training camp (PTC-)), and a post-season (POST-) timepoint, shortly after the completion of the season. All athletes were deemed eligible for participation in the study by the team physician. Participants were excluded if they had pre-existing injuries or sustained any injuries throughout the season that hindered their participation in the study or had if they had been diagnosed with a concussion within the last year.

2.2 Accelerometers

gForce Tracker (GFT) accelerometers (Artaflex Inc., Markham, Ontario, Canada) were used to quantify impact frequency for each player enrolled in this study. All players were assigned a GFT sensor with a corresponding ID number. Sensors were mounted inside player's helmets to the left crown and affixed with 3M Dual Lock^{TM} Velcro. GFT sensors are 50mm x 29mm x14mm in size and weigh 2 grams (Campbell et al., 2016). GFT sensors are equipped with a tri-axial accelerometer, a tri-axial gyroscope, a rechargeable lithium-polymer battery, and a built-in storage system with a capacity of 400 impacts. A trigger threshold of 15g was manually set to minimize impacts unrelated to contact (e.g., head movement). GFT sensors record data for 8 ms prior and 32 ms following a given impact to ensure the entire impact was collected.

Impact data was wirelessly transmitted back to the computer in real-time to facilitate impact monitoring. Observers were present at each session to place and activate sensors remotely at the start of each game or practice where helmets were worn. Any non-contact impact (e.g., dropping or kicking of helmets) were flagged to be removed later. Following each session, sensors were removed from helmets and stored data was uploaded to online GFT software. Devices were subsequently cleared and charged before the next session. Impact data was later filtered to remove erroneous impacts and further analyzed with processing scripts developed in-house. This allowed for each player's impact data to be summed and averaged throughout the season.

2.3 MR Data Acquisition

Images were acquired using a Siemens 3.0 T Magnetom Tim Trio system. Whole brain T1-weighted structural images were acquired using a gradient echo pulse sequence (MR-RAGE) with the following parameters: TR=1760 ms, TE=2.2 ms, TI=900 ms, 1 mm isotropic voxels. All DTI images were acquired using a diffusion-weighted fast spin-echo sequence with parameters: TR=7800 ms, TE=95 ms, FoV=256 mm, 60 axial slices with a thickness of 2 mm, and 2 mm isotropic voxels. Diffusion weighted images were acquired with a b-value of 1000 s/mm^2, in 30 directions. Three diffusion weighted images with no diffusion weighting (b=0 s/mm^2) were also acquired for normalization.

Pre-season scans were acquired between 4 and 72 days (mean 28 ± 22 days) prior to the first practice. Athletes were not participating in any contact sports prior to the start of the season. PTC scans were performed 2 to 22 days (mean 13 ± 5 days) after the last training-camp practice and prior to the first game of the regular season. Post-season scans were conducted 2 to 40 days (mean 29 ± 7 days) following the last game of the season.

2.4 Diffusion Data

2.4.1 Pre-processing of Diffusion Data

Diffusion-weighted images were processed using the FMRIB diffusion toolbox (FDT) that is part of the FSL (FMRIB's Software Library) software package.(Jenkinson, Beckmann, Behrens, Woolrich, & Smith, 2012) Pre-processing consisted of averaging the three b0 sets followed by the FSL (Smith et al., 2004) *top up* tool for estimation and correction of susceptibility-induced off-resonance fields. This was followed by using the *eddy* tool from FSL for correction of eddy current-induced distortions and subject movement. Brain extraction was preformed using the *Brain Extraction Tool* (*BET*) to remove non-brain tissue on the eddy corrected images. These images were used to calculate the 3x3 diffusion tensor parameters and diffusion tensor model at each brain voxel using the weighted least linear squares model with the *DTIFIT* tool within the FSL toolbox. This generated maps of the three eigenvalues and eigenvectors, from which FA, and MD maps were generated for each subject. Maps were then registered to a Montreal Neurological Institute (MNI) standard template (MNI152 2mm) using *FMRIB's Linear Image Registration Tool* (*FLIRT*)

(Greve & Fischl, 2009; Jenkinson, Bannister, Brady, & Smith, 2002; Jenkinson & Smith, 2001) and *FMRIB's Non-linear Image Registration Tool* (*FNIRT*) (Jenkinson et al., 2012; Jesper L. R. Andersson, 2007; Smith et al., 2004; Woolrich et al., 2009)

2.4.2 Probabilistic Tractography

Following initial preprocessing of the diffusion data, the *BEDPOSTX* (Bayesian estimation of Diffusion Parameters Obtained using Sampling Techniques)(Behrens, Berg, Jbabdi, Rushworth, & Woolrich, 2007) tool was used to model the crossing fibers within each brain voxel. The outputs from the *BEDPOSTX* tool were used to run probabilistic tractography for each WM tract of interest. Probabilistic tractography was conducted using AutoPtx (de Groot et al., 2013), an FSL plugin. AutoPtx uses previously defined scripts, along with binary seed, target, exclusion and stop masks, to run probabilistic tractography using FSL's *PROBTRACKX* tool (Behrens et al., 2007) in the subject's native space. In addition to selecting WM tracts, the number of individual streamlines or samples taken at each voxel can be defined for each structure. A list of WM tracts that were analyzed in this study and the number of streamlines used for each can be found in **Table 1.**

Table 1. List of WM tracts analyzed with AutoPtx, their abbreviations, if both left and right tracts were examined and the number of streamlines used.

WM tract	Abbreviation	Bilateral	Streamlines
Anterior Thalamic Radiation	ATR	Yes	5000
Cingulate gyrus of Cingulum	CGC	Yes	100000
Parahippocampal part of Cingulum	CGH	Yes	15000
Corticospinal Tract	CST	Yes	20000
Forceps Major	FMA	No	3000
Forceps Minor	FMI	No	3000
Inferior Fronto-occipital Fasciculus	IFO	Yes	22000

Inferior Longitudinal Fasciculus	ILF	Yes	6000
Posterior Thalamic Radiation	PTR	Yes	100000
Superior Longitudinal Fasciculus	SLF	Yes	2000
Superior Thalamic Radiation	STR	Yes	4000
Uncinate Fasciculus	UNC	Yes	6000

PROBTRACKX produces tractography results in the form of streamline density maps. These maps are 3D images that contain the number of streamlines that reach each voxel and are used to quantify the connectivity from the seed region or origin of the WM tract in question to the target or end region. The density maps produced using AutoPtx were normalized by using FSL's *fslmaths* command to divide the maps by their respective waytotals. The waytotal is a value that represents the total number of streamlines that have reached the target mask from the seed mask. A higher waytotal is produced when more streamlines reach their target and indicates a higher density and/or larger WM tract. Normalizing the images by waytotal allowed for differences across subjects in "trackability" to be accounted for and enabled inter-subject comparison. Fluctuations in "trackability" are typically due to factors such as variations in scan quality and motion, rather than biological or pathological differences. This is supported by the difference between waytotals between subjects varying by whole orders of magnitude, which is not typically observed when comparing diseased and healthy subjects.

After normalization, all tracts were registered to a standard MNI152 2mm template using the *FLIRT* and *FNIRT* tools as previously described for spatial alignment of all images. Following registration, all tracts were thresholded using the *fslmaths* tool to eliminate streamlines of low probability. Different thresholds were selected for each WM tract as the value range differed significantly between structures. The threshold selected for a specific WM tract was held constant for all subjects, as all images had been normalized prior to thresholding. A conservative threshold of less than 1% was used to avoid unintentionally eliminating streamlines that belonged to the tract in question. Probabilistic Tractography results of all WM tracts examined in this study can be found in **Figure 2**.

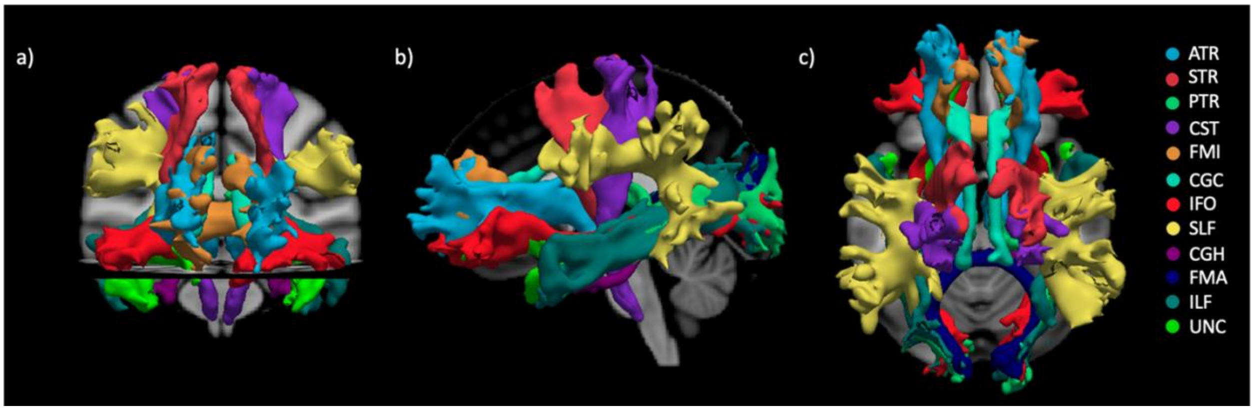

Figure 2. Probabilistic tractography outputs of all WM tracts including; the left and right ATR: anterior thalamic radiations, STR: superior thalamic radiations, PTR: posterior thalamic radiations, CST: corticospinal tracts, FMI: forceps minor, left and right CGC: cingulate gyri of cingulum, IFO: inferior fronto-occipital fasciculi, SLF: superior longitudinal fasciculi, CGH: parahippocampal parts of cingulum, FMA: forceps minor, ILF: inferior longitudinal fasciculi, and UNC: uncinate fasciculi from **A)** coronal, **B)** sagittal, and **C)** transverse orientations. WM tracts are overlaid on the standard 1mm Montreal Neurological Institute template.

All images were binarized using *fslmaths* to create a binary mask of the tract in question. Binary masks were overlaid on and multiplied by the corresponding subject's standardized FA map to produce a new image representing the FA values of the tract in question for that specific subject. The mean FA value of the resulting image was then calculated using FSL's *fslstats* tool for each subject and tract. The same process was repeated for MD, using the standardized MD maps.

2.5 Statistical Analysis

Statistical analysis of all data was carried out using SPSS Statistics (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Histograms and qq plots were used to visualize the distribution of the data. Subjects were first split into groups based on the cumulative frequency of impact exposure. The median impacts per session was calculated (10.07 impacts) and athletes which fell above this number were classified as “high exposure” (HE; n=11) and those below as “low exposure” (LE; n=11). Non-parametric t-tests (Mann-Whitney U tests) were then used to determine if there was a significant difference in impact frequency between exposure groups.

Mann-Whitney U tests were used to examine changes in diffusion metrics (FA, MD) between exposure groups (i.e., HE versus LE). Additional Mann-Whitney U tests were then used to assess changes in diffusion metrics for tracts where there was a significant difference between exposure groups at each of the three previously described timepoints (i.e., LE vs HE at PRE timepoint, LE vs HE at PTC timepoint, LE vs HE at POST timepoint). Non-parametric one-way repeated measures ANOVA tests (Friedman tests) were used to examine any changes in diffusion metrics over the three timepoints. Post-hoc tests were conducted when a significant p-value ($p<0.05$) was reported using a Bonferroni correction for multiple corrections. Significant results were then further investigated with planned comparisons using Wilcoxon signed-rank tests to determine any change in diffusion metrics over these timepoints in each exposure groups (i.e., LE group at PRE vs POST timepoint, HE group at PRE vs POST timepoint.).

Chapter 3

Results

3.1 Low and High Impact Exposure Groups Established Using Accelerometer Data

Data collected from helmet accelerometers were used to examine the frequency of subconcussive impacts over the football season for each athlete. The 22 athletes enrolled in this study received a total of 7943 impacts across the football season, with the total impacts per subject ranging from 33 to 778 (**Table 2**). After separation into two groups based on impact exposure, overall, the LE group received 2512 impacts over the course of the season, compared to 5658 for HE, which is an average of 7.20 ± 0.177 impacts per session for LE versus 14.62 ± 0.381 impacts per session for HE.

Table 2. Average number of impacts overall and by session type

ID	Position	Group	Game	TC	Practice	Total	Sum for season
Fbp.2	DB	Low	23.33	7.27	6.29	9.71	466
Fbp.8	WR	Low	13.71	7.00	5.10	7.18	273
Fbp.13	DB	Low	10.83	2.20	2.00	5.00	90
Fbp.14	DL	Low	14.38	8.88	6.50	9.06	290
Fbp.20	K	Low	3.14	2.00	1.80	2.54	33
Fbp.24	WR	Low	19.00	11.00	4.33	9.00	216
Fbp.26	WR	Low	11.57	5.13	3.11	4.90	206
Fbp.27	LB	Low	7.33	4.71	2.26	3.58	129
Fbp.29	DB	Low	15.71	6.00	5.83	7.64	298
Fbp.36	DL	Low	10.00	8.00	8.00	8.29	232
Fbp.48	LB	Low	14.86	10.78	5.20	9.00	279
Fbp.7	OL	High	25.50	12.91	10.19	13.39	509
Fbp.9	WR	High	26.88	8.57	6.58	11.10	433
Fbp.11	DB	High	40.13	9.60	6.36	12.93	595
Fbp.12	LB	High	20.13	13.44	4.61	10.43	422
Fbp.15	DL	High	26.00	21.73	12.26	16.91	778
Fbp.18	OL	High	12.00	14.55	15.55	15.00	495
Fbp.19	WR	High	26.20	26.78	9.95	17.00	561

Fbp.28	DL	High	36.67	19.11	19.29	21.23	552
Fbp.30	DB	High	27.86	3.75	4.78	11.17	268
Fbp.32	DB	High	33.00	7.40	4.00	11.18	369
Fbp.49	RB	High	45.25	9.67	4.75	17.45	506

DB, defensive back; DL, defensive line; K, kicker; LB, linebacker; OL, offensive line; RB, running back; TC, training camp; WR, wide receiver.

3.2 DTI and Probabilistic Tractography Reveal Changes in Fractional Anisotropy

Based on the exposure groups described above, we investigated changes in diffusion metrics at three timepoints over a single season of football. Probabilistic tractography was performed to recreate 22 tracts of interest (**Figure 2**) and outputs were used to examine changes in diffusion metrics (FA, MD). Probabilistic tractography outputs revealed significant changes in FA or MD in the tracts seen in **Figure 4.**

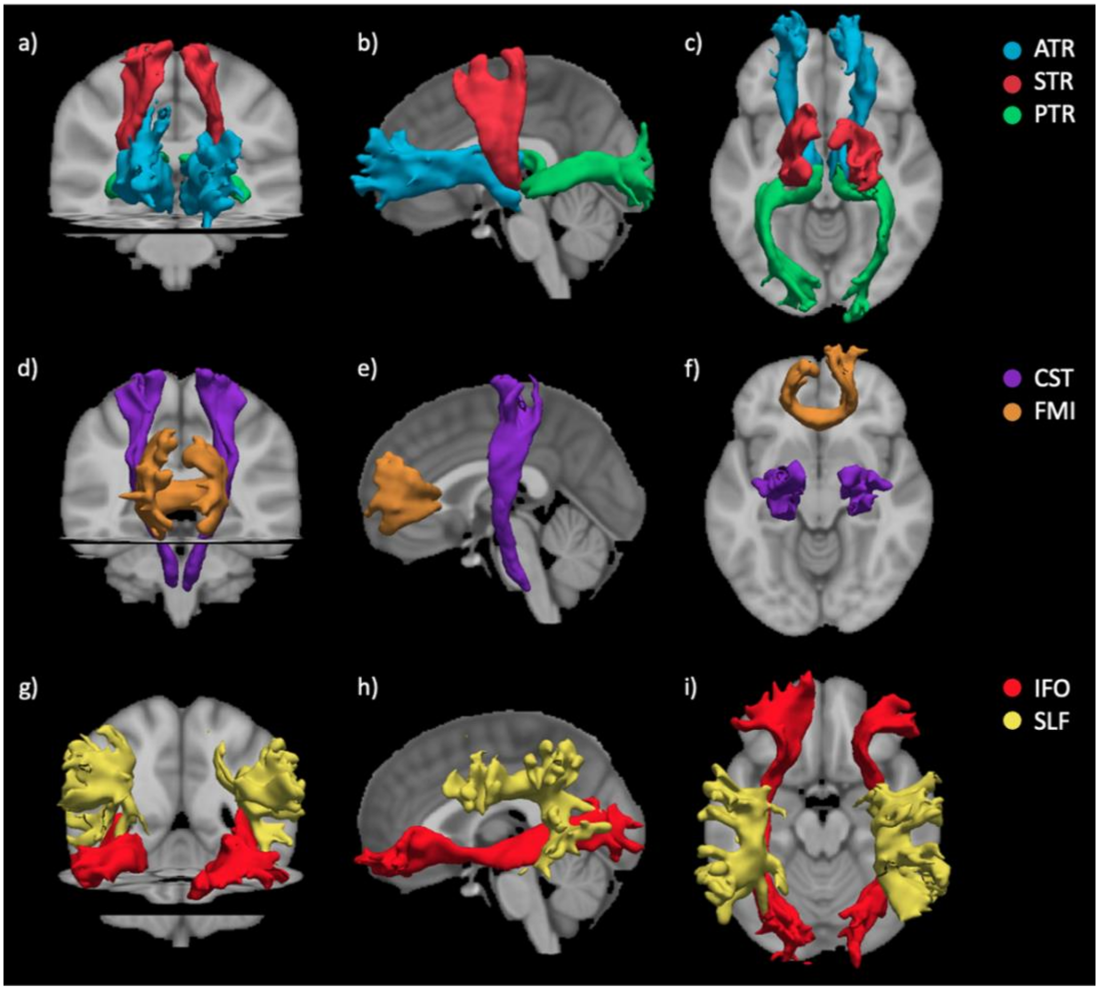

Figure 3. Probabilistic tractography outputs of WM tracts with altered FA or MD. A-C) The left and right ATR: anterior thalamic radiations, STR: superior thalamic radiations, and PTR: posterior thalamic radiations. **D-F)** The left and right CST: corticospinal tracts, and FMI: forceps minor. **G-I)** IFO: inferior fronto-occipital fasciculi, and SLF: superior longitudinal fasciculi. All WM tracts are shown in coronal (left), sagittal (center), and transverse (right) orientations. WM tracts are overlaid on the standard 1 mm Montreal Neurological Institute template.

Variations in FA were examined both between exposure groups and over the football season. Initial comparisons between high and low exposure groups using Mann-Whitney U tests revealed the HE group had several structures with decreased FA, namely the left and right corticospinal tracts (left: $p = 0.00016$, right: $p = 0.014$), superior thalamic radiations (left: $p =$

0.004, right: p = 0.007), and left anterior thalamic radiation (p = 0.013), but not the right. A summary of p-values for all FA comparisons can be found in **Appendix A.**

Additional Mann-Whitney U tests then were conducted for significant WM tracts to determine at what timepoint in the season FA changes were present between groups (**Figure 4**). The left corticospinal tract had significantly decreased FA in the HE group at both the PRE (p = 0.004) and POST timepoints (p = 0.019) when compared to the LE group, while the there was no significant difference between exposure groups at the PTC timepoint. The right corticospinal tract had significantly reduced FA in the HE group compared to the LE group at only the PRE timepoint (p = 0.019), as did the left anterior thalamic radiation (p = 0.04). Similar to the left corticospinal tract, the left superior thalamic radiation had significantly lower FA in the HE group than the LE group at both the PRE and POST timepoints (PRE: p = 0.008, POST: p = 0.029). There was no difference in FA between exposure groups in the right superior thalamic radiation when examined at each time point.

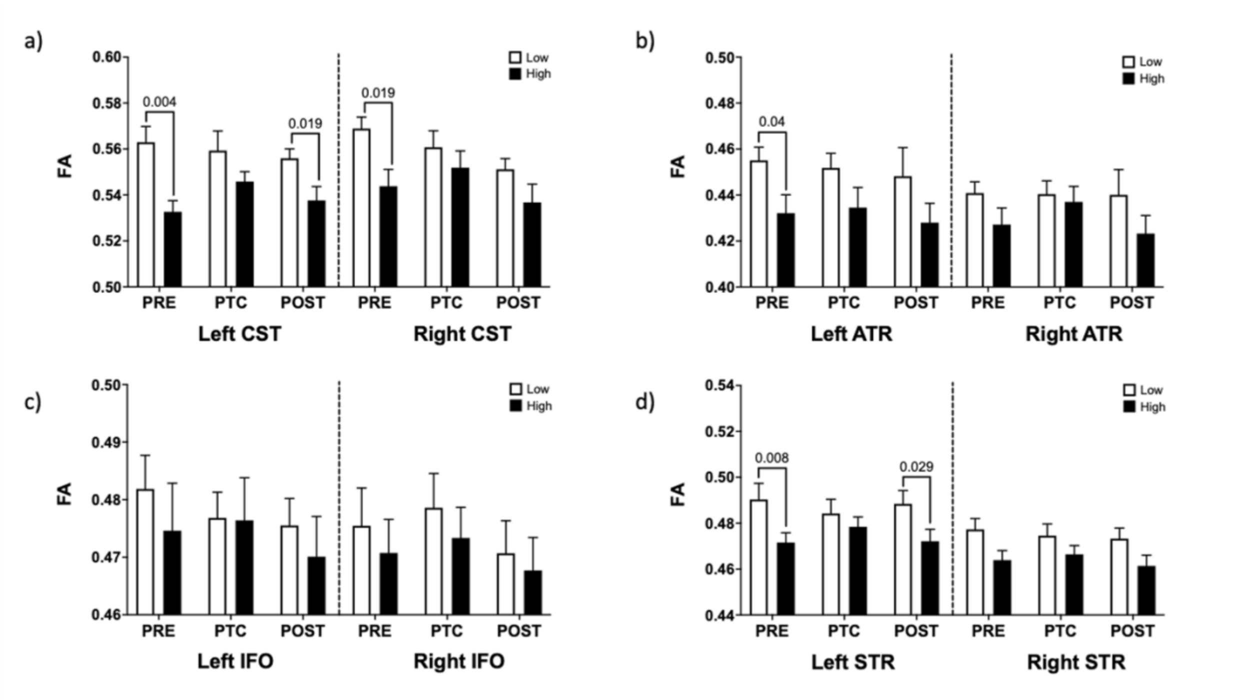

Figure 4. FA variations at PRE and POST between exposure groups. Data represents the mean ± SEM FA of high and low exposure groups at PRE, PTC and POST timepoints. Mann-Whitney U tests were used to examine differences between high and low exposure groups at each timepoint (n = 11). **A)** FA was significantly decreased in the left and right CST at the PRE timepoint in the high exposure group when compared to the low exposure group (left: p = 0.004, right: p = 0.019). Additionally, FA was significantly reduced at the POST timepoint in the high exposure group when compared to low exposure group in the left CST only (p = 0.019). **B)** FA was significantly diminished in the high exposure group compared to the low exposure group at the PRE timepoint in the left ATR only (p = 0.04). **C)** No significant differences were found at any timepoints for the left or right IFO. **D)** Only the left STR showed further significance, with reduced FA in the high exposure group compared to the low exposure group at both the PRE and POST timepoints (PRE: p = 0.008, POST: p = 0.029).

To explore the relationship between FA changes and season timepoints, Freidman tests with Bonferroni corrections for multiple comparisons were first conducted to compare the three season timepoints (**Figure 5**). A significant decrease in FA was observed from PTC to POST in both forceps minor (p = 0.0476), and the right posterior thalamic radiation (p = 0.02). Additionally, a significant reduction in FA was observed in the right corticospinal tract from the PRE to POST timepoint (p = 0.0138). There were no significant differences in FA over any of the season timepoints in the remaining WM structures. A summary of all p-values and descriptive statistics of FA measures can be found in **Appendix A**.

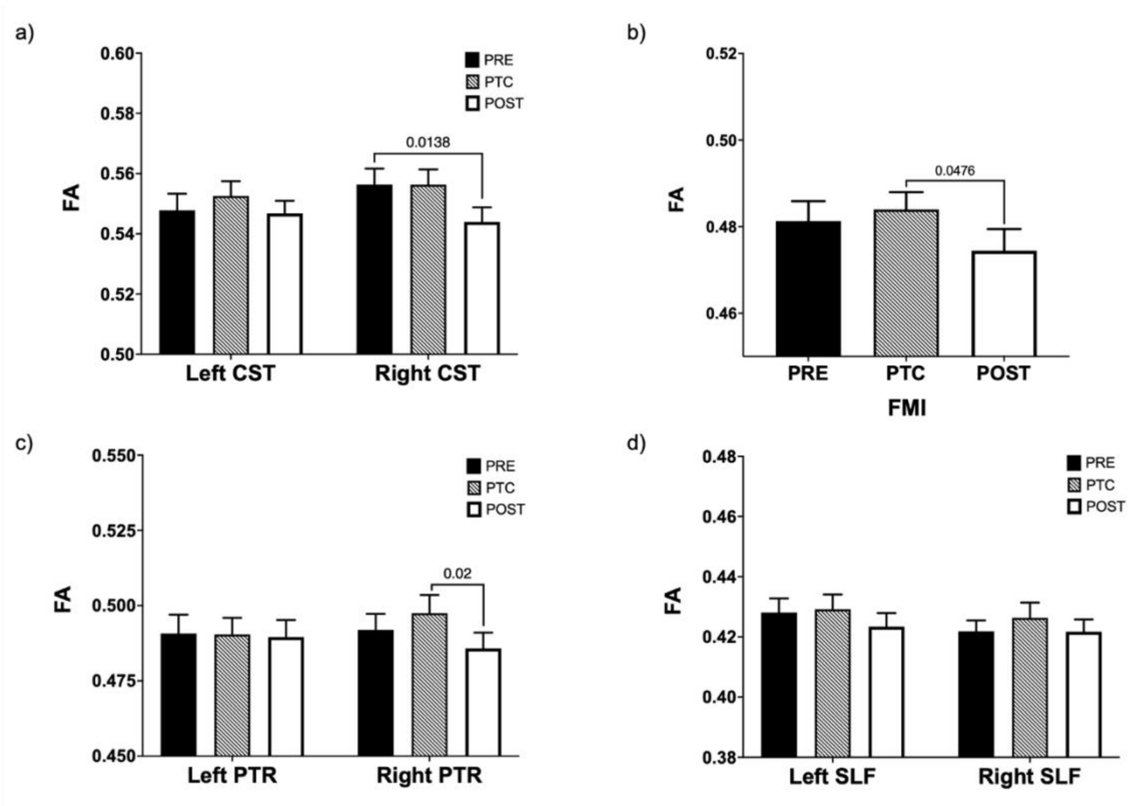

Figure 5. Changes in FA from PRE and PTC to POST. Data represents the mean ± SEM FA of PRE, PTC and POST timepoint groups. Results were compared by Friedman tests with a Bonferroni correction for multiple comparisons to analyze changes in FA over the PRE, PTC and POST timepoints (n = 22) within a single season of football. **A)** Significantly reduced FA was found from the PRE- to POST-season timepoints in the right CST (p = 0.0138), but not the left. **B)** FA was found to be significantly decreased from the PTC to POST timepoint in FMI (p = 0.0476). Comparisons between other timepoints were found to be non-significant. **C)** A significant reduction in FA was observed in the right PTR from the PTC to POST timepoint (p = 0.02). All

other comparisons in the right and left PTR were non-significant. **D)** No significant changes in FA were found between any timepoints in the left or right SLF.

To further examine the underlying role of the different exposure groups in FA reductions, Wilcoxon signed-rank tests were performed to investigate differences between individual exposure groups at time points that were significantly differed (**Figure 6**). The right corticospinal tract showed significantly decreased FA at POST compared to the PRE timepoint in the low exposure group ($p = 0.038$), while this trend was not observed for the high exposure group. A similar trend was observed in the right posterior thalamic radiation, with significantly lower FA in the low exposure group at POST compared to the PTC timepoint ($p = 0.016$). Lastly, forceps minor had significantly reduced FA in the high exposure group from PTC to POST timepoints ($p = 0.021$).

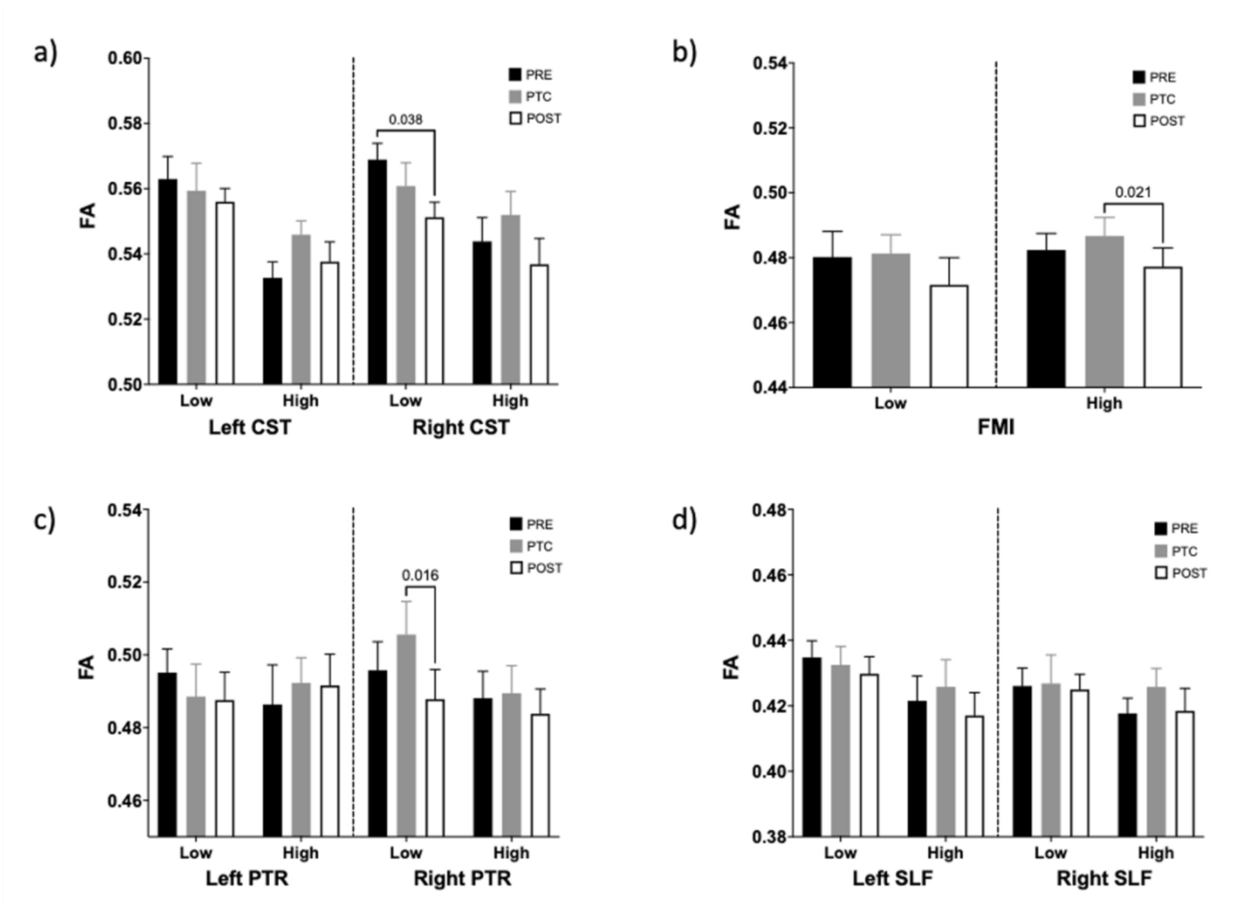

Figure 6. Low and high exposure groups show FA fluctuations over a football season. Data represents the mean ± SEM FA of high and low exposure groups at each timepoint (PRE, PTC, POST). Wilcoxon signed-rank tests were used to determine if there were differences in FA between timepoints for either exposure group (i.e., Low exposure PRE compared to low exposure POST) (n = 11). **A)** Significantly reduced FA was found in the right CST only in the low exposure group. The low exposure group showed reduced FA at POST compared to the PRE timepoint (p = 0.038). However, no significance was detected when comparing PRE to POST for the HE group. **B)** FMI was found to have significant FA decreases in the high exposure group only from the PTC to POST timepoint (p = 0.021). **C)** FA was found to be significantly reduced in the low exposure group from the PTC to POST timepoint in the right PTR only (p = 0.016). No significance was found for the high exposure group at this timepoint. **D)** No significant differences were found for the left or right SLF.

3.3 Variations in Mean Diffusivity Detected with DTI and Probabilistic Tractography

To initially compare the effects of impact exposure on MD, Mann-Whitney U tests were performed between the LE and HE groups. Within the HE group, several WM structures showed significantly increased MD when compared to the LE group. Specifically, both the left and right corticospinal tracts (left: $p = 0.001$, right: $p = 0.001$), the left anterior thalamic radiation ($p = 0.003$), inferior fronto-occipital fasciculus ($p = 0.021$), and superior thalamic radiation ($p = 0.008$) all showed increased MD in the HE group. A summary of all p-values for MD comparisons and descriptive statistics can be found in **Appendix A**.

Mann-Whitney U tests were also performed to examine MD at each time point throughout the season in WM tracts previously found to have significance (**Figure 7**). The left and right corticospinal tract had increased MD at the PRE timepoint only in the HE group in comparison to the LE group (left: $p = 0.006$, right: $p = 0.002$). The left anterior thalamic radiation followed a similar trend, with the HE group showing increased MD at the PRE timepoint when compared to the LE group ($p = 0.019$). Lastly, the inferior fronto-occipital fasciculus had increased MD in the HE group when compared to the LE group at the POST timepoint ($p = 0.047$).

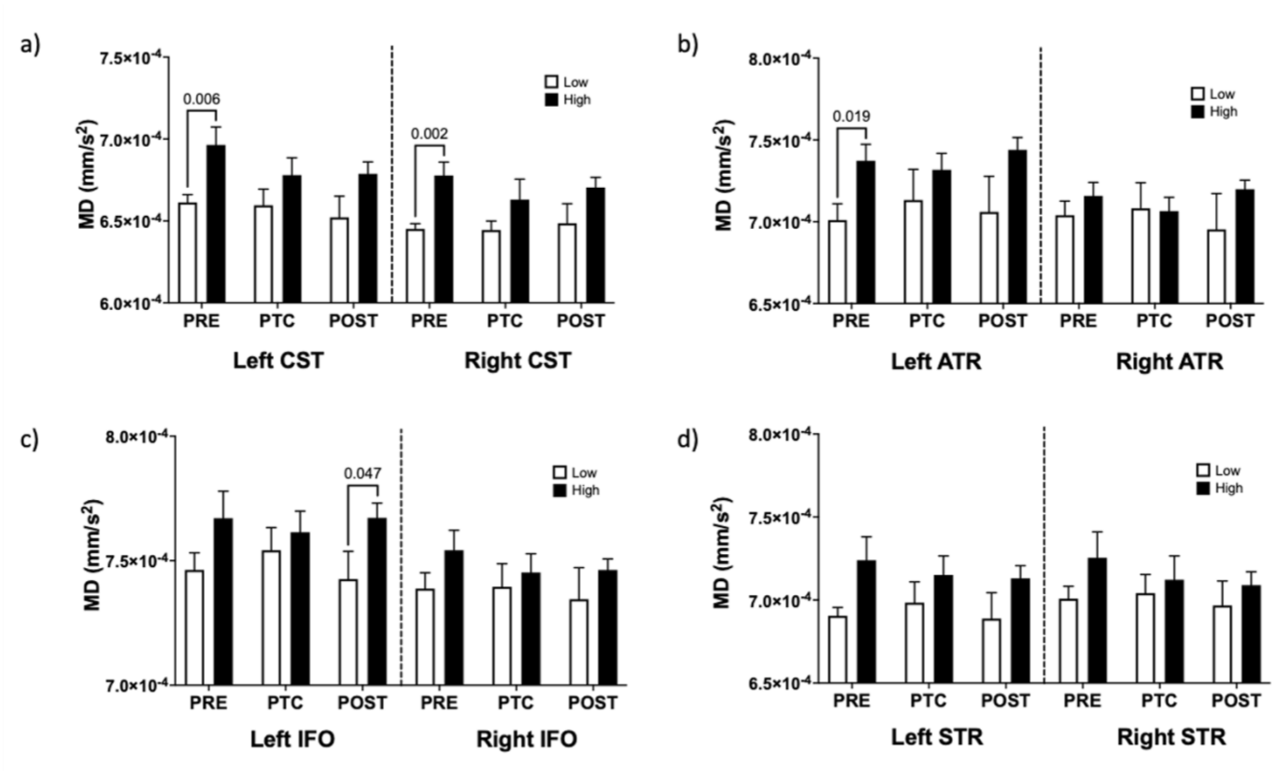

Figure 7. Changes in MD observed in the high exposure group at PRE and POST. Data represents the mean ± SEM MD of high and low exposure groups at each timepoint. Mann-Whitney U tests were used to further determine differences between exposure groups, by examining changes at each timepoint (n = 11). **A)** MD was found to be significantly elevated in the high exposure group compared to the low exposure group at the PRE timepoint, but not the PTC or POST timepoints in both the left and right CST (left: p = 0.006, right: p = 0.002). **B)** The left ATR was found to have significantly increased MD at the PRE timepoint in the high exposure group when compared to the low exposure group (p = 0.019). No significant differences were detected in the right ATR. **C)** The left IFO was found to have significantly increased MD in the high exposure group at the POST timepoint compared to the low exposure group (p = 0.047). No significant differences were found for the right IFO. **D)** No further significant differences were detected for the left or right STR.

The effect of the season timepoints on changes in MD were explored next. Friedman tests with a Bonferroni correction for multiple comparisons were used to compare differences in MD over each of the three season timepoints (**Figure 8**). Only the right superior longitudinal fasciculus showed significant changes in MD with significant decreases in this metric from the PRE to PTC timepoint (p = 0.031). A summary of all other p-values and descriptive statistics can be found in **Appendix A**.

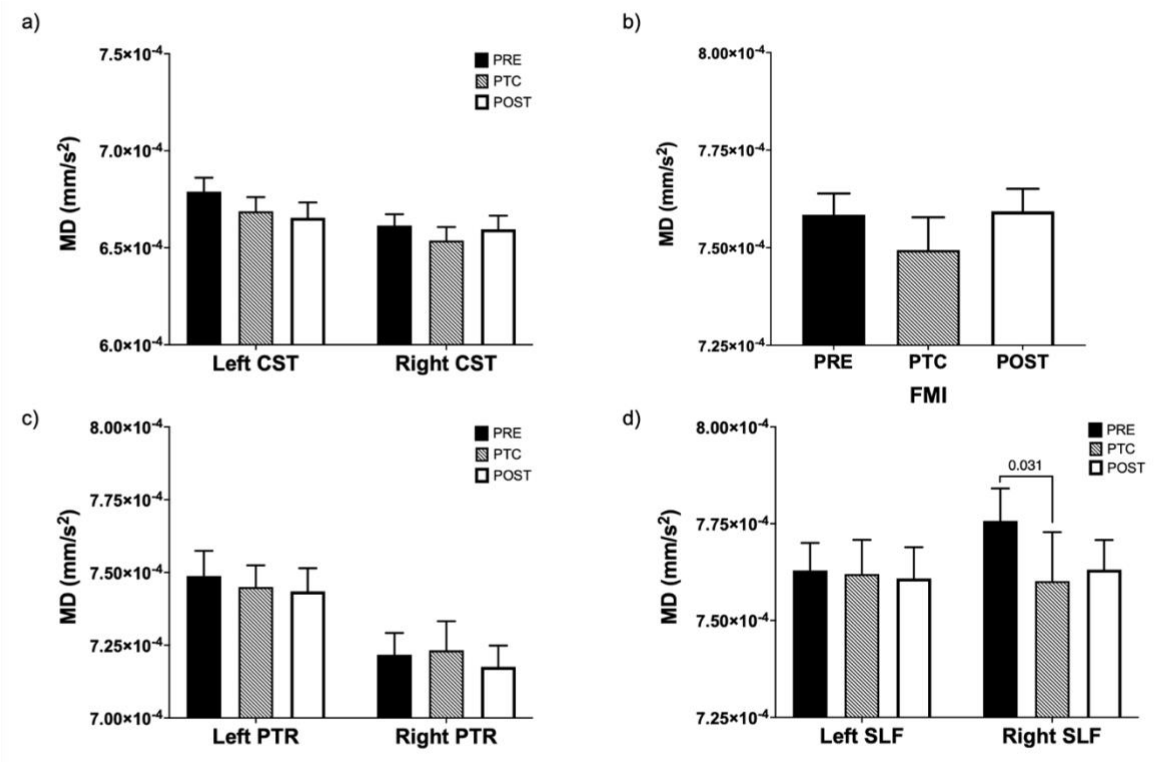

Figure 8. Decreased MD from only PRE to PTC timepoints in the right SLF. Data represents the mean ± SEM MD of PRE, PTC and POST timepoint groups. Results were compared by Friedman tests and using a Bonferroni correction for multiple comparisons to analyze changes in MD over the PRE, PTC and POST timepoints (n = 22) within a single season of football. **A)** Both the left and right CST were found to have no significant changes in MD across all timepoints. **B)** All comparisons were found to be non-significant for FMI. **C)** No significant changes in MD were observed between any timepoints in the left or right PTR. **D)** A significant reduction in MD was found from the PRE to PTC timepoint in the right SLF (p = 0.031). No other comparisons were significant for the left or right SLF.

We next examined the role of impact exposure groups in the previously observed decrease in MD from PRE to PTC in the right superior longitudinal fasciculus. Wilcoxon signed-rank tests were conducted to explore the MD variations in the LE and HE group over the significant timepoints (**Figure 9**). The right superior longitudinal fasciculus was found to have significantly decreased MD from PRE to PTC timepoints in the HE group only (p = 0.003).

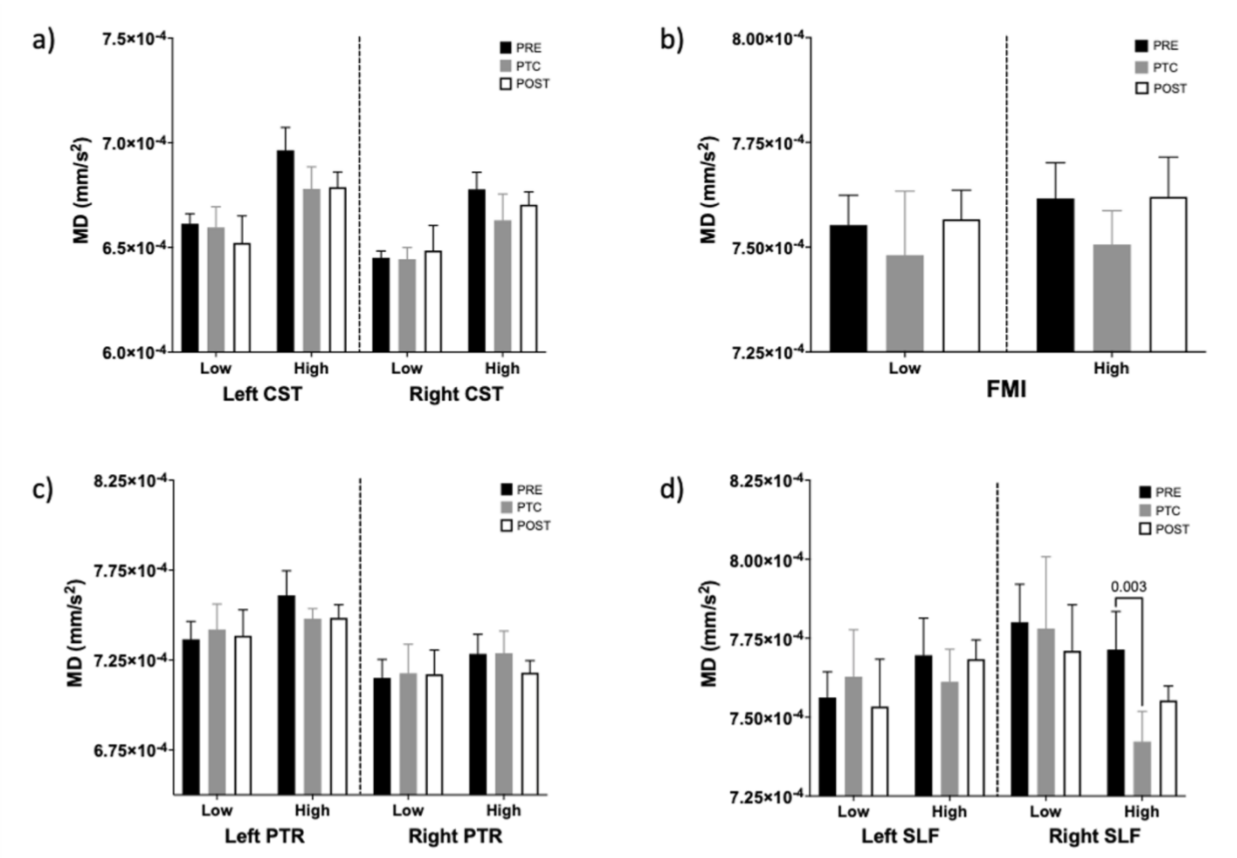

Figure 9. High exposure group shows MD variations from PRE to PTC timepoint in the right SLF. Data represents the mean ± SEM MD of high and low exposure groups for each timepoint (PRE, PTC, POST). Wilcoxon signed-rank tests were used to determine if changes in MD were present between timepoints for each exposure group (i.e., Low exposure PTC compared to low exposure at POST) (n = 11). **A)** No significance was found for the left or right CST for either exposure group. **B)** MD was not found to significantly vary from over any timepoints for either exposure groups in FMI. **C)** Neither the left nor right PTR revealed significant differences in MD

at over these timepoints for either exposure group. **D)** The right, but not left, SLF was found to have significantly decreased MD at the PTC timepoint when compared to the PRE timepoint in only the high exposure group ($p = 0.003$). No significant differences in MD were found in the low exposure group over these timepoints.

Chapter 4

Discussion

Mounting evidence suggests that continued exposure to asymptomatic repetitive head impacts may result in damage to the brain over time (Bazarian et al., 2012). The mechanisms underlying this phenomenon remain poorly understood, with a lack of consensus regarding specific damage that may occur, frequency at which SCI becomes harmful, and long-term implications and risks. DTI has proven to be a powerful method to study changes in WM microstructure and axonal integrity, particularly when applied to the study of head impacts. Furthermore, probabilistic tractography is a reliable and unique method to delineate WM tracts of interest to facilitate investigation of diffusivity measures. With the current literature reporting conflicting results (Asselin et al., 2020; Bazarian et al., 2014; Chun et al., 2015; McAllister et al., 2014; Slobounov et al., 2017), additional research is needed to elucidate the alterations in WM integrity that occur following SCI in contact sports such as football.

4.1 Diffusion metric differences between exposure groups

In the present study we investigated the effect of repetitive SCI exposure on WM integrity as detected by changes in DTI metrics (FA, MD) in a cohort of non-concussed collegiate football players over a single competitive season. When examining differences between the low and high exposure groups at each season timepoint, we identified several WM tracts with differences in diffusion measures at the PRE and POST timepoints. Decreased FA and increased MD were observed at the PRE timepoint in the HE group when compared to the LE group. Decreased FA in the HE group was found within the left and right corticospinal tracts, left anterior thalamic radiation, and left superior thalamic radiation compared to FA values in the LE group. Concurrently, the HE group showed increased MD in the left and right corticospinal tracts and the left anterior thalamic radiation when compared to the LE group. Prior to the PRE timepoint, athletes had not participated in contact sports since the conclusion of the previous football season. Therefore, these changes likely reflect axonal injury and compromised WM integrity as previously stated. This reduction in FA and increase in MD observed in the HE group at the PRE timepoint could reflect an additive or cumulative effect of repetitive head impacts due to greater SCI exposure in the HE group from the preceding season or seasons that did not resolve (Bazarian et al., 2014). These changes could also be driven by factors such as history of participation in contact sports like football (i.e., years played) (Stamm et al., 2015).

Prior studies have found evidence of similar diffusion metric changes with the same directionality that we observed (decreased FA, increased MD) that were still present 6 months following the conclusion of the football season in both concussed and non-concussed athletes (Bazarian et al., 2014; Henry et al., 2011; Lancaster et al., 2018). Therefore, these differences in WM integrity between exposure groups at the PRE timepoint suggest that the effects of previous head impacts may accumulate over an athlete’s life, with a greater number of impacts producing more pronounced changes and contributing to long lasting microstructural changes. This finding could be concerning as it may suggest that athletes with high exposure to repetitive head impacts, concussive or otherwise, may fail to recover following a single football season, causing progressive damage to WM tracts, persisting silently into subsequent seasons and potentially placing the brain at a higher risk compared to athletes with less or no exposure to SCI. Furthermore, prolonged, repetitive SCI exposure in contact athletes has been linked to increased risk for neurodegenerative diseases such as CTE, Alzheimer's disease, and Parkinson's disease (Lehman, Hein, Baron, & Gersic, 2012; McKee et al., 2009; B. I. Omalu et al., 2005; B. I. Omalu, Hamilton, Kamboh, DeKosky, & Bailes, 2010).

Additionally, there were significant differences between the HE and LE groups at the POST timepoint, the HE group was found to have reduced FA when compared to the LE group in both the left corticospinal and left superior thalamic radiation, while increased MD was seen in the left inferior fronto-occipital fasciculus of the HE group in contrast to the LE group. This loss of anisotropy and increase in mean diffusivity at the POST timepoint most likely represents the greater levels of SCI exposure in the HE group, resulting in compromised WM microarchitecture and axonal injury, leading to decreased axonal integrity, organization, and packing and increased extracellular space between axons. Notably, the absence of a control group (i.e., non-contact sport athletes) makes it difficult to draw conclusions from these findings with no baseline to compare to. Future studies should include non-contact sport athletes to better understand the effects of SCI frequency on contact sport athletes.

The failure of these metrics to stabilize between exposure groups up to a month following completion of the football season is in alignment with previous studies that found widespread alterations to diffusion metrics following a season of football in non-concussed athletes (Bahrami et al., 2016; Bazarian et al., 2014; Champagne, Coverdale, Germuska, & Cook, 2019; Kuzminski et al., 2018; McAllister et al., 2014). An example of one such study is the work conducted by McAllister and Colleagues (2014) which reported significantly increased MD in collegiate football and ice hockey players compared to non-contact athletes. These alterations in diffusion occurred over a single season of play, with a higher number of impacts found to be associated with increased MD (McAllister et al., 2014). Additionally, Kuzminski et al. (2018) found decreased FA in football players following a season of football, and also reported a relationship between this loss of anisotropy and impact frequency (Kuzminski et al., 2018). However, there are studies which have found changes in diffusion metrics of opposite directionality, or no significant changes at all (Slobounov et al., 2017). Taken together, findings from **Figure 4** and **Figure 7** suggest there may be an association between greater head impact exposure as seen in the HE group and compromised axonal integrity, present at least a month following the football season (POST timepoint).

Overall, the changes in FA and MD at the PRE and POST timepoints indicate that increased SCI exposure may cause axonal damage and loss of WM integrity, persisting beyond the end of the football season and into the next. As previously mentioned, the altered diffusion metrics detected in the HE group, are relative to those of the LE group only. The lack of a control group in this study limits the interpretation of our findings, as there is no baseline with which to compare. This could be ameliorated in future studies with the addition of a cohort of non-contact sport athletes of a comparable level (i.e., collegiate, high-school athletes). Additionally, the majority of longitudinal DTI studies have examined diffusivity changes at most up to 6 months following a single season (Bazarian et al., 2014; Davenport et al., 2014; Mayinger et al., 2018), and as such there remains a lack of studies investigating the long-term, chronic effects of repetitive SCI. Our findings highlight this limited understanding and emphasize a need for additional research in this field, particularly longitudinal studies examining DTI alterations over multiple seasons of football.

Taken together, comparisons between low and high exposure groups produced fairly consistent results for FA and MD. While the left and right corticospinal tracts and left anterior thalamic radiation all showed differences in both FA and MD at the PRE timepoint, the same effect was not seen in the left corticospinal tract at the POST timepoint, which was significant for FA, but not MD. Similar discrepancies were observed for the left anterior thalamic radiation and inferior fronto-occipital fasciculus, which showed significance for one metric, but no change in the other. While these findings suggest greater SCI exposure may affect WM tracts and accumulate over time, the apparent inconsistency between PRE and POST findings for both FA and MD highlights the need for additional research into the effects of SCI. These observed discrepancies could be a function of limited sample size (n = 22) and the heterogenous nature of neurological insults like SCI.

Though we did not specifically aim to establish a threshold for changes in WM integrity in this study, using DTI, several studies have found evidence of a cumulative effect of SCI (Asselin et al., 2020; Bahrami et al., 2016; Bazarian et al., 2012; Bazarian et al., 2014; Champagne, Peponoulas, et al., 2019; Davenport et al., 2014; Kuzminski et al., 2018), supporting the idea of a "dose-response relationship"(Montenigro et al., 2017). This was proposed by Montenigro et al. (2017) who investigated the relationship between cumulative head impacts and long-term clinical outcomes in former football players. The authors found the risk of later-life impairment remained fairly low at and below a given "threshold" or number of impacts, while, above this threshold, the risk of impairment increased in a dose-response fashion, roughly every 1000 impacts. The specific threshold varied between specific clinical outcomes ranging from 1801-7251 impacts, with the lowest thresholds observed in executive functioning and depression, however all measures followed the same dose-response trend. (Montenigro et al., 2017).

An increasing amount literature, in addition to the findings from this study, strongly suggest that increased exposure to SCI has the potential to cause long-term damage and heighten the risk of neurocognitive impairments. Despite the work done by Montenigro et al. (2017), the specific thresholds at which SCI exposure becomes harmful and inflicts irreparable damage remains elusive. The average collegiate football player may sustain up to 1400 subconcussive impacts per season (Bailes et al., 2013; Broglio et al., 2009; Stern et al., 2011) and in this study players sustained up to 778 impacts. As the duration of a collegiate football career is roughly 4-5 years and presumably began prior in adolescence, it is clear that these athletes potentially could reach the thresholds determined by Montenigro et al. (2017), although individual number of lifetime impacts would vary based on factors such as duration in the sport, position played and play time in games. As such, further characterization of a dose-response relationship and the threshold at which risk of long-term impairment occurs is needed to better understand the effects of repetitive SCI and the risks to contact sport athletes.

4.2 Diffusion metric fluctuations over the football season

When examining fluctuations in diffusion metrics over the football season and looking at all players together, FA was found to be decreased in the right corticospinal tract, forceps minor and the right posterior thalamic radiation showing POST timepoints with lower FA when compared to earlier season timepoints (**Figure 5**). Further investigation (**Figure 6**) showed the LE group to have decreased FA at the POST compared to the PRE timepoint in the right corticospinal tract and compared to PTC in the right posterior thalamic radiation. Additionally, the HE group had reduced FA at the POST timepoint compared to PTC in forceps minor. The most likely explanation for the decreased FA would be WM tract damage due to repetitive neurotrauma that accrued over the football season, in the absence of concussive head impacts, compromising axonal integrity and tissue microstructure, therefore resulting in a loss of diffusion anisotropy.

Interestingly, despite sustaining fewer impacts than the HE group, the LE group experienced decreases in FA over the football season as well. This may suggest that SCI exposure, even at a lower frequency, could still prove harmful to WM integrity. The HE group was found to have fewer WM tracts with decreased FA than the LE group. Many comparisons within the HE group showed non-significant trends which may be attributed to the small sample size of this study, as many comparisons for the HE group approached significance. Additionally, findings from **Figures 4**, **6**, and **7** provide evidence of compromised WM integrity, represented by reductions in FA or increases in MD in those with greater levels of SCI exposure, persisting at least one month after the football season. This suggests both the amount (impact exposure level) and duration of SCI exposure could affect WM microstructure and be important factors to consider regarding the dangers of repetitive SCI in the sport of football.

These findings align with many similar studies that found decreased FA in non-concussed football players, following a single competitive season (Bahrami et al., 2016; Bazarian et al., 2014; Champagne, Peponoulas, et al., 2019; Kuzminski et al., 2018; McAllister et al., 2014). For example, a study by Bahrami et al. (2016) found decreases in FA over a single football season, in a cohort of non-concussed athletes. Additionally, the authors reported a relationship between FA values and head impact sustained, with a higher number of head impacts associated with more pronounced decreases in FA (Bahrami et al., 2016). Conversely, previous research has also found instances of repeated SCI resulting in increased FA. Authors have attributed the increased FA to pathophysiological processes such as extracellular edema and axonal swelling, resulting in greater directionality of diffusion (Bazarian et al., 2014; Chun et al., 2015; Niogi & Mukherjee, 2010). The inconsistencies in the literature highlight a need for further research in this field to better characterize the effects of this type of neurotrauma on the brain.

Findings from **Figure 8** showed a significant decrease in MD from the PRE to PTC timepoint in the right superior longitudinal fasciculus. Further investigation found the HE group only to show significantly decreased MD from PRE to PTC timepoints in this structure (**Figure 9**). This reduction in MD is likely due to axonal swelling and cellular edema, causing impingement of the interstitial space, subsequently decreasing diffusion.

The observed decrease in MD in the right superior longitudinal fasciculus from the PRE to PTC timepoint in the HE group may be reflective of a more short-term response and associated change in diffusion metrics. This possibility is supported by a non-significant FA increase in the right superior longitudinal fasciculus over these same timepoints in the HE group (**Figure 6**), suggesting that the right superior longitudinal fasciculus underwent a different directionality of change in diffusion metrics than other WM tracts in this study. Previous research using DTI to examine WM changes in football players has indicated that SCI can produce both increases and decreases in FA and MD in differing brain regions over a single football season (Asselin et al., 2020; Bazarian et al., 2012; Bazarian et al., 2014). The authors concluded that the bidirectionality of changes in diffusion metrics were most likely reflective of multiple axonal injuries in various brain areas that could have occurred at different points over the season and thus, were at different stages in axonal injury and repair (Bazarian et al., 2012; Bazarian et al., 2014), which could explain the decrease in MD in the SLF. Additionally, past research has found similar changes at the acute and sub-acute timepoints following concussion (Bazarian et al., 2012; Henry et al., 2011; Maruta et al., 2020). While none of the subjects in the current study sustained a concussion during the season, this change could reflect a more acute response to an elevated level of head impact frequency that was not observed in the LE group. Alternatively, as athletes sometimes do not report concussion symptoms to avoid removal from play (Asken et al., 2016; Black, Sergio, & Macpherson, 2017; Garrick, 2005), although unlikely, it may be possible that this decrease in MD is being driven by an athlete whose concussion went unreported.

When considered collectively, these seemingly paradoxical changes could be due to an acute response to sudden onset of increased head impact frequency (i.e., start of the football season), followed by prolonged exposure to subconcussive impacts over several months, resulting in decreased FA and increased MD, thus implying compromise to WM integrity and axonal injury. It is possible that the change from the PRE to PTC timepoint, followed by a more chronic change of opposite directionality at the POST timepoint could be present non-significantly in other WM tracts. However, there are WM tracts that do not appear to follow this trend, which may be due to limitations of sample size, variable susceptibility of individual WM tracts, or differing timelines of pathophysiological processes. Regardless, additional research is needed to investigate the effect of repetitive SCI at differing timepoints over a season of football.

4.3 Structural Variability

Previous studies have shown widespread and variable changes in diffusion metrics following mTBI or repetitive subconcussion (Asselin et al., 2020; Bazarian et al., 2014; Chun et al., 2015; Henry et al., 2011; Mustafi et al., 2018; Niogi & Mukherjee, 2010).The inconsistencies in these results with respect to the affected WM tracts is most likely reflective of the heterogenous nature of head impacts (Crisco et al., 2011; Crisco et al., 2012). As the area in which a head impact may occur varies, it follows that so too would the subsequent WM alterations. In the case of SCI, this effect may be amplified, as pathophysiological changes are thought to accumulate silently with each head impact (Mainwaring et al., 2018; McAllister et al., 2014; Slobounov et al., 2017; Talavage et al., 2014), ultimately giving rise to these erratic findings.

However, some WM tracts seem to be more vulnerable than others. Such WM tracts can include but are not limited to the superior longitudinal fasciculus, the corona radiata, the corticospinal tract, the corpus callosum, the inferior fronto-occipital fasciculus, the anterior and posterior thalamic radiations and the internal capsule (Bazarian et al., 2012; Bazarian et al., 2014; Gajawelli et al., 2013; Herweh et al., 2016; Lancaster et al., 2016; Mayer et al., 2017; McAllister et al., 2014; Sasaki et al., 2014). This is consistent with findings of the present study as several of these WM tracts were found to have significant alterations in diffusion metrics. For example, the corticospinal tracts, left inferior fronto-occipital fasciculus, right superior longitudinal fasciculus and left anterior and right posterior thalamic radiations were all found to have variations in at least one diffusion metric. The cause of the seemingly greater susceptibility of these tracts remains unclear, however greater length and increased complexity of cortical connections and/or terminations may be a factor.

Overall, these results strongly suggest there is a detrimental effect of SCI exposure on WM integrity. Furthermore, our findings indicate that this effect and findings may differ based on factors such as timepoint and exposure level. Additionally, these results strongly suggest this compromise to neuronal integrity and WM microstructure may persist up to at least a month following the conclusion of the football season, and possibly into subsequent seasons. The lack of consensus and limited research regarding the timeline of insult and recovery from neurotrauma, particularly mild neurotrauma such as concussion and SCI, highlights the poor understanding of this injury and its pathophysiology. This emphasizes a need for additional research in this area, specifically the timeline of axonal repair in the absence of clinically detectable symptoms and longitudinal, multi-season studies to elucidate the long-term effects of repetitive SCI on neuronal function and brain health. Additionally, future studies should ideally use larger sample sizes to allow for greater statistical power and evaluation of possible interaction between within- and between-subjects factors (i.e., exposure group-timepoint interaction). However, as the long-term implications are still poorly established, steps to increase safety for football players by minimizing preventable head impacts should be considered. This could be done through limiting contact in practices and/or by optimizing tackling and blocking techniques to protect the head and decrease avoidable subconcussive impacts, mitigating the risk posed to the athlete (Champagne, Distefano, et al., 2019).

4.4 Limitations

4.4.1 Sample size

This study had several limitations. As previously discussed, our sample size was relatively small (n = 22) resulting in decreased statistical power. This increases the chance that our findings could be specific to this sample rather than representative of the population as a whole as the sample is not normally distributed. Moreover, a limited sample size could have hindered our ability to detect effects within certain tracts both between exposure groups and over season timepoints. Small sample sizes with reduced statistical power are a common problem in clinical neuroimaging studies. This, in combination with difficulty controlling for all possible variables (i.e., mid-season injuries) and correcting for multiple comparisons (Cremers, Wager, & Yarkoni, 2017), is a barrier that is frequently faced in these types of studies. Physiologic anatomical variations can further decrease the reliability of studies with a limited sample size. This may decrease the generalizability of the results by failing to establish a robust sample that is truly representative of the population (Poldrack et al., 2017).

As non-parametric statistics were used in this study, examining the interaction between exposure groups and season timepoints was not possible as would be if a two-way mixed ANOVA was used. Further studies should be done to account for pre-season differences in WM integrity between exposure groups, to better understand SCI. Additionally, increasing sample size to allow for the use of parametric statistics would allow for examination of the interaction between within- and between-subjects factors, which was not possible in the current study, due to limited sample size.

4.4.2 Probabilistic Tractography

Secondly, despite the proven advantages of probabilistic tractography, this method comes with inherent limitations. As probabilistic tractography estimates the orientation distribution per voxel, it is thought to produce more precise results than other methods, such as deterministic tractography, which instead estimates the average orientation (Van Hecke et al., 2016). However, probabilistic tractography is still prone to errors, particularly false detection of erroneous connections between structures (Jeurissen, Descoteaux, Mori, & Leemans, 2019; Jeurissen, Leemans, Tournier, Jones, & Sijbers, 2013; Van Hecke et al., 2016). These errors can be limited by introducing waypoint and seed masks to guide tractography algorithms and subsequently increase accuracy (Jeurissen et al., 2019). Unfortunately, implementation of these masks requires prior knowledge of the neuroanatomy in question. Furthermore, masks often have to be drawn manually which is not only laborious, but also prone to human error. We sought to minimize this limitation by using the AutoPtx plugin, which came with pre-drawn masks. However, as these masks were manually drawn by the creators of the plugin, they too could have been subject to some degree of error.

Lastly, an estimated 70% of all WM voxels contain fibers from at least 2 tracts (Jeurissen et al., 2013). This calls into question the validity of probabilistic tractography results as connectivity at the microstructural level is not particularly well defined. Axons often branch unpredictably and as such could enter or exit the anatomic boundaries of a given WM tract at any point. Furthermore, the presence of fibers from multiple WM tracts in a single voxel adds an additional level of complexity to this issue as fibers from different tracts could be simultaneously entering or exiting the confines of another. Overall, this makes defining the anatomic boundaries of a WM tract much more difficult and could decrease overall accuracy (Alexander et al., 2007; Hecke et al., 2016; Tournier et al., 2011). Researchers aim to minimize this potential source of error by using multiple masks to guide tractography algorithms (i.e., seed, waypoint, termination, exclusion) and thresholding probabilistic tractography outputs to remove low probability streamlines.

Chapter 5

Conclusions and Future Directions

5.1 Conclusions

The implications of SCI for contact sport athletes, particularly football players, have recently been of increasing concern with a growing body of evidence showing this form of neurotrauma can cause measurable change in the brain. Many of these studies have been conducted using DTI to explore the consequences of asymptomatic head impacts on the brain's WM tracts. Despite increasing research in this field, the effects of SCI exposure and the duration and long-term repercussions on the brain remain elusive. As such, the present study aimed to characterize the effects of repetitive SCI on WM integrity over a single season of collegiate football in players with low and high SCI exposure. We found that recurrent exposure to SCI can produce measurable change in WM integrity over a relatively short time period, which was observed as significant alterations in diffusion metrics.

Additionally, the results of this study indicate that higher levels of SCI exposure could cause greater compromise to WM integrity that may persist beyond the end of one football season and into the next. The decreased FA and increased MD observed in the present study are likely caused by axonal injury and altered WM microstructure due to a cumulative effect of repetitive SCI. Furthermore, this study has demonstrated FA decreases over the football season and fails to resolve at least one month after in athletes with low and high SCI exposure. As previously stated, this decrease in FA is likely due to compromise to WM integrity and axons that accrued silently over time. These findings are concerning, as the failure of diffusion metrics to normalize and damage to potentially persist could contribute to greater damage to WM tracts over larger time frames.

Lastly, this study showed significantly decreased MD in a single WM tract over a shorter period of time at the beginning of the football season in athletes with greater SCI exposure. This decrease conflicted with other findings showing increased MD and both changes were found in athletes with higher SCI exposure. This may suggest athletes with higher levels of SCI exposure undergo a more precipitous response (i.e., axonal swelling, interstitial edema) to a sudden onset or increase in head impacts. Although further research is necessary to better understand these seemingly paradoxical effects, this time interval could reflect a period of heightened vulnerability to further head impacts.

These results add to the increasing number of studies demonstrating that SCI, in non-concussed athletes, can cause measurable damage to WM tracts due to the cumulative nature of these impacts. This emphasizes the importance of minimizing exposure to ostensibly benign head impacts as the effects are not well understood and could help to mitigate the risk posed to athletes. Furthermore, the long-term implications of SCI are not well established, but are thought to elevate risk for neurocognitive impairment or development of neurodegenerative diseases, such as CTE, later in life (McKee, Alosco, et al., 2016; Montenigro et al., 2017). As such, reducing the amount of head impacts an athlete sustains may help to diminish the risk of long-term effects in the future. Although there has been substantial progress into the effects of SCI on the brain, there is still a need for further research to better characterize and understand this type of neurotrauma, particularly the point at which it becomes harmful and the duration of WM injury.

5.2 Future Directions

This study provides further insight into the effects of repetitive SCI exposure on WM integrity over a single season of football but does not explore the effects of SCI over multiple seasons in the same athletes. These results justify future studies to better understand the cumulative nature of SCI, and if athletes accrue damage that carries from one season to the next.

Although results from this study showed that changes in diffusion metrics persisted at least one month after the football season, it did not examine changes at other crucial timepoints. Therefore, studies investigating additional timepoints, such as those immediately following or multiple months after the end of a season, could provide valuable information into the pathophysiological timeline of SCI, resolution of WM injury and duration of these effects.

Additional studies should be completed with a control group to better understand the effects of SCI on WM integrity in contact sport athletes. This would help to further elucidate the effects of impact exposure frequency (i.e., high and low exposure groups) in comparison to a group of non-contact athletes. Furthermore, use of a control group could help to account for the effects of physical activity on the brain, which has been previously demonstrated to produce measurable change in diffusion metrics (Clark et al., 2019; Haeger, Costa, Schulz, & Reetz, 2019; Islam et al., 2020).

Lastly, while this study investigated the effects of SCI on FA and MD, there are other diffusion metrics that were not included in the analysis. Despite less frequent use in DTI research, changes in AD and RD often drive observed changes in FA and MD (Kulikova et al., 2015; Lebel et al., 2012; Sala et al., 2012; Tian & Ma, 2017) and allow for better understanding of factors causing diffusion disruption. As such, future studies using additional diffusion measures such as AD and RD, along with FA and MD could help to provide a more robust picture of how SCI affects WM integrity.

References

Abbas, K., Shenk, T. E., Poole, V. N., Breedlove, E. L., Leverenz, L. J., Nauman, E. A., . . . Robinson, M. E. (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 Connect, 5*(2), 91-101. doi:10.1089/brain.2014.0279

Alexander, A. L., Lee, J. E., Lazar, M., & Field, A. S. (2007). Diffusion tensor imaging of the brain. *Neurotherapeutics, 4*(3), 316-329. doi:10.1016/j.nurt.2007.05.011

Arfanakis, K., Haughton, V. M., Carew, J. D., Rogers, B. P., Dempsey, R. J., & Meyerand, M. E. (2002). Diffusion tensor MR imaging in diffuse axonal injury. *AJNR Am J Neuroradiol, 23*(5), 794-802. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12006280

Asken, B. M., McCrea, M. A., Clugston, J. R., Snyder, A. R., Houck, Z. M., & Bauer, R. M. (2016). "Playing Through It": Delayed Reporting and Removal From Athletic Activity After Concussion Predicts Prolonged Recovery. *J Athl Train, 51*(4), 329-335. doi:10.4085/1062-6050-51.5.02

Assaf, Y., & Pasternak, O. (2008). Diffusion tensor imaging (DTI)-based white matter mapping in brain research: a review. *J Mol Neurosci, 34*(1), 51-61. doi:10.1007/s12031-007-0029-0

Asselin, P. D., Gu, Y., Merchant-Borna, K., Abar, B., Wright, D. W., Qiu, X., & Bazarian, J. J. (2020). Spatial regression analysis of MR diffusion reveals subject-specific white matter changes associated with repetitive head impacts in contact sports. *Sci Rep, 10*(1), 13606. doi:10.1038/s41598-020-70604-y

Bahrami, N., Sharma, D., Rosenthal, S., Davenport, E. M., Urban, J. E., Wagner, B., . . . Maldjian, J. A. (2016). Subconcussive Head Impact Exposure and White Matter Tract Changes over a Single Season of Youth Football. *Radiology, 281*(3), 919-926. doi:10.1148/radiol.2016160564

Bailes, J. E., Petraglia, A. L., Omalu, B. I., Nauman, E., & Talavage, T. (2013). Role of subconcussion in repetitive mild traumatic brain injury. *J Neurosurg, 119*(5), 1235-1245. doi:10.3171/2013.7.JNS121822

Basser, P. J., Mattiello, J., & LeBihan, D. (1994). MR diffusion tensor spectroscopy and imaging. *Biophys J, 66*(1), 259-267. doi:10.1016/S0006-3495(94)80775-1

Bazarian, J. J., Zhong, J., Blyth, B., Zhu, T., Kavcic, V., & Peterson, D. (2007). Diffusion tensor imaging detects clinically important axonal damage after mild traumatic brain injury: a pilot study. *J Neurotrauma, 24*(9), 1447-1459. doi:10.1089/neu.2007.0241

Bazarian, J. J., Zhu, T., Blyth, B., Borrino, A., & Zhong, J. (2012). Subject-specific changes in brain white matter on diffusion tensor imaging after sports-related concussion. *Magn Reson Imaging, 30*(2), 171-180. doi:10.1016/j.mri.2011.10.001

Bazarian, J. J., Zhu, T., Zhong, J., Janigro, D., Rozen, E., Roberts, A., . . . Blackman, E. G. (2014). Persistent, long-term cerebral white matter changes after sports-related repetitive head impacts. *PLoS One, 9*(4), e94734. doi:10.1371/journal.pone.0094734

Behrens, T. E., Berg, H. J., Jbabdi, S., Rushworth, M. F., & Woolrich, M. W. (2007). Probabilistic diffusion tractography with multiple fibre orientations: What can we gain? *Neuroimage, 34*(1), 144-155. doi:10.1016/j.neuroimage.2006.09.018

Bernick, C., Banks, S. J., Shin, W., Obuchowski, N., Butler, S., Noback, M., . . . Modic, M. (2015). Repeated head trauma is associated with smaller thalamic volumes and slower processing speed: the Professional Fighters' Brain Health Study. *Br J Sports Med, 49*(15), 1007-1011. doi:10.1136/bjsports-2014-093877

Black, A. M., Sergio, L. E., & Macpherson, A. K. (2017). The Epidemiology of Concussions: Number and Nature of Concussions and Time to Recovery Among Female and Male Canadian Varsity Athletes 2008 to 2011. *Clin J Sport Med, 27*(1), 52-56. doi:10.1097/JSM.0000000000000308

Blennow, K., Hardy, J., & Zetterberg, H. (2012). The neuropathology and neurobiology of traumatic brain injury. *Neuron, 76*(5), 886-899. doi:10.1016/j.neuron.2012.11.021

Broglio, S. P., Sosnoff, J. J., Shin, S., He, X., Alcaraz, C., & Zimmerman, J. (2009). Head impacts during high school football: a biomechanical assessment. *J Athl Train, 44*(4), 342-349. doi:10.4085/1062-6050-44.4.342

Campbell, K. R., Warnica, M. J., Levine, I. C., Brooks, J. S., Laing, A. C., Burkhart, T. A., & Dickey, J. P. (2016). Laboratory Evaluation of the gForce Tracker, a Head Impact Kinematic Measuring Device for Use in Football Helmets. *Ann Biomed Eng, 44*(4), 1246-1256. doi:10.1007/s10439-015-1391-7

Cantu, R. C. (1996). Head injuries in sport. *Br J Sports Med, 30*(4), 289-296. doi:10.1136/bjsm.30.4.289

Champagne, A. A., Coverdale, N. S., Germuska, M., & Cook, D. J. (2019). Multi-parametric analysis reveals metabolic and vascular effects driving differences in BOLD-based cerebrovascular reactivity associated with a history of sport concussion. *Brain Inj, 33*(11), 1479-1489. doi:10.1080/02699052.2019.1644375

Champagne, A. A., Distefano, V., Boulanger, M. M., Magee, B., Coverdale, N. S., Gallucci, D., . . . Cook, D. J. (2019). Data-informed Intervention Improves Football Technique and Reduces Head Impacts. *Med Sci Sports Exerc, 51*(11), 2366-2374. doi:10.1249/MSS.0000000000002046

Champagne, A. A., Peponoulas, E., Terem, I., Ross, A., Tayebi, M., Chen, Y., . . . Cook, D. J. (2019). Novel strain analysis informs about injury susceptibility of the corpus callosum to repeated impacts. *Brain Commun, 1*(1), fcz021. doi:10.1093/braincomms/fcz021

Choe, M. C. (2016). The Pathophysiology of Concussion. *Curr Pain Headache Rep, 20*(6), 42. doi:10.1007/s11916-016-0573-9

Chu, Z., Wilde, E. A., Hunter, J. V., McCauley, S. R., Bigler, E. D., Troyanskaya, M., . . . Levin, H. S. (2010). Voxel-based analysis of diffusion tensor imaging in mild traumatic brain injury in adolescents. *AJNR Am J Neuroradiol, 31*(2), 340-346. doi:10.3174/ajnr.A1806

Chun, I. Y., Mao, X., Breedlove, E. L., Leverenz, L. J., Nauman, E. A., & Talavage, T. M. (2015). DTI Detection of Longitudinal WM Abnormalities Due to Accumulated Head Impacts. *Dev Neuropsychol, 40*(2), 92-97. doi:10.1080/87565641.2015.1020945

Clark, C. M., Guadagni, V., Mazerolle, E. L., Hill, M., Hogan, D. B., Pike, G. B., & Poulin, M. J. (2019). Effect of aerobic exercise on white matter microstructure in the aging brain. *Behav Brain Res, 373*, 112042. doi:10.1016/j.bbr.2019.112042

Cortes, N., Lincoln, A. E., Myer, G. D., Hepburn, L., Higgins, M., Putukian, M., & Caswell, S. V. (2017). Video Analysis Verification of Head Impact Events Measured by Wearable Sensors. *Am J Sports Med, 45*(10), 2379-2387. doi:10.1177/0363546517706703

Cremers, H. R., Wager, T. D., & Yarkoni, T. (2017). The relation between statistical power and inference in fMRI. *PLoS One, 12*(11), e0184923. doi:10.1371/journal.pone.0184923

Crisco, J. J., Wilcox, B. J., Beckwith, J. G., Chu, J. J., Duhaime, A. C., Rowson, S., . . . Greenwald, R. M. (2011). Head impact exposure in collegiate football players. *J Biomech, 44*(15), 2673-2678. doi:10.1016/j.jbiomech.2011.08.003

Crisco, J. J., Wilcox, B. J., Machan, J. T., McAllister, T. W., Duhaime, A. C., Duma, S. M., . . . Greenwald, R. M. (2012). Magnitude of head impact exposures in individual collegiate football players. *J Appl Biomech, 28*(2), 174-183. doi:10.1123/jab.28.2.174

Davenport, E. M., Whitlow, C. T., Urban, J. E., Espeland, M. A., Jung, Y., Rosenbaum, D. A., . . . Maldjian, J. A. (2014). Abnormal white matter integrity related to head impact exposure in a season of high school varsity football. *J Neurotrauma, 31*(19), 1617-1624. doi:10.1089/neu.2013.3233

de Groot, M., Vernooij, M. W., Klein, S., Ikram, M. A., Vos, F. M., Smith, S. M., . . . Andersson, J. L. (2013). Improving alignment in Tract-based spatial statistics: evaluation and optimization of image registration. *Neuroimage, 76*, 400-411. doi:10.1016/j.neuroimage.2013.03.015

Deary, I. J., Ritchie, S. J., Munoz Maniega, S., Cox, S. R., Valdes Hernandez, M. C., Luciano, M., . . . Bastin, M. E. (2019). Brain Peak Width of Skeletonized Mean Diffusivity (PSMD) and Cognitive Function in Later Life. *Front Psychiatry, 10*, 524. doi:10.3389/fpsyt.2019.00524

DiCesare, C. A., Green, B., Yuan, W., Diekfuss, J. A., Barber Foss, K. D., Dudley, J., . . . Myer, G. D. (2020). Machine Learning Classification of Verified Head Impact Exposure Strengthens

Associations with Brain Changes. *Ann Biomed Eng, 48*(12), 2772-2782. doi:10.1007/s10439-020-02662-2
Elbin, R. J., Sufrinko, A., Schatz, P., French, J., Henry, L., Burkhart, S., . . . Kontos, A. P. (2016). Removal From Play After Concussion and Recovery Time. *Pediatrics, 138*(3). doi:10.1542/peds.2016-0910
Gajawelli, N., Lao, Y., Apuzzo, M. L., Romano, R., Liu, C., Tsao, S., . . . Law, M. (2013). Neuroimaging changes in the brain in contact versus noncontact sport athletes using diffusion tensor imaging. *World Neurosurg, 80*(6), 824-828. doi:10.1016/j.wneu.2013.10.020
Garrick, J. G. (2005). Unreported concussion in high school football players. *Clin J Sport Med, 15*(5), 385; author reply 385. doi:10.1097/01.jsm.0000181438.48892.1b
Giza, C. C., & Hovda, D. A. (2001). The Neurometabolic Cascade of Concussion. *J Athl Train, 36*(3), 228-235. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/12937489
Giza, C. C., & Hovda, D. A. (2014). The new neurometabolic cascade of concussion. *Neurosurgery, 75 Suppl 4*, S24-33. doi:10.1227/NEU.0000000000000505
Glenn, T. C., Kelly, D. F., Boscardin, W. J., McArthur, D. L., Vespa, P., Oertel, M., . . . Martin, N. A. (2003). Energy dysfunction as a predictor of outcome after moderate or severe head injury: indices of oxygen, glucose, and lactate metabolism. *J Cereb Blood Flow Metab, 23*(10), 1239-1250. doi:10.1097/01.WCB.0000089833.23606.7F
Greve, D. N., & Fischl, B. (2009). Accurate and robust brain image alignment using boundary-based registration. *Neuroimage, 48*(1), 63-72. doi:10.1016/j.neuroimage.2009.06.060
Haeger, A., Costa, A. S., Schulz, J. B., & Reetz, K. (2019). Cerebral changes improved by physical activity during cognitive decline: A systematic review on MRI studies. *Neuroimage Clin, 23*, 101933. doi:10.1016/j.nicl.2019.101933
Hecke, W. V., Emsell, L., & Sunaert, S. (2016). *Diffusion Tensor Imaging - A Practical Handbook*: Springer.
Hellewell, S. C., Nguyen, V. P. B., Jayasena, R. N., Welton, T., & Grieve, S. M. (2020). Characteristic patterns of white matter tract injury in sport-related concussion: An image based meta-analysis. *Neuroimage Clin, 26*, 102253. doi:10.1016/j.nicl.2020.102253
Henry, L. C., Tremblay, J., Tremblay, S., Lee, A., Brun, C., Lepore, N., . . . Lassonde, M. (2011). Acute and chronic changes in diffusivity measures after sports concussion. *J Neurotrauma, 28*(10), 2049-2059. doi:10.1089/neu.2011.1836
Herweh, C., Hess, K., Meyding-Lamade, U., Bartsch, A. J., Stippich, C., Jost, J., . . . Hahnel, S. (2016). Reduced white matter integrity in amateur boxers. *Neuroradiology, 58*(9), 911-920. doi:10.1007/s00234-016-1705-y
Horsfield, M. A., & Jones, D. K. (2002). Applications of diffusion-weighted and diffusion tensor MRI to white matter diseases - a review. *NMR Biomed, 15*(7-8), 570-577. doi:10.1002/nbm.787
Huppi, P. S., & Dubois, J. (2006). Diffusion tensor imaging of brain development. *Semin Fetal Neonatal Med, 11*(6), 489-497. doi:10.1016/j.siny.2006.07.006
Inglese, M., Makani, S., Johnson, G., Cohen, B. A., Silver, J. A., Gonen, O., & Grossman, R. I. (2005). Diffuse axonal injury in mild traumatic brain injury: a diffusion tensor imaging study. *J Neurosurg, 103*(2), 298-303. doi:10.3171/jns.2005.103.2.0298
Islam, M. R., Luo, R., Valaris, S., Haley, E. B., Takase, H., Chen, Y. I., . . . Wrann, C. D. (2020). Diffusion tensor-MRI detects exercise-induced neuroplasticity in the hippocampal microstructure in mice. *Brain Plast, 5*(2), 147-159. doi:10.3233/BPL-190090
Jenkinson, M., Bannister, P., Brady, M., & Smith, S. (2002). Improved optimization for the robust and accurate linear registration and motion correction of brain images. *Neuroimage, 17*(2), 825-841. doi:10.1016/s1053-8119(02)91132-8
Jenkinson, M., Beckmann, C. F., Behrens, T. E., Woolrich, M. W., & Smith, S. M. (2012). Fsl. *Neuroimage, 62*(2), 782-790. doi:10.1016/j.neuroimage.2011.09.015
Jenkinson, M., & Smith, S. (2001). A global optimisation method for robust affine registration of brain images. *Med Image Anal, 5*(2), 143-156. doi:10.1016/s1361-8415(01)00036-6

Jesper L. R. Andersson, M. J., Stephen Smith. (2007). *Non-linear registration aka Spatial Normalisation*. FMRIB Technial Report. Oxford, United Kingdom.
Jeurissen, B., Descoteaux, M., Mori, S., & Leemans, A. (2019). Diffusion MRI fiber tractography of the brain. *NMR Biomed, 32*(4), e3785. doi:10.1002/nbm.3785
Jeurissen, B., Leemans, A., Tournier, J. D., Jones, D. K., & Sijbers, J. (2013). Investigating the prevalence of complex fiber configurations in white matter tissue with diffusion magnetic resonance imaging. *Hum Brain Mapp, 34*(11), 2747-2766. doi:10.1002/hbm.22099
Koerte, I. K., Lin, A. P., Willems, A., Muehlmann, M., Hufschmidt, J., Coleman, M. J., . . . Shenton, M. E. (2015). A review of neuroimaging findings in repetitive brain trauma. *Brain Pathol, 25*(3), 318-349. doi:10.1111/bpa.12249
Kulikova, S., Hertz-Pannier, L., Dehaene-Lambertz, G., Buzmakov, A., Poupon, C., & Dubois, J. (2015). Multi-parametric evaluation of the white matter maturation. *Brain Struct Funct, 220*(6), 3657-3672. doi:10.1007/s00429-014-0881-y
Kuzminski, S. J., Clark, M. D., Fraser, M. A., Haswell, C. C., Morey, R. A., Liu, C., . . . Petrella, J. R. (2018). White Matter Changes Related to Subconcussive Impact Frequency during a Single Season of High School Football. *AJNR Am J Neuroradiol, 39*(2), 245-251. doi:10.3174/ajnr.A5489
Lancaster, M. A., Olson, D. V., McCrea, M. A., Nelson, L. D., LaRoche, A. A., & Muftuler, L. T. (2016). Acute white matter changes following sport-related concussion: A serial diffusion tensor and diffusion kurtosis tensor imaging study. *Hum Brain Mapp, 37*(11), 3821-3834. doi:10.1002/hbm.23278
Langlois, J. A., Rutland-Brown, W., & Wald, M. M. (2006). The epidemiology and impact of traumatic brain injury: a brief overview. *J Head Trauma Rehabil, 21*(5), 375-378. doi:10.1097/00001199-200609000-00001
Lebel, C., Gee, M., Camicioli, R., Wieler, M., Martin, W., & Beaulieu, C. (2012). Diffusion tensor imaging of white matter tract evolution over the lifespan. *Neuroimage, 60*(1), 340-352. doi:10.1016/j.neuroimage.2011.11.094
Lebel, C., Walker, L., Leemans, A., Phillips, L., & Beaulieu, C. (2008). Microstructural maturation of the human brain from childhood to adulthood. *Neuroimage, 40*(3), 1044-1055. doi:10.1016/j.neuroimage.2007.12.053
MacFarlane, M. P., & Glenn, T. C. (2015). Neurochemical cascade of concussion. *Brain Inj, 29*(2), 139-153. doi:10.3109/02699052.2014.965208
Mainwaring, L., Ferdinand Pennock, K. M., Mylabathula, S., & Alavie, B. Z. (2018). Subconcussive head impacts in sport: A systematic review of the evidence. *Int J Psychophysiol, 132*(Pt A), 39-54. doi:10.1016/j.ijpsycho.2018.01.007
Martin, G. (2016). Traumatic brain injury: The first 15 milliseconds. *Brain Inj, 30*(13-14), 1517-1524. doi:10.1080/02699052.2016.1192683
Martland, H. (1928). Punch Drunk. *Journal of the American Medical Association*. doi:doi:10.1001/jama.1928.02700150029009
Maruta, J., Mallott, J. M., Sulioti, G., Ghajar, J., Palacios, E. M., & Mukherjee, P. (2020). Concussion Disrupts Normal Brain White Matter Microstructural Symmetry. *Front Neurol, 11*, 548220. doi:10.3389/fneur.2020.548220
Mayer, A. R., Ling, J., Mannell, M. V., Gasparovic, C., Phillips, J. P., Doezema, D., . . . Yeo, R. A. (2010). A prospective diffusion tensor imaging study in mild traumatic brain injury. *Neurology, 74*(8), 643-650. doi:10.1212/WNL.0b013e3181d0ccdd
Mayer, A. R., Ling, J. M., Dodd, A. B., Meier, T. B., Hanlon, F. M., & Klimaj, S. D. (2017). A prospective microstructure imaging study in mixed-martial artists using geometric measures and diffusion tensor imaging: methods and findings. *Brain Imaging Behav, 11*(3), 698-711. doi:10.1007/s11682-016-9546-1

Mayinger, M. C., Merchant-Borna, K., Hufschmidt, J., Muehlmann, M., Weir, I. R., Rauchmann, B. S., . . . Bazarian, J. J. (2018). White matter alterations in college football players: a longitudinal diffusion tensor imaging study. *Brain Imaging Behav, 12*(1), 44-53. doi:10.1007/s11682-017-9672-4

McAllister, T. W., Ford, J. C., Flashman, L. A., Maerlender, A., Greenwald, R. M., Beckwith, J. G., . . . Jain, S. (2014). Effect of head impacts on diffusivity measures in a cohort of collegiate contact sport athletes. *Neurology, 82*(1), 63-69. doi:10.1212/01.wnl.0000438220.16190.42

McCrory, P., Meeuwisse, W. H., Aubry, M., Cantu, B., Dvorak, J., Echemendia, R. J., . . . Turner, M. (2013). Consensus statement on concussion in sport: the 4th International Conference on Concussion in Sport held in Zurich, November 2012. *Br J Sports Med, 47*(5), 250-258. doi:10.1136/bjsports-2013-092313

McCrory, P., Meeuwisse, W. H., Dvorak, J., Echemendia, R. J., Engebretsen, L., Feddermann-Demont, N., . . . Sills, A. K. (2017). 5th International Conference on Concussion in Sport (Berlin). *Br J Sports Med, 51*(11), 837. doi:10.1136/bjsports-2017-097878

McKee, A. C., Alosco, M. L., & Huber, B. R. (2016). Repetitive Head Impacts and Chronic Traumatic Encephalopathy. *Neurosurg Clin N Am, 27*(4), 529-535. doi:10.1016/j.nec.2016.05.009

McKee, A. C., Cairns, N. J., Dickson, D. W., Folkerth, R. D., Keene, C. D., Litvan, I., . . . group, T. C. (2016). The first NINDS/NIBIB consensus meeting to define neuropathological criteria for the diagnosis of chronic traumatic encephalopathy. *Acta Neuropathol, 131*(1), 75-86. doi:10.1007/s00401-015-1515-z

McKee, A. C., Cantu, R. C., Nowinski, C. J., Hedley-Whyte, E. T., Gavett, B. E., Budson, A. E., . . . Stern, R. A. (2009). Chronic traumatic encephalopathy in athletes: progressive tauopathy after repetitive head injury. *J Neuropathol Exp Neurol, 68*(7), 709-735. doi:10.1097/NEN.0b013e3181a9d503

McKee, A. C., & Daneshvar, D. H. (2015). The neuropathology of traumatic brain injury. *Handb Clin Neurol, 127,* 45-66. doi:10.1016/B978-0-444-52892-6.00004-0

McKee, A. C., Daneshvar, D. H., Alvarez, V. E., & Stein, T. D. (2014). The neuropathology of sport. *Acta Neuropathol, 127*(1), 29-51. doi:10.1007/s00401-013-1230-6

McKee, A. C., Stern, R. A., Nowinski, C. J., Stein, T. D., Alvarez, V. E., Daneshvar, D. H., . . . Cantu, R. C. (2013). The spectrum of disease in chronic traumatic encephalopathy. *Brain, 136*(Pt 1), 43-64. doi:10.1093/brain/aws307

Messe, A., Caplain, S., Pelegrini-Issac, M., Blancho, S., Montreuil, M., Levy, R., . . . Benali, H. (2012). Structural integrity and postconcussion syndrome in mild traumatic brain injury patients. *Brain Imaging Behav, 6*(2), 283-292. doi:10.1007/s11682-012-9159-2

Metwalli, N. S., Benatar, M., Nair, G., Usher, S., Hu, X., & Carew, J. D. (2010). Utility of axial and radial diffusivity from diffusion tensor MRI as markers of neurodegeneration in amyotrophic lateral sclerosis. *Brain Res, 1348,* 156-164. doi:10.1016/j.brainres.2010.05.067

Miles, L., Grossman, R. I., Johnson, G., Babb, J. S., Diller, L., & Inglese, M. (2008). Short-term DTI predictors of cognitive dysfunction in mild traumatic brain injury. *Brain Inj, 22*(2), 115-122. doi:10.1080/02699050801888816

Millspaugh, J. (1937). Dementia pugilistica. *US Navy Medical Bullitin*(35), 297-303.

Montenigro, P. H., Alosco, M. L., Martin, B. M., Daneshvar, D. H., Mez, J., Chaisson, C. E., . . . Tripodis, Y. (2017). Cumulative Head Impact Exposure Predicts Later-Life Depression, Apathy, Executive Dysfunction, and Cognitive Impairment in Former High School and College Football Players. *J Neurotrauma, 34*(2), 328-340. doi:10.1089/neu.2016.4413

Moon, D. W., Beedle, C. W., & Kovacic, C. R. (1971). Peak head acceleration of athletes during competition--football. *Med Sci Sports, 3*(1), 44-50. doi:10.1249/00005768-197100310-00008

Murugavel, M., Cubon, V., Putukian, M., Echemendia, R., Cabrera, J., Osherson, D., & Dettwiler, A. (2014). A longitudinal diffusion tensor imaging study assessing white matter fiber tracts after sports-related concussion. *J Neurotrauma, 31*(22), 1860-1871. doi:10.1089/neu.2014.3368

Mustafi, S. M., Harezlak, J., Koch, K. M., Nencka, A. S., Meier, T. B., West, J. D., . . . Wu, Y. C. (2018). Acute White-Matter Abnormalities in Sports-Related Concussion: A Diffusion Tensor Imaging

Study from the NCAA-DoD CARE Consortium. *J Neurotrauma, 35*(22), 2653-2664. doi:10.1089/neu.2017.5158
Niogi, S. N., Luther, N., Kutner, K., Shetty, T., McCrea, H. J., Barnes, R., . . . Hartl, R. (2019). Increased sensitivity to traumatic axonal injury on postconcussion diffusion tensor imaging scans in National Football League players by using premorbid baseline scans. *J Neurosurg*, 1-9. doi:10.3171/2019.3.JNS181864
Niogi, S. N., & Mukherjee, P. (2010). Diffusion tensor imaging of mild traumatic brain injury. *J Head Trauma Rehabil, 25*(4), 241-255. doi:10.1097/HTR.0b013e3181e52c2a
Omalu, B., Bailes, J., Hamilton, R. L., Kamboh, M. I., Hammers, J., Case, M., & Fitzsimmons, R. (2011). Emerging histomorphologic phenotypes of chronic traumatic encephalopathy in American athletes. *Neurosurgery, 69*(1), 173-183; discussion 183. doi:10.1227/NEU.0b013e318212bc7b
Poldrack, R. A., Baker, C. I., Durnez, J., Gorgolewski, K. J., Matthews, P. M., Munafo, M. R., . . . Yarkoni, T. (2017). Scanning the horizon: towards transparent and reproducible neuroimaging research. *Nat Rev Neurosci, 18*(2), 115-126. doi:10.1038/nrn.2016.167
Povlishock, J. T., & Katz, D. I. (2005). Update of neuropathology and neurological recovery after traumatic brain injury. *J Head Trauma Rehabil, 20*(1), 76-94. doi:10.1097/00001199-200501000-00008
Reid, S. E., Tarkington, J. A., Epstein, H. M., & O'Dea, T. J. (1971). Brain tolerance to impact in football. *Surg Gynecol Obstet, 133*(6), 929-936. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/5117389
Sala, S., Agosta, F., Pagani, E., Copetti, M., Comi, G., & Filippi, M. (2012). Microstructural changes and atrophy in brain white matter tracts with aging. *Neurobiol Aging, 33*(3), 488-498 e482. doi:10.1016/j.neurobiolaging.2010.04.027
Sasaki, T., Pasternak, O., Mayinger, M., Muehlmann, M., Savadjiev, P., Bouix, S., . . . Koerte, I. K. (2014). Hockey Concussion Education Project, Part 3. White matter microstructure in ice hockey players with a history of concussion: a diffusion tensor imaging study. *J Neurosurg, 120*(4), 882-890. doi:10.3171/2013.12.JNS132092
Shenton, M. E., Hamoda, H. M., Schneiderman, J. S., Bouix, S., Pasternak, O., Rathi, Y., . . . Zafonte, R. (2012). A review of magnetic resonance imaging and diffusion tensor imaging findings in mild traumatic brain injury. *Brain Imaging Behav, 6*(2), 137-192. doi:10.1007/s11682-012-9156-5
Shultz, S. R., MacFabe, D. F., Foley, K. A., Taylor, R., & Cain, D. P. (2012). Sub-concussive brain injury in the Long-Evans rat induces acute neuroinflammation in the absence of behavioral impairments. *Behav Brain Res, 229*(1), 145-152. doi:10.1016/j.bbr.2011.12.015
Slobounov, S. M., Walter, A., Breiter, H. C., Zhu, D. C., Bai, X., Bream, T., . . . 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
Smith, S. M., Jenkinson, M., Woolrich, M. W., Beckmann, C. F., Behrens, T. E., Johansen-Berg, H., . . . Matthews, P. M. (2004). Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage, 23 Suppl 1*, S208-219. doi:10.1016/j.neuroimage.2004.07.051
Soares, J. M., Marques, P., Alves, V., & Sousa, N. (2013). A hitchhiker's guide to diffusion tensor imaging. *Front Neurosci, 7*, 31. doi:10.3389/fnins.2013.00031
Solomon, G. S., & Zuckerman, S. L. (2015). Chronic traumatic encephalopathy in professional sports: retrospective and prospective views. *Brain Inj, 29*(2), 164-170. doi:10.3109/02699052.2014.965205
Steenerson, K., & Starling, A. J. (2017). Pathophysiology of Sports-Related Concussion. *Neurol Clin, 35*(3), 403-408. doi:10.1016/j.ncl.2017.03.011
Stern, R. A., Riley, D. O., Daneshvar, D. H., Nowinski, C. J., Cantu, R. C., & McKee, A. C. (2011). Long-term consequences of repetitive brain trauma: chronic traumatic encephalopathy. *PM R, 3*(10 Suppl 2), S460-467. doi:10.1016/j.pmrj.2011.08.008

Stojanovski, S., Nazeri, A., Lepage, C., Ameis, S., Voineskos, A. N., & Wheeler, A. L. (2019). Microstructural abnormalities in deep and superficial white matter in youths with mild traumatic brain injury. *Neuroimage Clin, 24*, 102102. doi:10.1016/j.nicl.2019.102102

Svaldi, D. O., Joshi, C., McCuen, E. C., Music, J. P., Hannemann, R., Leverenz, L. J., . . . Talavage, T. M. (2020). Accumulation of high magnitude acceleration events predicts cerebrovascular reactivity changes in female high school soccer athletes. *Brain Imaging Behav, 14*(1), 164-174. doi:10.1007/s11682-018-9983-0

Talavage, T. M., Nauman, E. A., Breedlove, E. L., Yoruk, U., Dye, A. E., Morigaki, K. E., . . . Leverenz, L. J. (2014). Functionally-detected cognitive impairment in high school football players without clinically-diagnosed concussion. *J Neurotrauma, 31*(4), 327-338. doi:10.1089/neu.2010.1512

Tayebi, M., Holdsworth, S. J., Champagne, A. A., Cook, D. J., Nielsen, P., Lee, T. R., . . . Shim, V. (2021). The role of diffusion tensor imaging in characterizing injury patterns on athletes with concussion and subconcussive injury: a systematic review. *Brain Inj, 35*(6), 621-644. doi:10.1080/02699052.2021.1895313

Tian, L., & Ma, L. (2017). Microstructural Changes of the Human Brain from Early to Mid-Adulthood. *Front Hum Neurosci, 11*, 393. doi:10.3389/fnhum.2017.00393

Tournier, J. D., Mori, S., & Leemans, A. (2011). Diffusion tensor imaging and beyond. *Magn Reson Med, 65*(6), 1532-1556. doi:10.1002/mrm.22924

Van Hecke, W., Emsell, L., & Sunaert, S. (2016). *Diffusion tensor imaging : a practical handbook*. New York ; Heidelberg: Springer.

Vinciguerra, C., Giorgio, A., Zhang, J., Nardone, V., Brocci, R. T., Pasto, L., . . . De Stefano, N. (2020). Peak width of skeletonized mean diffusivity (PSMD) and cognitive functions in relapsing-remitting multiple sclerosis. *Brain Imaging Behav*. doi:10.1007/s11682-020-00394-4

Woolrich, M. W., Jbabdi, S., Patenaude, B., Chappell, M., Makni, S., Behrens, T., . . . Smith, S. M. (2009). Bayesian analysis of neuroimaging data in FSL. *Neuroimage, 45*(1 Suppl), S173-186. doi:10.1016/j.neuroimage.2008.10.055

Wu, Y. C., Harezlak, J., Elsaid, N. M. H., Lin, Z., Wen, Q., Mustafi, S. M., . . . McAllister, T. W. (2020). Longitudinal white-matter abnormalities in sports-related concussion: A diffusion MRI study. *Neurology, 95*(7), e781-e792. doi:10.1212/WNL.0000000000009930

Appendix A

WM Tracts with Non-significant Variations in FA

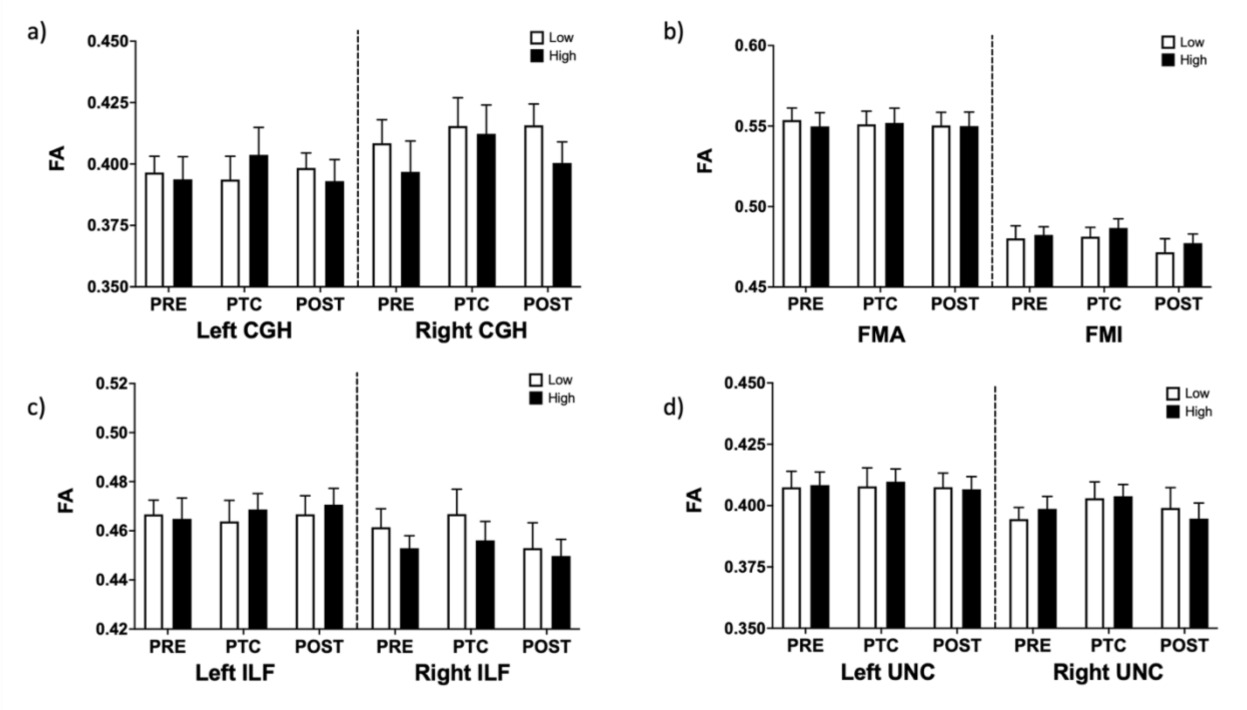

Figure 1. No significant change in FA between HE and LE groups at season timepoints. Data represents the mean ± SEM FA of high and low exposure groups at PRE-, PTC and POST-season timepoints. Mann-Whitney U tests were used to examine differences between high and low exposure groups at each timepoint (n = 11). No significant differences were found at any timepoints in the **A)** left and right CGH, **B)** FMA and FMI, **C)** left and right ILF or **D)** left and right UNC.

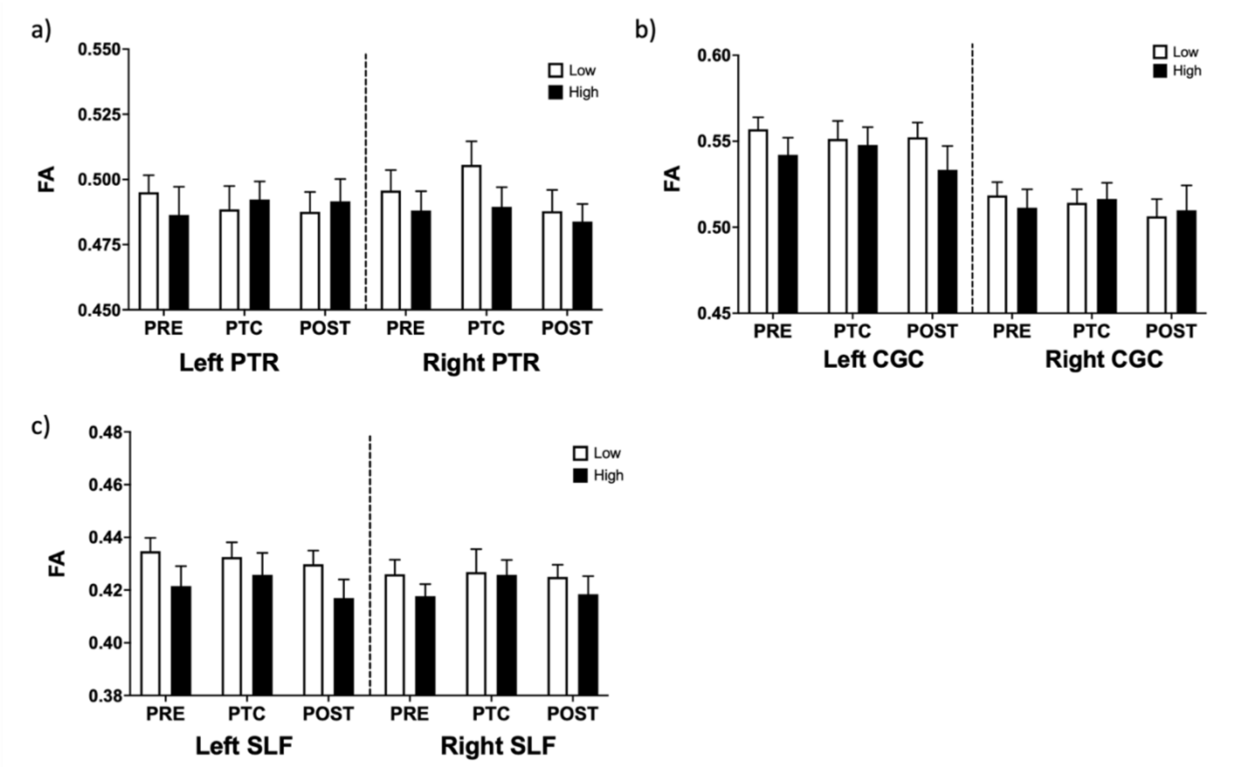

Figure 2. Non-significant alterations in FA between HE and LE groups at season timepoints. Data represents the mean ± SEM FA of high and low exposure groups at PRE-, PTC and POST-season timepoints. Mann-Whitney U tests were conducted to investigate variations between exposure groups at each season timepoint (n = 11). No significant alterations were found at any timepoints in the **A)** left and right PTR, **B)** left and right CGC or **C)** left and right SLF.

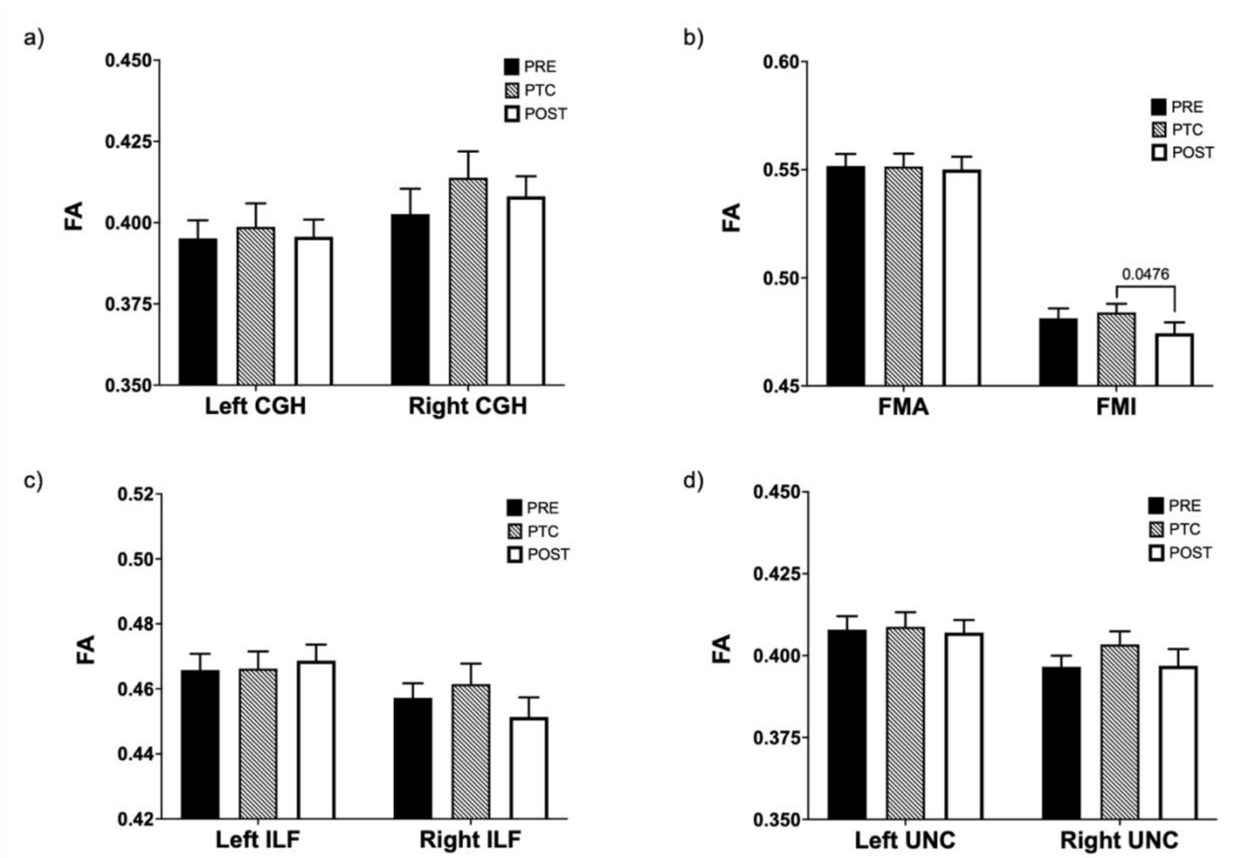

Figure 3. Significant FA reductions in FMI from PTC to POST-season timepoints. Data represents the mean ± SEM FA of PRE-, PTC and POST-season timepoint groups. Results were compared by Friedman tests with a Bonferroni correction for multiple comparisons to assess changes in FA over the PRE-, PTC and POST-season timepoints ($n = 22$) for a single football season. **A)** No significant changes in FA for the left or right CGH over any season timepoints. **B)** Significantly decreased FA from PTC to POST-season timepoints in FMI ($p = 0.0476$) but not FMA. **C)** No significant alterations in FA in the left or right ILF within season timepoints. **D)** The left and right UNC did not show any significant changes in FA over any timepoint.

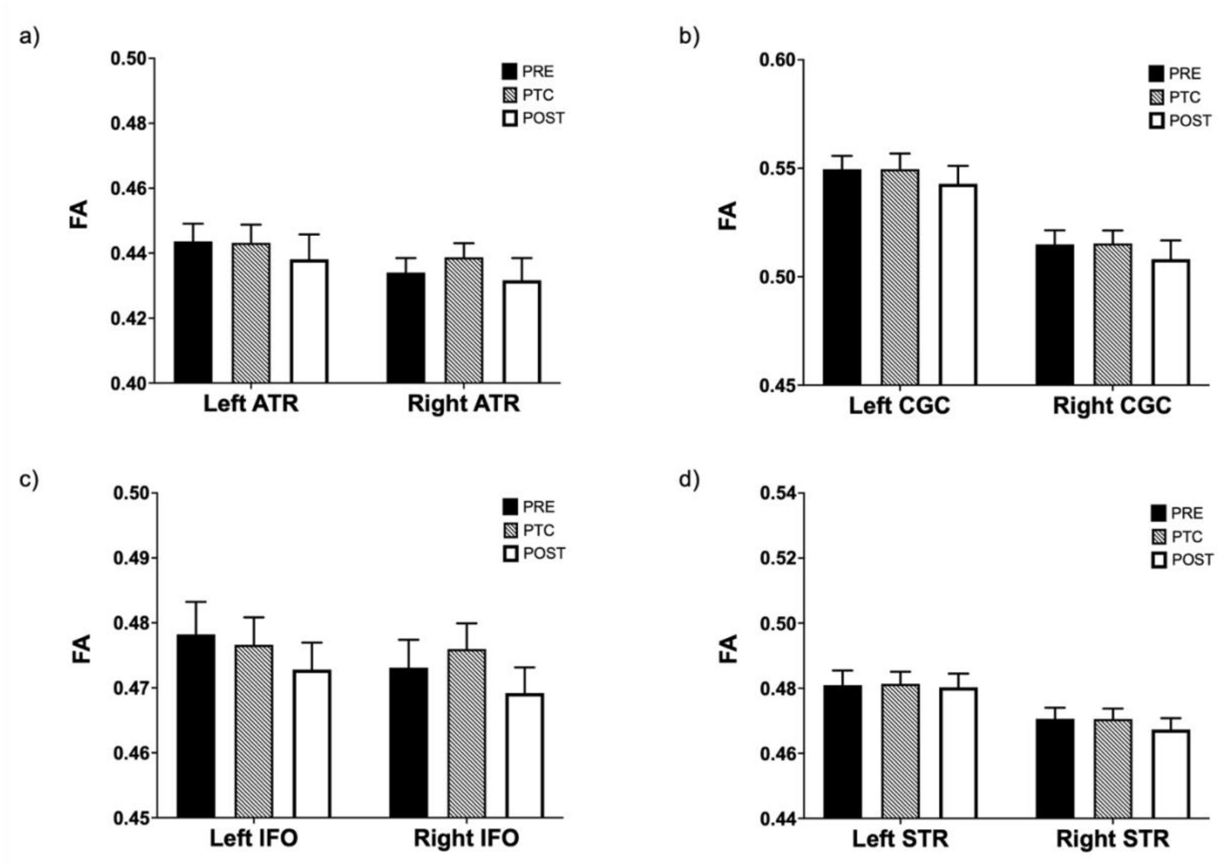

Figure 4. No significant FA changes between any season timepoints. Data represents the mean ± SEM FA of PRE-, PTC and POST-season timepoint groups. Results were compared by Friedman tests with a Bonferroni correction for multiple comparisons to analyze changes in FA over the PRE-, PTC and POST-season timepoints (n = 22) for a single season of football. No significant changes in FA were found at any timepoints for the **A)** left and right ATR, **B)** left and right CGC, **C)** left and right IFO or **D)** left and right STR.

Appendix B

WM Tracts with Non-significant Fluctuations in MD

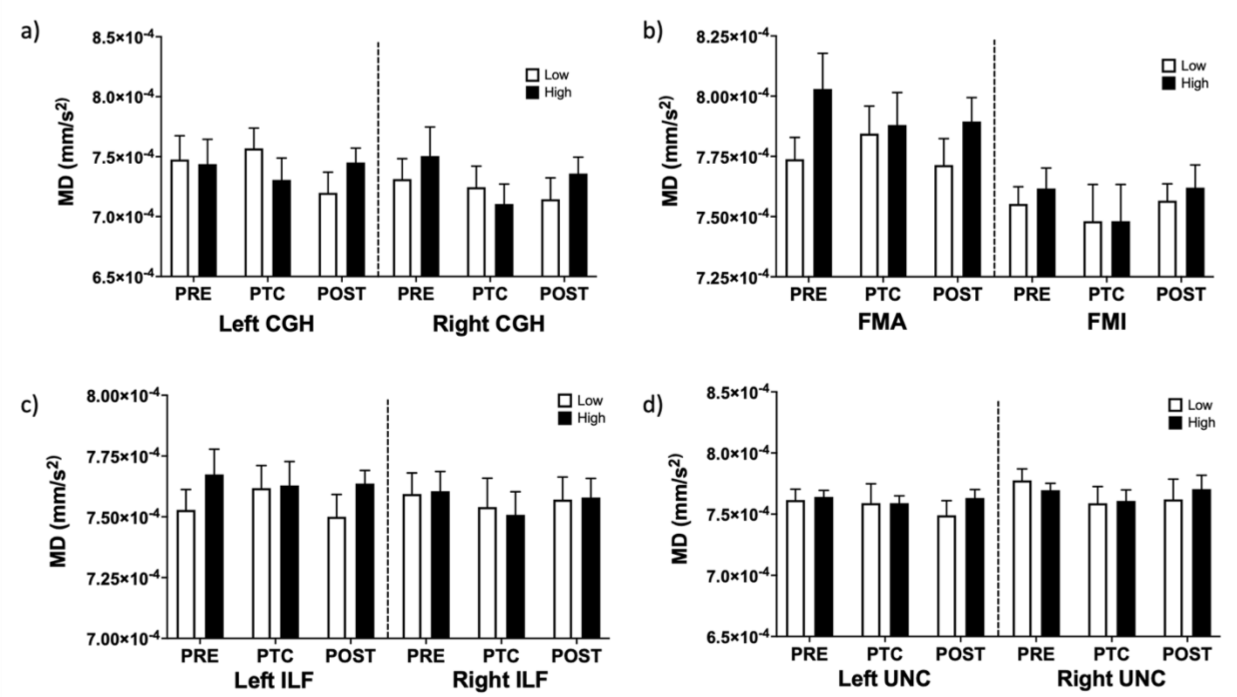

Figure 1. No significant difference in MD observed in either exposure group over season timepoints. Data represents the mean ± SEM MD of high and low exposure groups at each timepoint. Mann-Whitney U tests were used to further determine differences between exposure groups, by examining changes at each timepoint (n = 11). No significant alterations in MD were detected for either exposure group in **A)** the left and right CGH, **B)** FMA, FMI, **C)** the left or right ILF or **D)** the left or right UNC.

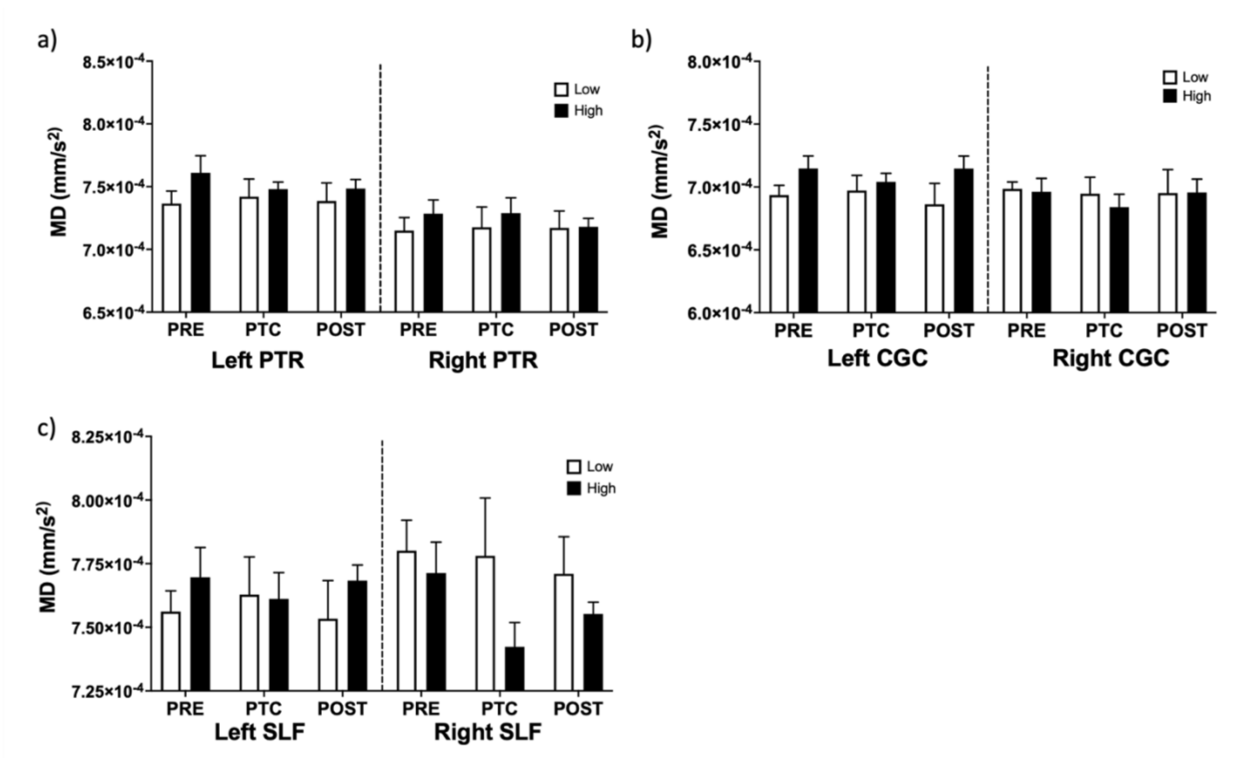

Figure 2. No significant changes in MD found in either exposure group. Data represents the mean ± SEM MD of high and low exposure groups at each timepoint. Mann-Whitney U tests were used to further determine differences between exposure groups, by examining changes at each timepoint (n = 11). No significant differences between exposure groups were found for the **A)** left and right PTR, **B)** left and right CGC or **C)** the left or right SLF.

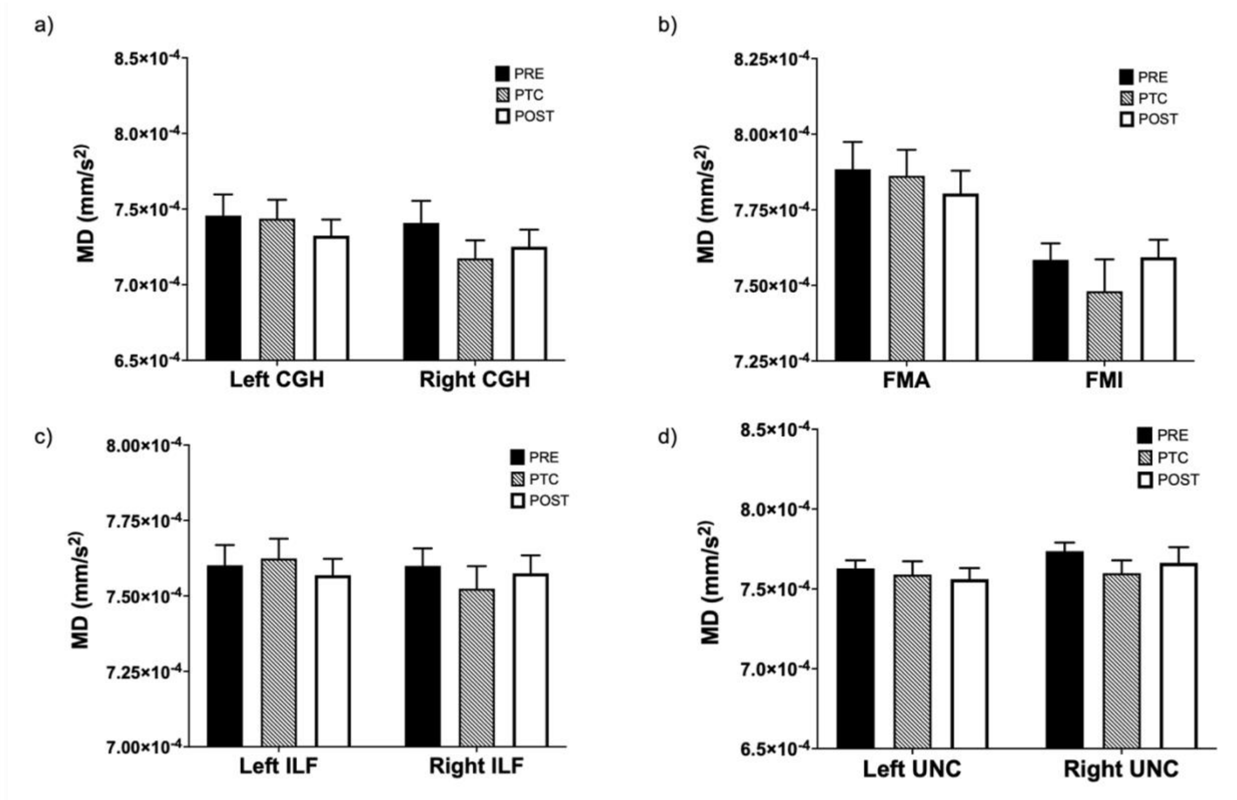

Figure 3. No significant differences in MD found over any season timepoint. Data represents the mean ± SEM MD of PRE, PTC and POST timepoint groups. Results were compared by Friedman tests and using a Bonferroni correction for multiple comparisons to analyze changes in MD over the PRE, PTC and POST timepoints (n = 22) within a single season of football. No significant MD alterations over any season timepoints were found for **A)** the left and right CGH, **B)** FMA or FMI, **C)** the left and right ILF, or **D)** the left or right UNC.

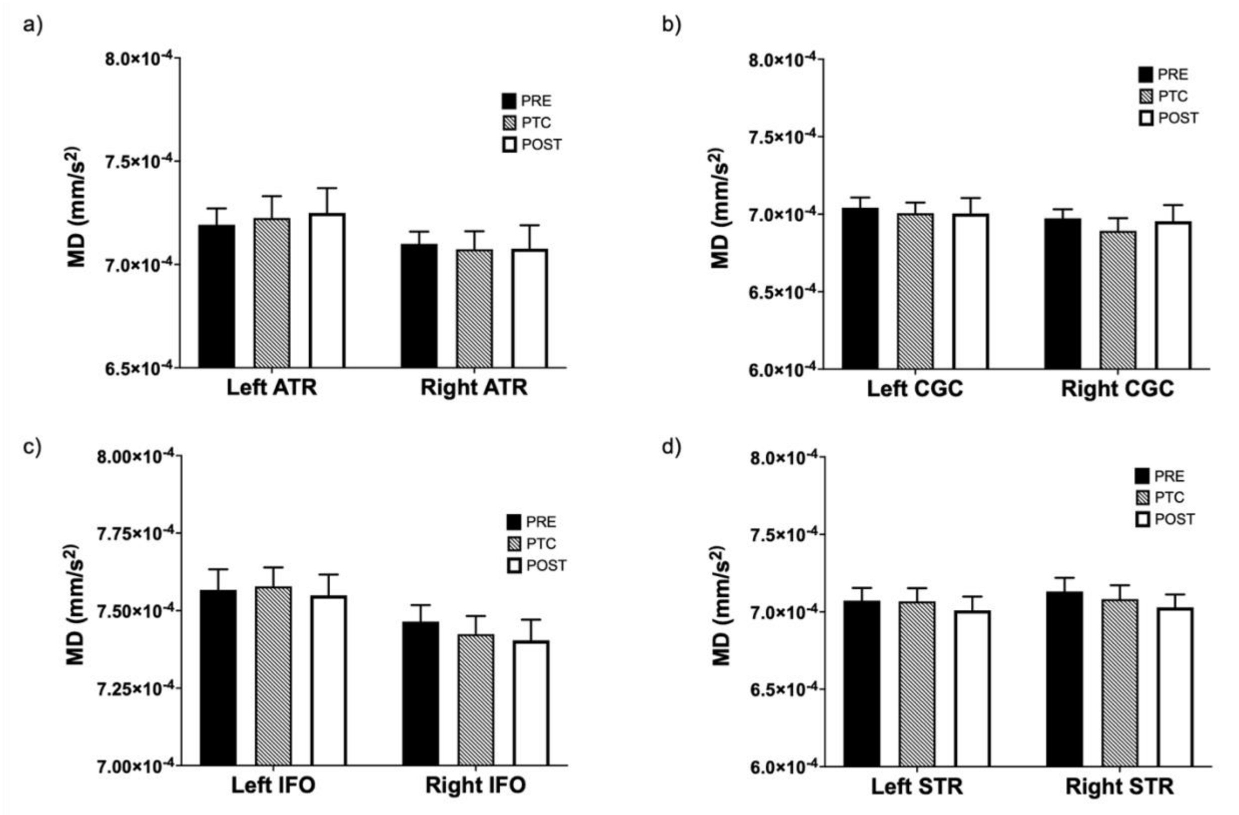

Figure 4. No significance observed in MD differences over season timepoints. Data represents the mean ± SEM MD of PRE, PTC and POST timepoint groups. Results were compared by Friedman tests and using a Bonferroni correction for multiple comparisons to assess variations in MD over the PRE, PTC and POST timepoints (n = 22) within a single season of football. Friedman tests revealed no significant changes in MD within any season timepoints for the left and right **A)** ATR, **B)** CGC, **C)** IFO or **D)** STR.

Appendix C

Descriptive Statistics of FA and MD for All WM Tracts

Table 1. Descriptive statistics of FA measures separated by timepoint and exposure group

	Low-PRE		Low-PTC		Low-POST		High-PRE		High-PTC		High-POST	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
lh.atr	0.455	0.019	0.452	0.021	0.448	0.041	0.432	0.027	0.435	0.029	0.428	0.028
rh.atr	0.441	0.016	0.440	0.019	0.440	0.036	0.427	0.024	0.437	0.022	0.423	0.026
lh.cgc	0.557	0.023	0.551	0.035	0.552	0.028	0.542	0.033	0.548	0.034	0.533	0.046
rh.cgc	0.518	0.026	0.514	0.026	0.506	0.033	0.511	0.035	0.516	0.031	0.510	0.048
lh.cgh	0.397	0.022	0.394	0.031	0.398	0.02	0.394	0.031	0.404	0.037	0.393	0.029
rh.cgh	0.408	0.032	0.415	0.038	0.416	0.029	0.397	0.042	0.412	0.039	0.4	0.028
lh.cst	0.563	0.021	0.559	0.025	0.556	0.012	0.533	0.015	0.546	0.013	0.538	0.018
rh.cst	0.569	0.015	0.561	0.021	0.551	0.014	0.544	0.022	0.552	0.022	0.537	0.024
fma	0.554	0.025	0.551	0.027	0.550	0.027	0.550	0.028	0.552	0.030	0.550	0.029
fmi	0.480	0.026	0.481	0.019	0.472	0.028	0.482	0.017	0.487	0.019	0.477	0.019
lh.ifo	0.482	0.019	0.477	0.015	0.476	0.016	0.475	0.027	0.476	0.024	0.470	0.023
rh.ifo	0.475	0.022	0.479	0.020	0.471	0.019	0.471	0.019	0.473	0.018	0.468	0.019
lh.ilf	0.467	0.019	0.464	0.029	0.467	0.025	0.465	0.028	0.469	0.022	0.471	0.022
rh.ilf	0.461	0.025	0.467	0.034	0.453	0.034	0.453	0.017	0.456	0.025	0.450	0.022
lh.ptr	0.495	0.022	0.489	0.030	0.488	0.025	0.486	0.036	0.492	0.023	0.492	0.029
rh.ptr	0.496	0.026	0.506	0.030	0.488	0.027	0.488	0.025	0.489	0.025	0.484	0.023
lh.slf	0.435	0.017	0.433	0.019	0.430	0.017	0.422	0.025	0.426	0.028	0.417	0.023
rh.slf	0.426	0.018	0.427	0.029	0.425	0.015	0.418	0.015	0.426	0.019	0.418	0.023
lh.str	0.490	0.023	0.484	0.020	0.488	0.019	0.472	0.014	0.478	0.014	0.472	0.017
rh.str	0.477	0.016	0.475	0.017	0.473	0.015	0.464	0.014	0.466	0.013	0.461	0.015
lh.unc	0.407	0.022	0.408	0.025	0.407	0.019	0.408	0.018	0.410	0.017	0.407	0.017
rh.unc	0.395	0.016	0.403	0.022	0.399	0.027	0.399	0.017	0.404	0.016	0.395	0.021

Table 2. Descriptive Statistics of MD Measures ($x10^{-3}$ mm/s^2) divided into exposure and timepoint groups

	Low-PRE		Low-PTC		Low-POST		High-PRE		High-PTC		High-POST	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
lh.atr	0.701	0.033	0.713	0.062	0.706	0.072	0.737	0.033	0.732	0.033	0.744	0.025
rh.atr	0.704	0.029	0.708	0.052	0.695	0.073	0.716	0.027	0.707	0.028	0.720	0.018
lh.cgc	0.694	0.026	0.697	0.040	0.686	0.055	0.715	0.033	0.704	0.023	0.715	0.033

rh.cgc	0.699	0.018	0.695	0.043	0.695	0.062	0.696	0.035	0.684	0.034	0.696	0.035
lh.cgh	0.748	0.066	0.757	0.056	0.720	0.057	0.744	0.069	0.731	0.061	0.745	0.040
rh.cgh	0.731	0.057	0.724	0.059	0.714	0.059	0.750	0.080	0.710	0.055	0.736	0.045
lh.cst	0.661	0.014	0.660	0.030	0.652	0.039	0.696	0.033	0.678	0.032	0.679	0.022
rh.cst	0.645	0.010	0.644	0.016	0.649	0.036	0.678	0.024	0.663	0.037	0.670	0.018
fma	0.774	0.030	0.784	0.038	0.771	0.036	0.803	0.049	0.788	0.045	0.789	0.033
fmi	0.755	0.024	0.748	0.051	0.757	0.023	0.762	0.028	0.751	0.027	0.762	0.031
lh.ifo	0.746	0.023	0.754	0.030	0.743	0.037	0.767	0.036	0.761	0.028	0.767	0.020
rh.ifo	0.739	0.021	0.740	0.031	0.735	0.042	0.754	0.027	0.745	0.025	0.746	0.015
lh.ilf	0.753	0.028	0.762	0.031	0.750	0.030	0.767	0.034	0.763	0.033	0.764	0.018
rh.ilf	0.759	0.029	0.754	0.039	0.757	0.031	0.761	0.027	0.751	0.031	0.758	0.026
lh.ptr	0.737	0.033	0.742	0.047	0.739	0.048	0.761	0.045	0.748	0.019	0.748	0.024
rh.ptr	0.715	0.034	0.718	0.053	0.717	0.045	0.728	0.036	0.729	0.041	0.718	0.022
lh.slf	0.756	0.027	0.763	0.049	0.753	0.050	0.770	0.039	0.761	0.034	0.768	0.020
rh.slf	0.780	0.040	0.778	0.075	0.771	0.048	0.771	0.040	0.742	0.032	0.755	0.015
lh.str	0.691	0.017	0.698	0.041	0.689	0.052	0.724	0.047	0.715	0.038	0.713	0.025
rh.str	0.701	0.025	0.704	0.037	0.697	0.049	0.725	0.052	0.712	0.047	0.709	0.026
lh.unc	0.762	0.029	0.759	0.052	0.749	0.040	0.764	0.018	0.759	0.020	0.763	0.023
rh.unc	0.778	0.031	0.759	0.045	0.762	0.055	0.770	0.019	0.761	0.030	0.770	0.038

Appendix D

Summary of P-values for All WM Tracts and Diffusivity Measures

Table 1. Summary of P values for FA Measures

	Impact Exposure	Timepoint
lh.atr	0.013*	0.057
rh.atr	0.072	0.28
lh.cgc	0.267	0.385
rh.cgc	0.873	0.483
lh.cgh	0.803	0.727
rh.cgh	0.23	0.108
lh.cst	0.00016***	0.607
rh.cst	0.014*	0.012*
fma	0.852	0.554
fmi	0.405	0.032*
lh.ifo	0.327	0.055
rh.ifo	0.359	0.055
lh.ilf	0.974	0.422
rh.ilf	0.284	0.17
lh.ptr	0.803	0.834
rh.ptr	0.216	0.025*
lh.slf	0.142	0.142
rh.slf	0.314	0.422
lh.str	0.004**	0.834
rh.str	0.007**	0.113
lh.unc	0.832	0.554
rh.unc	0.542	0.244

Table 2. Summary of P values for MD measures

	Impact Exposure	Timepoint
lh.atr	0.003**	0.65
rh.atr	0.419	0.727
lh.cgc	0.053	0.834
rh.cgc	0.493	0.385
lh.cgh	0.949	0.566
rh.cgh	0.691	0.293
lh.cst	0.001***	0.678

rh.cst	0.001***	0.17
fma	0.088	0.351
fmi	0.878	0.632
lh.ifo	0.021*	0.966
rh.ifo	0.164	0.483
lh.ilf	0.147	0.566
rh.ilf	0.822	0.339
lh.ptr	0.14	0.664
rh.ptr	0.223	0.786
lh.slf	0.296	0.61
rh.slf	0.089	0.028*
lh.str	0.008**	0.422
rh.str	0.168	0.861
lh.unc	0.305	0.108
rh.unc	0.419	0.607