

**The effect of subconcussive impacts on white matter integrity in the  
corpus callosum and corticospinal tracts**

by

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## **Abstract**

Over the course of a football season the athletes are subject to a large number of impacts ranging in magnitude. With these impacts failing to cause any clinical signs of concussion, they often get disregarded as non-injurious. Recent evidence shows that the cumulative effects of these impacts may cause microstructural damage within the brain. This study set out to examine the effects of these subconcussive impacts on two specific white matter tracts in the brain: the corpus callosum (CC) and the corticospinal tract (CST). 20 Canadian University level football players were followed for one season. These players were examined using diffusion tensor imaging at three time points: prior to any team sessions (preseason), following training camp (post-training camp), and following the last session of the season (post-season). Four DTI measures were analyzed: fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD), in order to assess white matter integrity. Players were separated into two groups based on impact exposure throughout the season. Results showed that the high exposure group had significantly lower FA (main effect of Group) in the CC and the bottom-left section of the CST. There was a significant decrease in FA over time in the top section of the left CST. There were no observed changes in MD, AD or RD. The changes found in this study indicate that despite no outward symptoms of injury (SRC), “silent” changes are occurring within the brain’s microstructures as a result of receiving numerous subconcussive impacts during a season of football.

## **Co-Authorship**

This research project was completed under the supervision of Dr. D.J. Cook of Queen's University. Data collection was facilitated by my colleague Dr. Allen Champagne.

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## **List of Abbreviations**

AD – Axial diffusivity  
CC – Corpus callosum  
CST – Corticospinal tract  
CTE – Chronic traumatic encephalopathy  
DB – Defensive back  
DL – Defensive line  
DTI – Diffusion tensor imaging  
EPI – Echo planar imaging  
FA – Fractional anisotropy  
GFT – gForce Tracker  
GM – Grey matter  
GSI – GADD severity index  
HIC – Head injury criterion  
HITS – Head impact telemetry system  
LA – Linear acceleration  
MD – Mean diffusivity  
MRI – Magnetic resonance imaging  
mTBI – Mild traumatic brain injury  
NCAA – National Collegiate Athletic Association  
NFL – National Football League  
NFT – Neurofibrillary tangles  
OL – Offensive line

PCS = Post-concussion syndrome

QB - Quarterback

RA – Rotational Acceleration

RB – Running back

RD – Radial Diffusivity

RHI – Repeated head impacts

ROI – Region of interest

SRC – Sport-related concussion

TBI – Traumatic brain injury

WM – White matter

WR – Wide receiver

# Chapter 1

## Introduction and Literature Review

### 1.1 Head impacts in football

Over the course of a collegiate football season players will experience a large number of impacts of varying magnitudes to all parts of their body. These impacts may be caused by contact with other players, contact with the ground, or potentially contact with a stationary object (e.g., the goal post). While impacts to the body and limbs may cause musculoskeletal injuries, the most potentially injurious impacts occur to the players' head and neck. Recently, there is growing concern about the short- and long-term consequences of sports-related concussion (SRC), and more specifically, about the consequences of cumulative repetitive subconcussive impacts (Slobounov et al., 2017).

SRC can be characterized as a mild traumatic brain injury (mTBI), induced by biomechanical forces (Mccrory et al., 2017). SRC are either caused by a direct blow to the head or neck, or elsewhere on the body with a resulting impulsive force on the head. SRC often produces the onset of short-term deficiencies in neurological function, that may or may not resolve spontaneously; these symptoms may evolve over the following minutes to hours. According to the US Centers for Disease Control and Prevention, it is estimated that approximately 300 000 SRC occur every year in the United States as a result of playing contact sports (Thurman, Branche, & Sniezek, 1998). On top of that, there remains a large number of SRC which go undiagnosed, and unreported. In contact sports, concussions account for a large percentage of total injuries, accounting for 8% of all football-related

injuries (Dick, 2003). In the US, of all contact sports, football has the highest number of concussions, due to the extremely large number of participants each year at the high school and collegiate levels (Dick, 2003; Powell & Barber-Foss, 1999). The direct relationship between impacts to the head, and mTBI is currently not well understood. The National Football League (NFL) suggested that risk of concussion is directly associated with the peak linear acceleration of the head during an impact (Pellman et al., 2003). Another suggestion was that the location and direction of the impact is a large factor in the mechanisms of concussive injury (Pellman et al., 2003). Guskiewicz and Mihalik (2011) hypothesized that a concussive threshold is difficult to establish due to the varying magnitudes and locations of concussive impacts, as well as other factors, such as frequency of impacts, and concussion history.

### **1.1.1 Long term effects of repeated head impacts**

A growing concern with regards to concussions and subconcussive (an injury from any impact that does not cause a diagnosed SRC) injuries is the lasting and long-term effects on the brain. In the short-term, concussion symptoms consist of sensitivity (noise and light), dizziness, fogginess, irritability, headache and fatigue, and in most cases these symptoms subside in a matter of days or weeks (Hall, Hall, & Chapman, 2005). Post-concussion syndrome (PCS) is used to describe a condition arising after a concussion, with the patient experiencing concussion-like symptoms for an extended period of time. The American Psychiatric Association's (APA) current criteria for PCS is that three or more symptoms must persist at least 3 months following the head injury (Hall et al., 2005). Recent research shows that most people suffering from PCS will fully recover within 3 to 6

months, with only a small portion of people reported symptoms beyond 1 year after injury (Hall et al., 2005; Evans, 1992; Mittenberg & Strauman, 2000). In addition to PCS, there are also significant worries surrounding the degenerative effects of repetitive head impacts, and the long-term outcomes that may result, such as chronic traumatic encephalopathy (CTE).

### **1.1.2 Chronic traumatic encephalopathy**

CTE is a neurodegenerative disease that is believed to be the result of repetitive brain trauma that occurs during both contact sports and military participation (McKee et al., 2009). To date, CTE has mostly been found in persons who have sustained repeated brain traumas (McKee et al., 2009; Gavett, Stern, & McKee, 2011). Interestingly, CTE has also been found in autopsies performed on patients with no history of mTBI or repeated head impacts. While the increased focus on research in the area of CTE is relatively novel, the fact that contact sports are associated with neurodegenerative disease has been known for some time. In a 1928, Martland published a paper on a common phenomenon in boxers which he termed “punch drunk”. This symptom spectrum appeared to be the result of repeated blows to the head, and in particular the boxers who took significant head impacts as a part of their fighting strategy (Martland, 1928). Eventually, the term “punch drunk” was updated to be called dementia pugilistica by Millspaugh (1937), which described the syndrome characterized by the confusion and motor deficits commonly found in boxers. Finally, in the 1960’s, the term CTE surfaced when it became apparent that dementia pugilistica was not solely found in the boxing community. From this point forward, CTE

was used to describe the range of symptoms and deterioration that arises from repetitive brain injuries (McKee et al., 2009).

While SRC and PCS are defined by temporary neuronal and axonal damage, CTE is a degenerative disease that presents many years following head trauma (Gavett et al., 2011). Most commonly, CTE onset occurs in midlife, usually long after the athlete has retired from their sport. Common early signs of CTE include personality and behavioural changes, such as, irritability and a short-temper (Gavett et al., 2011). In some cases, cognitive decline may be the first signs to arise, with poor memory and executive function the two most common deficits (McKee et al., 2009). While these represent the early signs of CTE, the later stages of the disease bring forth movement, speech and ocular abnormalities, while cognition tends to worsen with time (McKee et al., 2009). A small number of cases with documented CTE have developed dementia prior to their death, however the relative infrequency may in part be due to the number of CTE patients who either commit suicide, die in accidents or from drug overdose at an early age, not allowing the dementia to develop (McKee et al., 2009; Omalu, Hamilton, Kamboh, DeKosky, & Bailes, 2010).

In terms of gross pathology, CTE is represented by general brain atrophy, with a reduced brain weight. Microscopically, it is represented by the accumulation of the phosphorylated tau protein as neurofibrillary tangles (NFTs) within the various cortices, diencephalon, brain stem, and spinal cord, among others (Baugh et al., 2012). It is also common to see significant neuronal loss in the hippocampus, entorhinal cortex, and amygdala, while other areas of the brain show a lesser degree of neuronal loss (McKee et al., 2009). NFTs are often distributed in multifocal patches in superficial cortical areas.

This distribution was first described in 1992 (Hof et al., 1992), wherein CTE NFTs tend to distribute within layer II and the upper portion of layer III in cortical areas, and tend to be more dense than those associated with Alzheimer's Disease (AD).

## **1.2 Measuring head impacts using accelerometers**

With the growing concern surrounding the long-term effects of concussions and repeated head impacts, understanding the mechanism of injury becomes paramount in injury treatment and, most importantly, prevention. In order to quantify the biomechanical properties of sport-related head impacts, researchers have been using devices to track and log any impacts sustained by the players. The earliest research using head impact biomechanics in vivo was performed in 1971, using accelerometers to measure head acceleration during football games (Moon, Beedle, & Kovacic, 1971). Since this time, there have been numerous studies that employ accelerometers to estimate impact tallies, magnitude, and severity, with the goal of better understanding the biomechanical properties of sport-related head injuries.

### **1.2.1 Accelerometer measurements**

Helmet accelerometers are able to measure both the linear (LA) and rotational accelerations (RA) caused by impacts to the head. LA and RA are calculated as the resultant vector of the sum of the three-dimensional LA/RAs recorded by the sensor (O'Connor, Rowson, Duma, & Broglio, 2017). LA is measured in gravitational units (G), which is the acceleration due to gravity, equalling  $9.81 \text{ m/s}^2$ . RA is measured in terms of speed of rotation (in radians), in units of  $\text{rad/s}^2$  (O'Connor et al., 2017). LA and RA are

known to be closely related to one another, as each measure can greatly affect the other ( Pellman et al., 2003; Rowson et al., 2012). The magnitude of LA and RA are dependent on the distance between the point of impact and the centre of gravity of the head. As the point of impact moves further from the centre of gravity, the resultant RA will increase relative to the LA, while forces in line with the centre of gravity will yield a larger LA (Stemper & Pintar, 2012). Recent research has shown that both linear and rotational forces influence concussive injury (Guskiewicz & Mihalik, 2011; Post & Blaine Hoshizaki, 2015).

Due to the overlapping influence and correlations of LA and RA, researchers have since created weighted impact scores that attempt to assess impact severity based on a combination of various accelerometer-based metrics. These metrics include the Gadd Severity Index (GSI; Gadd, 1966), Head Injury Criterion (HIC; Versace, 1971), and the Head Impact Telemetry severity profile (HITsp). The GSI can provide an accurate estimate of short-duration impacts, however it lacked the ability to estimate long-duration injuries that may produce diffuse brain injury. HIC was then designed to account for these inadequacies, however it was still limited in its ability to evaluate long-duration impacts (Prasad & Mertz, 2010). Originally, HIC and GSI were designed to assess the risk of skull fractures with moderate to severe TBI, making them less sensitive for assessing the risk of concussion or mTBI. In need of a more accurate injury estimator, Greenwald and colleagues (2008) created the HITsp metric that is thought to be more sensitive to mTBI. HITsp combines LA, RA, impact location and impact duration using a weighted principal component analysis. To date, this metric has been shown to be a more accurate estimator of concussion, when compared to LA, RA or HIC (Greenwald et al., 2008).



## 1.2.2 Sensor types

A study performed in 1971 was thought to be the first use of accelerometers to measure the impacts of football players in real time (Moon et al., 1971). To record impacts, three separate accelerometers were mounted to the players' heads. These three sensors could retrieve real-time linear acceleration at three impact locations (one location per accelerometer): back, top and the right side of the head. This system also required the player to carry a telemetry package on their chest so the transmission could reach the sideline receiver to record and maintain the incoming data.

Head impact systems have evolved into a number of different forms, each suited to specific measurements, or specific sports. It is now possible to track impacts with significant accuracy using one mounted accelerometer, which gives the option of placing the sensor in more places based on space and need. X2 Biosystems (X2 Biosystems, Inc, Seattle, WA) have developed multiple products in which accelerometers can be used in helmetless sports. The X-Patch uses a triaxial accelerometer mounted behind the player's ear (adhesively), while the X-Guard is an embedded accelerometer in a custom-fit mouthguard. Both of these systems aim to produce highly accurate data by directly analyzing forces imparted on the head, as opposed to forces imparted on a helmet which are then transferred to the head (O'Connor et al., 2017). Both the X-Patch and X-Guard systems record LA at 1 kHz, which RA is recorded at 850 Hz. The output from these devices includes peak LA, peak RA, HIC, impact location, and direction of peak LA. The X2 systems track a 100-millisecond trace, 10 milliseconds prior to the impact, and 90 milliseconds postimpact when a recorded impact exceeds 10g (the minimum threshold for

recording) (O'Connor et al., 2017). These sensors systems do have their own limitations, as skin movement, mouthguard fit, and saliva build-up may cause accuracy issues (King, Hume, Brughelli, & Gissane, 2015).

While some accelerometers do not require attachment to a helmet, many do, and these are optimal for helmet-based sports such as football and hockey. Many sensors now are mounted to the players' helmets, as opposed to directly to the head of the athlete. The most common helmet-mounted accelerometer system is the head impact telemetry system (HITS) (Simbex, LLC, Lebanon, NH). The HITS system uses 6 single-axis accelerometers that fit tightly inside the helmet. In order for an impact to be recorded, 1 of the 6 accelerometers must record an impact above 1.14g, however this threshold may be lowered slightly (O'Connor et al., 2017). Similar to the X2 systems, LA is recorded at 1 kHz, however the impact trace is 40 milliseconds, 8 ms preimpact and 32 ms postimpact. This device produces a final output consisting of peak LA, peak RA, 40-millisecond LA time trace, impact location, HIC, GSI and HITsp. A sensor technology that is growing in popularity is the gForceTracker system, which is a helmet-mounted accelerometer. GForceTracker sensors are able to measure impact forces in real-time, recording linear acceleration (gForce), rotational velocity (degrees/sec), impact location, as well as the HIC and GADD indices.

### **1.3 Subconcussive impacts**

While the research surrounding the effects of concussive impacts is vast, subconcussive impacts are a relatively new concept, and are not well understood. The term subconcussive impacts is used to describe impacts that produce damage within the brain,

without showing any of the common acute symptoms most associated with concussion or traumatic brain injury (TBI) (Koerte et al., 2015). The overall effects and detriments related to repetitive subconcussive impacts are not fully known, however, recent evidence shows varying results as to how the impacts affect the brain's structure and integrity. Many studies have used helmet-mounted accelerometers to examine the frequency and magnitude of head impacts in sports such as football, hockey, rugby, and soccer (Cortes et al., 2015). These studies have shown that over the course of a football season, players are exposed to a vast number of subconcussive blows, with the number varying based on position, playing time, and session type (Crisco et al., 2010; Crisco et al., 2011). Evidence suggests that the damage caused by these impacts have a cumulative effect depending on frequency and magnitude of the impacts (McAllister et al., 2013).

The first study to examine the forces imparted during football games was performed by Moon and colleagues (1971). The results of this study showed extraordinarily high impact magnitudes, with many being recorded greater than 1000g (1000 m/sec<sup>2</sup>). The researchers noted in their discussion that the data was heavily biased toward the largest recorded impacts. They also assumed that hundreds of smaller impacts would have occurred during games that were not noted in this paper. Despite recognizing that the impacts recorded in this study are far beyond the skull's threshold for injury, helmets provide a large amount of protection, making the impacts viable. The researchers then concluded that impacts recorded over 1000g occur regularly in football games and are not sufficient to produce head injuries.

Recent research has shown that the peak magnitude values presented by Moon and colleagues are inaccurately high. In 2012, Crisco and colleagues performed a study with

the purpose of analyzing the magnitude of recorded head impacts in collegiate football players. In this study, 254 players from three teams participated over the course of two National Collegiate Athletic Association (NCAA) seasons (2007 and 2008). Using the HIT system, the players were tracked during both practices and games to assess all football-related impacts. Players were then assigned to a position group based on their primary playing position. In total there was eight groups: defensive line (DL), linebacker (LB), defensive back (DB), offensive line (OL), running back (RB), wide receiver (WR), quarterback (QB) and special teams (ST). The team-wide 50<sup>th</sup> and 95<sup>th</sup> percentile for peak linear and rotational velocity was calculated and group differences were assessed based on these values. Over the two seasons, a total of 184,358 impacts were recorded to be above the 10g minimum threshold. Independent of player or position, the 50<sup>th</sup> percentile value for peak LA was found to be 20.3g, while the 95<sup>th</sup> percentile was found to be 62.2g. The results of this study found that football impacts are heavily skewed towards lower magnitudes. It was also found that player position was a significant estimator of impact magnitude, as was impact location.

In a previous study (2011) Crisco and colleagues followed 314 collegiate level football players for three full seasons. The purpose of this study was to examine the frequency and magnitude of all impacts received at the collegiate football level with the HIT system during both practices and games. Just as in the 2012 study, the players were grouped by position (eight groups). The groups were then analyzed to determine if certain playing positions are at a greater risk compared to others by receiving a greater frequency or magnitude of impacts. For this study, the researchers chose to use a minimum threshold of 10g impacts, to eliminate any non-impact events (e.g., running, jumping, etc.). Team-

wide results show that the 314 football players sustained a total 286,636 head impacts over the course of three full seasons, with one player sustaining as many as 2492 impacts. For all positions, it was found that the players were exposed to a greater number of impacts per session during games when compared to practices. It was discovered that the QB group sustained the least number of impacts, while the DL group sustained the most. When assessing impact magnitudes, it was found that the RB group received the impacts with the highest magnitudes. Despite the fact that they received the largest number of impacts, the OL and DL groups sustained the lowest magnitudes of all position groups.

These findings were replicated in a prior study by Mihalik and colleagues (2007). In this study, 72 collegiate levels football players were followed for the 2005 and 2006 NCAA football seasons. Again, the players in this study were outfitted with the HIT accelerometer system, and a 10g minimum threshold was used to delete any negligible impacts sustained by the players. It was found that these players were exposed to a total of 57,024 impacts were recorded over the 10g threshold over the two full seasons. It was found that the OL group received the most impacts, while the WR group received the least. When assessing impact magnitudes, the researchers found that the DL group sustained the highest magnitude impacts on average, followed closely by the OL group. The group with the lowest average magnitude impacts was the DB group. The results indicating that the OL and DL sustained the greatest number of impacts is in concordance with the vast majority of previous literature, however the fact the OL group also sustained the greatest magnitude impacts differs from most other results. One confounding factor in these results could be that the group analyses were done as an average of all impacts within that group,

whereas other studies, like that of Crisco and colleagues, tend to use a per-player average within the groups.

#### **1.4 Diffusion tensor imaging (DTI)**

Diffusion tensor imaging (DTI) is a relatively new neuroimaging concept; by tracking the diffusion of water within the brain, DTI can visualize and assess structures of the brain (O'Donnell & Westin, 2011). The diffusion tensor was introduced in magnetic resonance imaging (MRI) in 1994 (Basser, Mattiello, & Lebihan, 1994), although this wasn't the first time scientists used diffusion as a method of analyzing the brain and its components. Originally, in order to measure anisotropic diffusion axon direction must already be known to the researchers, meaning only specific samples (e.g., axons of a giant squid) could be analyzed (Beaulieu & Allen, 1994b). DTI is a highly sensitive, non-invasive method for examining the movement of water within the tissues of the brain using MRI technology and is a marker of white matter integrity (Soares, Marques, Alves, & Sousa, 2013). Using the magnetic field gradients of an MRI, DTI creates an image that is sensitive to the diffusion of water in one particular direction. This process is then repeated in a number of directions to create an estimated three-dimensional model (the diffusion tensor) at each voxel (O'Donnell & Westin, 2011). This model is then used to describe the magnitude, the degree of anisotropy, and the orientation of diffusion (Alexander, Lee, Lazar, & Field, 2007). Using the acquired diffusion anisotropy and diffusion directions, white matter tractography can then be used to estimate connectivity patterns in white matter (Alexander et al., 2007).

Diffusion is a molecular transport process, in which material is transferred from one spatial location to another over some period of time (Alexander et al., 2007). This random

movement is typically driven thermally, and has been termed Brownian motion (Beaulieu, 2002). In a pure liquid, diffusion is typically influenced by molecular weight, intermolecular interactions (viscosity), and temperature. However, the microstructure of a cell influences the overall diffusion due to physical barriers, and individualized compartments (e.g., axons, glial cells, neurons, intracellular, extracellular) (Beaulieu, 2002). In a pure liquid, when there are no disturbances or barriers to diffusion, diffusion occurs equally in all directions and is termed *isotropic* diffusion. When diffusion occurs in a sample that contains structural barriers (e.g., axons) and is constrained to occur in certain directions compared to others, this is termed *anisotropic* diffusion (Beaulieu, 2002).

Most commonly, DTI images are acquired using the pulsed-gradient spin echo (PGSE) sequence with a single-shot, echo planar imaging (EPI) readout (Alexander et al., 2007). The simplest configuration of this sequence consists of a pair of large gradient pulses on either side of the 180° pulse (Alexander et al., 2007). The first of the two gradient pulses presents a phase-shift by demagnetizing the sample. The second pulse then re-phases and spins that have moved as a result of diffusion during the second pulse will then be subjected to a different field strength (Hagmann et al., 2006). Any non-diffusing molecules' (stationary) phases will cancel entirely, causing no signal attenuation (Alexander et al., 2007). If any change is detected, it will result in a decreased signal intensity; longer displacement results in a larger phase-shift, and thus further decreased signal. With this, the resultant image will show low signal intensity in areas of the brain where diffusion has occurred along the applied diffusion gradient (Hagmann et al., 2006).

#### **1.4.1 DTI acquisition parameters**

During DTI, each diffusion gradient will detect movement of molecules in that gradient's direction. In order to attain a complete and accurate representation of the brain, it is important to repeat this process in various directions to ensure that all fibers are tracked, regardless of which direction they travel. To measure a diffusion tensor, a minimum of 6 diffusion encoding directions are needed (Alexander et al., 2007), while adding directions will increase the spatial accuracy of the image, however it will also increase scanning time. The required level of spatial resolution will be dependent on the application of the results. For most clinical applications a coarse resolution may be sufficient with a small number of encoded directions and a decreased scan-time. However if accurate quantifications are required, spatial resolution becomes paramount in ensuring good results (Alexander et al., 2007) It has been found that using 30 encoded directions is a good compromise between scanning time and image accuracy, as increasing the number of directions beyond 30 failed to substantially improve tensor orientation or mean diffusivity (MD; the mean amount of diffusion occurring in that voxel) estimates (Derek K. Jones, 2004). In order to accurately estimate the diffusion tensor, high b-values are required, as well as one minimally T2 weighted low b-image ( $b = 0 \text{ s/mm}^2$ ), according to Mukherjee and colleagues (2008). Most DTI studies will use a b-value between  $700 - 1000 \text{ s/mm}^2$ , while the clinical standard is  $1000 \text{ s/mm}^2$ . DTI is reliant on spatial resolution to properly track fibers and typically  $2 - 2.5 \text{ mm}$  voxels are recommended, with an interleaved acquisition to minimize the interactions of nearby fibers (Alexander et al., 2007). Using a 3.0T scanner, it is possible to attain high quality DTI data in less than 10 minutes using a  $2.5 \text{ mm}$  resolution and 64 encoded directions (Alexander, Lee, Wu, & Field, 2006; Jones et al., 2002).



### 1.4.2 DTI results and metrics

The diffusion tensor ( $\mathbf{D}$ ) is represented by a 3x3 covariance matrix,

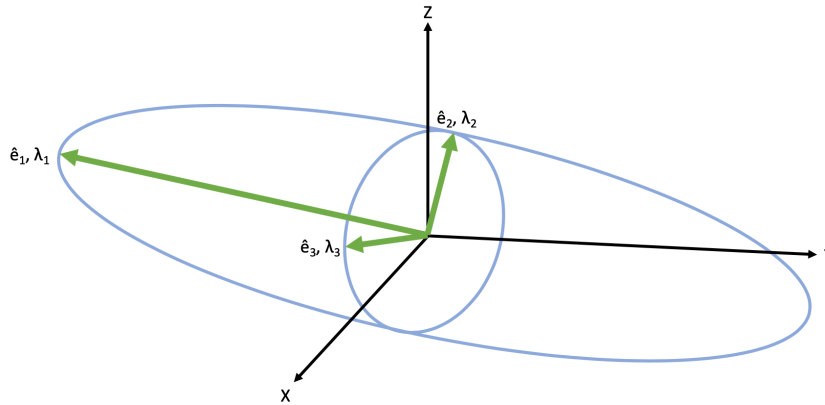
$$\mathbf{D} = \begin{bmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{yx} & D_{yy} & D_{yz} \\ D_{zx} & D_{zy} & D_{zz} \end{bmatrix} \quad (1)$$

which describes the diffusion displacements in a three-dimensional space based on diffusion time (Alexander et al., 2007). Within the tensor there are three mutually perpendicular directions ( $D_{xx}$ ,  $D_{yy}$ ,  $D_{zz}$ ), as well as the cross-terms represented by the other values (e.g.,  $D_{yz}$ ,  $D_{xy}$ , etc.). This matrix can then be diagonalized such that the non-diagonal terms become equal to zero, yielding a new matrix:

$$\mathbf{D} = \begin{bmatrix} \lambda_1 & 0 & 0 \\ 0 & \lambda_2 & 0 \\ 0 & 0 & \lambda_3 \end{bmatrix} \quad (2)$$

With this, the three remaining (diagonal) terms are call  $\lambda_1$ ,  $\lambda_2$ , and  $\lambda_3$  and these values are known as the eigenvalues for the diffusion tensor. Eigenvalues represent the magnitude by which diffusion is occurring in each direction (Chepuri et al., 2002). Each eigenvalue comes with a corresponding eigenvector ( $\hat{e}_1$ ,  $\hat{e}_2$ ,  $\hat{e}_3$ ), which not only represents the magnitude (as eigenvalues do), but also reflects the direction of maximal and minimal diffusion (Soares et al., 2013). The eigenvalues are also the means by which the main DTI metrics are calculated, and the basis on which DTI results are formed. With these values, it is also possible to visualize the diffusion tensor in the form of an ellipsoid, wherein the

eigenvectors define the directions of the axes, and the eigenvalues represent the magnitudes (Figure 1) (Alexander et al., 2007).



**Figure 1.** A schematic demonstrating an ellipsoid visualization (in three dimensions) using a diffusion tensor’s eigenvalues ( $\lambda_1, \lambda_2, \lambda_3$ ) and corresponding eigenvectors ( $\hat{e}_1, \hat{e}_2, \hat{e}_3$ ) to determine the magnitude and direction of diffusion.

When analyzing the results of a DTI study, it is necessary to first calculate the appropriate metrics using the values found within the tensor. The most commonly used indices of diffusion are fractional anisotropy (FA) and mean diffusivity (MD) (Soares et al., 2013). MD is simply the average of the three eigenvectors (Equation 3), and is used as a measure of the total diffusion occurring at that voxel, regardless of directionality (O’Donnell & Westin, 2011).

$$\hat{\lambda} = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3} \quad (3)$$

FA is a normalized value used to measure what fraction of the diffusion in that voxel is anisotropic. FA is calculated by comparing each eigenvalue to the mean of all eigenvalues ( $\hat{\lambda}$ ), as below:

$$\mathbf{FA} = \sqrt{\frac{3}{2}} \sqrt{\frac{(\lambda_1 - \hat{\lambda})^2 + (\lambda_2 - \hat{\lambda})^2 + (\lambda_3 - \hat{\lambda})^2}{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}} \quad (4)$$

Since FA is a measure that compares the difference between eigenvalues, it becomes independent of absolute magnitude (Hagmann et al., 2006). While FA and MD are the most common DTI measures, there exist two metrics which help to explain the differences found in the main two: radial diffusivity ( $D_r$ ) and axial diffusivity ( $D_a$ ). Axial diffusivity is equal to the largest eigenvalue (Equation 5b), representing the diffusion occurring in the principal diffusion direction. Radial diffusivity is calculated by averaging the two smallest eigenvalues (Equation 5a), representing the average diffusion occurring perpendicular to the principal diffusion direction (Hagmann et al., 2006).

$$\begin{aligned} \text{a) } D_r &= (\lambda_2 + \lambda_3) / 2 \\ \text{b) } D_a &= \lambda_1 \end{aligned} \quad (5)$$

### 1.4.3 Microstructural effects on anisotropic diffusion

The lack of isotropic diffusion occurring in white matter tracts is undoubtedly associated with the arrangement of myelinated fibers. All axonal systems tend to consist of the same microstructural factors: the myelin sheath around the axons, the axonal membrane and the microtubules and neurofilaments of the neurofibrils (Beaulieu, 2002). All of these structures are longitudinally oriented, imparting directional barriers against diffusion. Within a barrier-free environment, water molecules are free to diffuse in any direction, however, within these tracts the transverse diffusion is restricted by the hydrophobic myelin (Chepuri et al., 2002). Along with the physical structures, there are a few more

factors that affect the water molecules' ability to diffuse perpendicular to the tract: tight packing of axons, thicker myelin (less permeable), or the altered radii of the axons (Chepuri et al., 2002). Another factor that may affect DTI values are the surrounding features of the brain; if the tract of interest is near grey matter (GM) or cerebrospinal fluid (CSF) the resulting metrics may be altered. Also, if the tract is in an area with many crossing WM tracts, this create conflicting measurements (Alexander, Hasan, Lazar, Tsuruda, & Parker, 2001).

Early research showed that the directional organization of WM is the basis by which anisotropic diffusion occurs, with myelin appearing to be the modulating factor for the amount of anisotropy (Beaulieu & Allen, 1994a). Song and colleagues (2002) recently examined diffusion in a strain of mice with demyelinated axons. In this study, the researchers examined 8 white matter tracts: the anterior commissure, cerebral peduncle, corpus callosum, external capsule, fornix, optic nerve, optic tract and trigeminal nerve. The relative anisotropy of all examined fiber tracts was lower in the experimental mice compared to the respective tracts of the controls. However, only 4 of these tracts had a statistically significant difference, those tracts being the anterior commissure, cerebral peduncle, optic nerve and optic tract. Importantly, total diffusivity and parallel diffusivity ( $D_a$ ) did not significantly differ between experimental mice and the controls. However, the perpendicular diffusivity ( $D_r$ ) was found to be significantly higher in the mutant mice in every examined tract, with the exception of the corpus callosum and the external capsule. More recent studies have confirmed the results of Song and colleagues, however, some studies have also shown demyelination to cause a decrease in  $D_a$  (Tyszka, Readhead, Bearer, Pautler, & Jacobs, 2006).

While it appears that demyelination has a significant effect on  $D_r$ , some suggest that  $D_a$  is a better representation of axonal damage (Sun et al., 2006). In 2006, Sun and colleagues examined the effects of axonal damage and demyelination on the diffusion in white matter tracts in mice. Using a cuprizone treatment, they were able to achieve demyelination, and remyelination following the removal of cuprizone from the diet. The mice with axonal damage had a decrease in parallel diffusivity ( $D_a$ ) in the corpus callosum, and an increase in perpendicular diffusivity ( $D_r$ ) during the demyelination phase.  $D_a$  recovered to baseline levels when the cuprizone treatment was halted and remyelination occurred.  $D_r$  showed signs of returning to baseline levels as well, however it appeared as though the time frame of the study did not allow for full recovery of this measure.

Another significant cause of changes in DTI metrics is thought to be neuroinflammation, though there is less research on the relationship. In general, it is believed that the increase in tissue water associated with inflammation results in an increased MD (Tievsky, Ptak, & Farkas, 1999; Werring, Clark, Barker, Thompson, & Miller, 1999). For example, chronic lesions caused by multiple sclerosis (MS) had highly elevated MD, whereas chronic and acute lesions had elevated MD, but to a much lesser degree (Tievsky et al., 1999). In another 1999 study (Werring et al., 1999), researchers found that MS lesions showed the greatest MD elevation, whereas inflammatory lesions showed a significant decrease in FA.

While all of the DTI metrics are each derived from the same main components (eigenvalues), each one appears to be sensitive to difference mechanisms of injury. With this, it is important to study each metric individually to assess how the structure and composition of the tracts are changing over time. FA and MD are most commonly assessed

as they are seen as measures that capture large-scale changes, while  $D_r$  and  $D_a$  are often used as complimentary measures which better describe the underlying workings of FA and MD, more thoroughly explaining the diffusion that is, and isn't occurring within the tracts.

## **1.5 DTI in sports**

### **1.5.1 DTI and sport-related concussion**

Despite the evident neurological symptoms after SRC, typical neuroimaging protocols show no abnormalities (Lancaster et al., 2016). Among other magnetic resonance imaging (MRI) techniques, DTI has been shown to be effective in tracking the structural changes in the brain's white matter associated with SRC. Furthermore, there is evidence that these white matter structural changes continue beyond symptom resolution (Dimou & Lagopoulos, 2014; Gardner et al., 2012; Meier et al., 2015). In mTBI, it is relatively agreed upon that in the chronic stages, anisotropic diffusion decreases, however in the sub-acute or acute stages of mTBI, the direction of change (increase or decrease) in anisotropic diffusion has been reported to both increase and increase (Bouix et al., 2013; Mayer et al., 2010). Some studies have shown decreased FA (Arfanakis et al., 2002; Inglese et al., 2005), while others have noted increased FA (Churchill, Caverzasi, Graham, Hutchison, & Schweizer, 2017; Henry et al., 2011; Murugavel et al., 2014) in certain parts of the brain. Among these mixed results, there are also studies which have found no FA changes after mTBI (Zhang et al., 2010).

In 2014, Murugavel and colleagues performed a longitudinal DTI study with the goal of examining white matter changes over time following SRC. Following the concussive injury, brain scans were attained after 2 days, 2 weeks and 2 months. This study

consisted of 21 male varsity athletes, all of which were medically diagnosed to have a concussion (with no loss of consciousness). The control group of this study consisted of 16 age-matched varsity athletes who participate in noncontact sports, and no reported history of SRC. In order to analyze the results between-group t-tests were performed using tract-based spatial statistics (TBSS) on the skeletonized WM tracts for all four diffusion measures (FA, MD, RD, and AD). Results of this study show that RD values were significantly higher 2 days post-injury, compared to 2 weeks post-injury. Conversely, FA values were found to increase from 2 days to 2 weeks post-injury. It was also noted that the clusters in which these RD and FA changes were found were co-localized.

Another study examining the effects of SRC on white matter using DTI was performed by Bazarian and colleagues (2007). In this study, 6 subjects who experienced isolated mTBI were compared against 6 matched controls. All subjects were tested within 72 hours post-injury, the tests consisted of a DTI scan, PCS assessments, and neurobehavioral testing. For the DTI assessment, both FA and MD were examined within five pre-determined regions of interest (ROI): the anterior internal capsule (AIC), posterior internal capsule (PIC), anterior corpus callosum (ACC), posterior corpus callosum (PCC), and external capsule (EC). Using voxel-based morphometry, a full brain analysis was completed, and yielded no significant differences in both FA and MD. Ultimately, the results of this study indicate that the patients who suffered a mTBI had decreased MD and increased FA compared to controls. The ROI analysis suggested that there was axonal injury in the internal capsule and the PCC. It was estimated by the authors that these changes were the result of axonal swelling, which is an early step in the process of axonal injury.

Despite the many studies examining the effects of SRC and mTBI on the microstructure of the brain, the resulting changes are still debated. What is known, is that these traumatic events cause structural changes in most cases, and the acute and sub-acute effects may differ from what is seen chronically. With knowledge of these results, researchers have since turned their focus to the structural effects of non-concussive events, of which there are no associated behavioural changes. It was hypothesized that these sub-concussive impacts may create similar effects to mTBI and SRC, by way of a cumulative effect.

### **1.5.2 DTI and repeated subconcussive impacts**

With the recent emphasis on analyzing the long-term effects of sub-concussive impacts, researchers have begun to examine the microstructural changes associated with participation in contact sports. These athletes do not present with any clinical symptoms of TBI; however, the underlying mechanisms of brain injury create concern for their short- and long-term well-being. Players receive a vast number of impacts over the course of any football-related sessions, cumulating to a large number of head impacts sustained over the course of one full season. DTI, having been shown to be an effective method of measuring concussive changes, is now being used to analyze the short- and long-term effects of subconcussive impacts sustained in various contact sports, with an emphasis in North American on football players.

In a research study performed by Bazarian and colleagues (2014) assessed white matter changes related to repetitive head impacts (RHI) over the course of a college-level football season. The players underwent a DTI scan prior to the beginning of the season



(time 1), after the final game of the season (time 2), and again after six months of non-contact rest (time 3) and helmets were outfitted with the HITS system. Athletes had changes in both FA and MD between time 1 and time 2 scans compared to the controls. As well, in voxels where FA decreased over time, MD was found to increase. Also, the percentage of voxels with decreased FA (between time 1 and time 2) were positively correlated with several helmet impact measures. In a similar study, Slobounov and colleagues (2017) examined collegiate football players before and after one full season of NCAA football using a wide range of advanced neuroimaging techniques, including DTI. Interestingly, this study did not find any changes to white matter integrity between the two time points.

With recent studies showing that RHI cause significant changes in white matter integrity, further research into the area is needed to better understand the causes for these changes. It is now important to better understand why these changes occur, how the kinematics of these impacts influences the changes, and whether the athletes may be at a substantial risk as a result of their sports.

## **1.6 Research Objectives and Hypothesis**

This study examined the effects of subconcussive impacts, sustained over the course of one season of Canadian university-level football, on the structural integrity of white matter in the brain. Specifically, this study examined the effects on two specific white matter tracts: the corpus callosum and the corticospinal tract. These tracts will be divided into three sections (thirds; six sections total for the CST's two parallel tracts) to assess changes occurring in specific regions.

This research study consisted of three primary objectives. Firstly, we sought to examine the effects of subconcussive impacts on the two chosen white matter tracts (CC and CST). The second aim of this study was to determine if certain regions of these tracts are more susceptible to damage than others. Finally, the third objective was to examine the effects of increased impact exposure, determining if receiving a greater number of impacts during a season increases risk of injury.

It was hypothesized that the CC will prove to be more susceptible to DTI changes as a result of the subconcussive impacts. This hypothesis is based on the structure and location of the CC, with its midline location increasing its vulnerability to the shearing and twisting forces associated with head impacts. The CST's location should allow it to remain relatively unharmed as a result of the biomechanical forces exerted during head impacts. Secondly, we hypothesized that certain areas of either tract would be at a greater risk of injury compared to other segments. Specifically, we believed that the front section of the CC and the top section of the CST would experience greater changes than the rest of their respective tracts. Our third hypothesis estimates that players experiencing a larger number of head impacts will be at a greater risk of injury and will therefore experience greater DTI changes as a result.

# Chapter 2

## Materials and Methods

### 2.1 Participants

This study was approved by the Queen's University Research Ethics Board, and all subjects provided informed consent for their participation. Twenty-five male collegiate-level football players were consented over the span of two seasons. All of these players completed a neuroimaging protocol at 3 time points: before the season (prior to any contact practices), following training camp (prior to the first regular season game), and shortly after the conclusion of the season. Three of these players were removed from the study due to poor on field participation. One player was removed due to a corruption issue with their imaging data, another player was removed due to a possible scanner malfunction, as they were found to be a significant outlier in various respects. This resulted in a total of twenty players from two separate football seasons included in the final analysis (18 – 22 years; mean  $20.3 \pm 1.26$  years).

### 2.2 Neuroimaging acquisition

Preseason scans were performed between two and 72 days (mean  $23.9 \pm 23.97$  days) prior to the first on-field session and athletes were not participating in any contact sport during this period. The post-training camp images were acquired within four to 24

days (mean  $14 \pm 5.6$  days) following the last practice session before the first regular season game. The postseason images were acquired approximately one month following the last game of the season (mean  $33.8 \pm 6.93$  days).

All neuroimaging was performed at Queen's University using a Siemens 3.0T Magnetom Tim Trio system with a 32 channel receiver head coil. DTI parameters were as follows: slice thickness = 2mm, 60 axial slices, TR = 7800ms, TE = 95ms, FOV = 256mm, 128x128 acquisition matrix, voxel size = 2mm, echo spacing = 0.84ms. Diffusion weighting gradients were applied in 30 anterior-posterior phase-encoding directions, with a b-value of 1000 sec/mm<sup>2</sup>. A high-resolution T1-weighted MPRAGE image was also acquired at the beginning of each imaging session.

### **2.3 Image pre-processing and registration**

The diffusion-weighted images were pre-processed using the FMRIB Diffusion Toolbox (FDT), a portion of FSL's software package (FMRIB Software Library v6.0). Four initial steps were taken to pre-process the images: averaging the four  $b_0$  values, eddy current and motion correction, and the removal of non-brain tissue. First, the images were corrected for any susceptibility induced distortions using *topup* (Andersson et al. 2003; Smith et al., 2004). Next, the *eddy* function (Jesper et al., 2016) was used to correct for eddy current-induced distortions and any potential subject movement during the scan. Following the eddy corrections, using the *BET* function (Smith, 2002) non-brain regions were removed. Finally, using the corrected images, the *DTIFIT* function was run to calculate the 3x3 diffusion tensor parameters at each voxel. These newly calculated eigenvectors allowed an FA and MD map to be created for each subject. Maps were then

registered to the high-resolution T1-weighted anatomical image and then transformed to MNI152 standard space using linear (FLIRT) and nonlinear (FNIRT) registration.

## **2.4 Along-tract analysis**

The DTI metrics were then determined in segments of each tract using custom MATLAB scripts. First, to isolate the corpus callosum and corticospinal tracts, an FSL atlas was used to create a mask of the entire tract. From this, slices of the tract (perpendicular to the length of the tract) were created by averaging the FA values of the voxels contained in that slice's area, creating evenly spaced and weighted slices. These averaged values were then assessed to determine if the mask contained grey matter (FA values of 0), and if found, that slice was deleted from the tract. In the end, the CC consisted of 50 FA values along the entirety of the tract, with each value representing the mean FA for that slice. The CST contained 62 slices in each of the two hemispheres. This process was then repeated to determine slice-by-slice MD. The slice-by-slice data was then combined for segments to make it more interpretable. The CC was evenly split into thirds (Figure 2), with the middle section containing one more slice than the anterior and posterior portions (16 slices, 17 slices, 16 slices). The CST was split into three segments based on the anatomy of the tract (Figure 3), with the top section having 24 slices, the middle section having 21 slices and the bottom section containing 17 slices. Each of the tract's slices contained within each segment were then averaged to create one value for each segment. An overall average of each tract was also calculated. This process was replicated for FA, MD and RD with each metric having the same output.

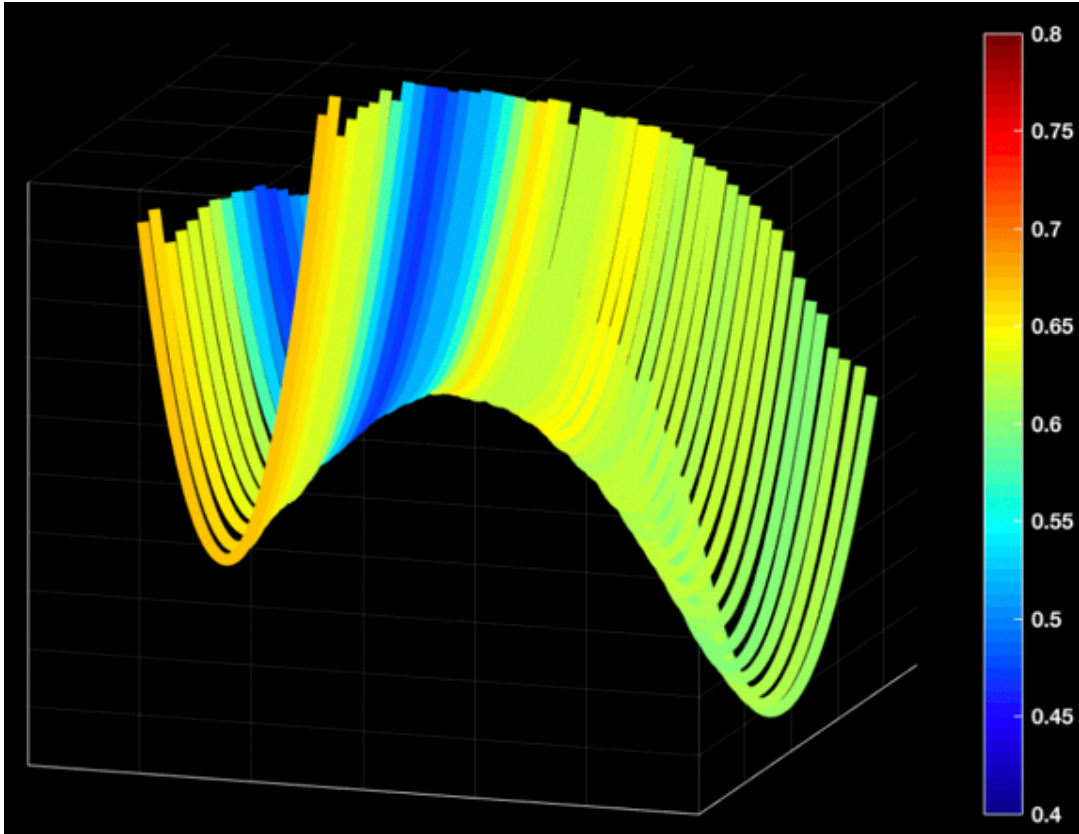


Figure 2. A 3-D visualization of the slices of the corpus callosum. This image is from the anterior, with the posterior of the tract on the left side of the image. The colours represent the values, FA in this example, where red represents a higher value.

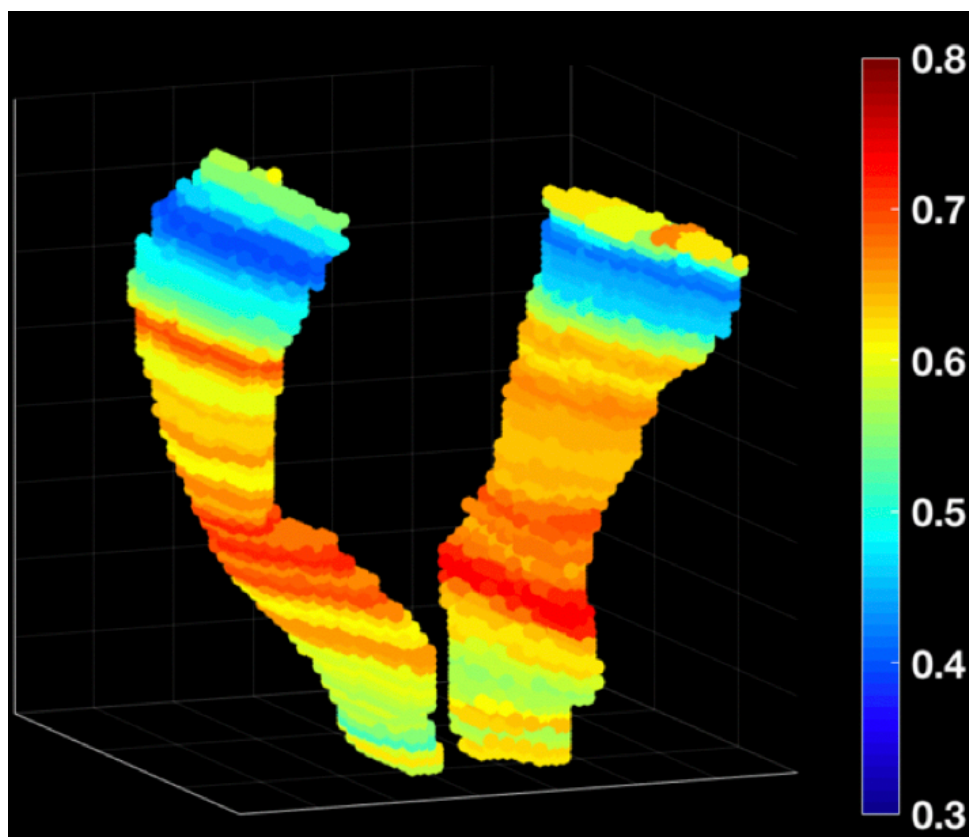


Figure 3. A 3-D visualization of the slices of the corticospinal tract. This is an anterior view showing both of the parallel tracts (right tract on the left side of the image). The colours represent the DTI matrix values, FA in this case, with

## 2.5 Helmet Accelerometers

GForceTracker (GFT; Artaflex Inc., Markham, Ontario, Canada) provided helmet mounted accelerometers for each of the players enrolled in this study. The GFT3S is a small sensor that measures 50 mm long, 29 mm wide, and 14 mm high, weighing 2 grams (Campbell et al., 2016). Each sensor contained a tri-axial linear accelerometer, a tri-axial gyroscope and a rechargeable battery, with a built-in storage system capable of recording up to 400 time-stamped impacts. The three axes of the accelerometer had a range of  $\pm 200$  g with a 1 g resolution. The gyroscope, measuring rotational velocity in degrees per second ( $^{\circ}/s$ ) had a range of  $\pm 2000$   $^{\circ}/s$ . For this study, the sensors were manually programmed to

record any impact that exceeded a 15 g linear velocity, with an alarm set to ping the computer when an impact over 100 g occurred. GFT sensors record 8 ms of data prior to the threshold, as well as 32 ms of data afterwards. Linear acceleration was sampled at 3000 Hz, and was filtered using a low pass anti-aliasing filter of 300 Hz. Rotational velocity was sampled at 800 Hz, with a low pass anti-aliasing filter of 100 Hz (Campbell et al., 2016).

The GFT sensors were able to record each impact's peak linear acceleration, as well as rotational velocity (Campbell et al., 2016). The GFT software was then able to calculate the impact locations using azimuth and elevation coordinates. The locations were then categorized as front, back, right, left, top or bottom. Azimuth angles between 315° and 45° were categorized as front impacts, while angles between 135° and 225° were impacts to the back of the head. Azimuth angles within 45° and 135° and between 205° and 35° were right and left impacts. Finally, the impacts were categorized as either top or bottom if the elevation angle was above 45° (top) or less than 45° (bottom), with this location overriding any location set by the azimuth angle (Campbell et al., 2016).

## **2.6 Collection of head impact data**

Using adhesive velcro the sensors were fastened to the inside of the helmet shell to the left of the crown air bladder. The sensors remained in the helmets for the duration of the season, with the exception of when one needed to be removed for replacement, or technical issues. Training camp during season one consisted of 12 sessions and 11 sessions in season two. The first regular season consisted of 36 sessions, 9 of which were games (one playoff game, eight regular season), and 27 practices. The second regular season had 26 total sessions, 8 games and 18 practices.



Prior to the commencement of each session, a USB hub was plugged into the research computer, acting as a wireless connection device to all of the active sensors. This hub was able to remotely turn on/off the sensors, while also providing real-time data to the computer as impacts occurred on the field. At each session, there was a minimum of one researcher designated to observe the practice/game and oversee the impacts as they occurred. With a high vantage point, the researcher was always able to have a good view of the field. The aim of this researcher was to eliminate any helmet impact that did not occur as a result of an impact to the players head (e.g., dropping of the helmet). When the researcher determined that an impact was not legitimate, they would note the player's study number, the time of the impact, and a reason for why the impact shouldn't be included in the final analysis. To go with this, the start and end time of each session was recorded to eliminate any impacts that may have occurred prior to the start or following the conclusion of the session. Following the conclusion of each session, the on-field researcher would wirelessly download the impact data from each sensor using the GFT hub. GFT processed the uploaded data and generated a session report for each player each day. Only GFT and Queen's research associates were able to access these session reports.

## **2.7 Head impact analysis**

All impact data was downloaded from the GFT server as a Microsoft Excel spreadsheet (Microsoft, Redmond, WA). Each player that sustained an impact in the selected session had an excel file assigned to them, with all of the pertinent information related to their head impacts. Prior to the final analysis, all impacts that did not occur as a result of a head impact required were deleted using the notes from the researcher present at

the field. Using custom MATLAB scripts, each player's impacts were tallied and summed in order to analyze that player's exposure throughout the season. Players were evaluated based on their total impact exposure (number of impacts from start to finish), impacts during training camp, and the cumulative magnitude of these impacts (sum of total gForce, gForceSum). Players were then ranked based on their exposure to head impacts and grouped based on exposure using the median number of impacts. The 10 players found to be above the median number of impacts were categorized as 'High Exposure' (HE), while the remaining 10 players who sustained fewer impacts than the median were assigned to the 'Low Exposure' (LE) group. In order to compare the two groups, five measurements taken by the accelerometers were used to examine the differences in magnitude and tally of head impacts sustained over the season between the two groups. Firstly, total number of impacts from start of training camp to the final game was used to separate the players. Next the impacts were analyzed to assess the linear and rotational magnitude accumulated over the entire season. This was calculated by summing all impacts for each player. This led to two measures, cumulative linear acceleration (GFsum), and cumulative rotational velocity (RRsum). These measures were then analyzed on a per-impact basis, to determine the magnitude (linear and rotational) of the average impact of each player. This led to another two measures, average linear acceleration per impact (LA/impact), and average rotational velocity per impact (RR/impact).

## **2.8 Statistical Analysis**

All data was managed and stored using Microsoft Excel spreadsheets, while all statistical tests were completed using The Statistical Package for Social Sciences (SPSS

version 25.0) (IBM Corp.). To determine if there was a relationship between the number of impacts sustained and cumulative impact forces (rotational and linear) a Spearman Rank correlation was performed. The two exposure groups were compared using a two-way repeated measures ANOVA, with a between-subject factor of exposure group and within-subject factor of time point. This ANOVA was performed for both tracts at each of the previously described segments. This analysis examined all of the four DTI metrics (FA, MD, AD, RD) at all three time points. To assess the differences in impacts between the two exposure groups, independent-samples t-tests were used to compare each of the five accelerometer measurements including impact totals, cumulative linear acceleration, cumulative rotational velocity, as well as per-impact measures from both the linear and rotational components.

# Chapter 3

## Results

Of the 20 subjects involved in the study 10 reported a previous concussion (medically diagnosed), while three other players reported having an undiagnosed concussion prior to the commencement of the current football season. The number of prior reported concussions varied from one to four (mean  $0.75 \pm 1.02$ ), while the time since the player's most recent concussion ranged from one to eight years prior to the current season ( $4.2 \pm 2.44$  years). Of the players who reported undiagnosed concussions, none had a prior diagnosed concussion, and none reported more than one.

### 3.1 Impact analysis

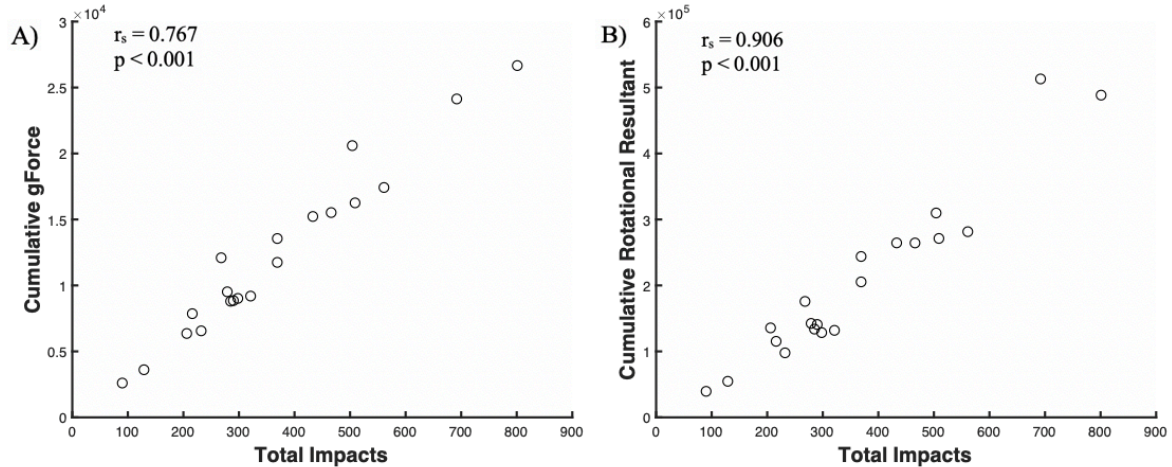
In total, the 20 players enrolled in this study received 7318 impacts across all sessions (Table 1). On average the players sustained  $366 \pm 181$  impacts (median 310), with the least affected player sustaining 90 impacts, while the most impacts recorded in one player was 801. Players sustained an average of  $96 \pm 62$  impacts in training camp (median 85), and  $270 \pm 138$  impacts (median 244) during the regular season. The cumulative sum of the gForce (GFsum) sustained throughout the season was also assessed; on average the players sustained  $11834 \pm 6659$  g (median 9355) over the entire season. In terms of cumulative rotational velocity; the players sustained an average of  $206901.37 \pm 126591^\circ/\text{s}$  (median 159092). In assessing the correlation between the total number of impacts and

cumulative linear acceleration, there was a strong correlation ( $r_s = 0.767$ ;  $p < 0.001$ ; Figure 4A). Total cumulative rotational velocity was also correlated to the total number of impacts sustained ( $r_s = 0.906$ ;  $p < 0.001$ ; Figure 4B).

**Table 1.** Impact breakdown for each participant for the whole season, training camp and regular season

Subject	Position	Exposure Group	Impacts	TC Impacts	S Impacts	GFsum
fbp.15	DL	High	801	262	539	26664.0
fbp.11	FS	High	692	96	596	24135.1
fbp.19	TE	High	561	241	320	17423.0
fbp.7	OL	High	509	142	367	16527.3
fbp.49.s2	RB	High	504	115	389	20596.7
fbp.2	DB	High	466	80	386	15522.6
fbp.9	WR	High	433	60	373	15226.0
fbp.12	LB	High	369	121	248	11746.4
fbp.32.s2	FS	High	369	91	278	13555.5
fbp.25	DB	High	321	82	239	9192.2
fbp.29	DB	Low	298	48	250	9016.2
fbp.14.s2	DL	Low	290	96	194	8850.0
fbp.8	TE	Low	285	82	203	8801.8
fbp.48.s2	LB	Low	279	107	172	9517.1
fbp.30.s2	DB	Low	268	40	228	12096.6
fbp.36.s2	DL	Low	232	68	164	6557.4
fbp.24	WR	Low	216	88	128	7859.3
fbp.26	WR	Low	206	41	165	6358.6
fbp.27	LB	Low	129	33	96	3609.0
fbp.13.s2	DB	Low	90	18	72	2601.5

**Abbreviations** – DB: Defensive back, DL: Defensive lineman, FS: Free safety, LB: Linebacker, OL: Offensive lineman, TE: Tight end, WR: Wide receiver, TC: Training camp, S: Regular season, GFsum: cumulative gForce  
 Players with '.s2' in their subject name are players from the second season of football



**Figure 4.** Correlation between cumulative linear acceleration (gForce; A), rotational acceleration (B) and the total number of impacts sustained over a full season.

### 3.2 Comparing the exposure groups

Players who sustained more impacts than the overall median (per player), were placed into the high exposure (HE) group and the remaining 10 players were deemed low exposure (LE) players. Various aspects of the head impact data were statistically analyzed to determine the differences between the groups (Table 2). On average, HE players sustained  $503 \pm 150$  impacts, while LE players were subject to  $229 \pm 71$  impacts over the course of the entire season ( $t(18) = 5.2$ ,  $p < 0.001$ ). When assessing cumulative linear acceleration (gForce), there was a significant difference between the two groups ( $t(18) = 4.9$ ,  $p < 0.001$ ), where the HE players received a greater cumulative gForce compared to the LE players. There was also a significant difference when assessing the cumulative rotational velocity between the two groups ( $t(18) = 4.6$ ,  $p < 0.001$ ) where HE players were exposed to higher cumulative rotational velocity compared to the LE players. When analyzing the magnitude of impacts, both linear and rotationally, on a per-impact basis, there were no significant differences between the two groups (Table 2).

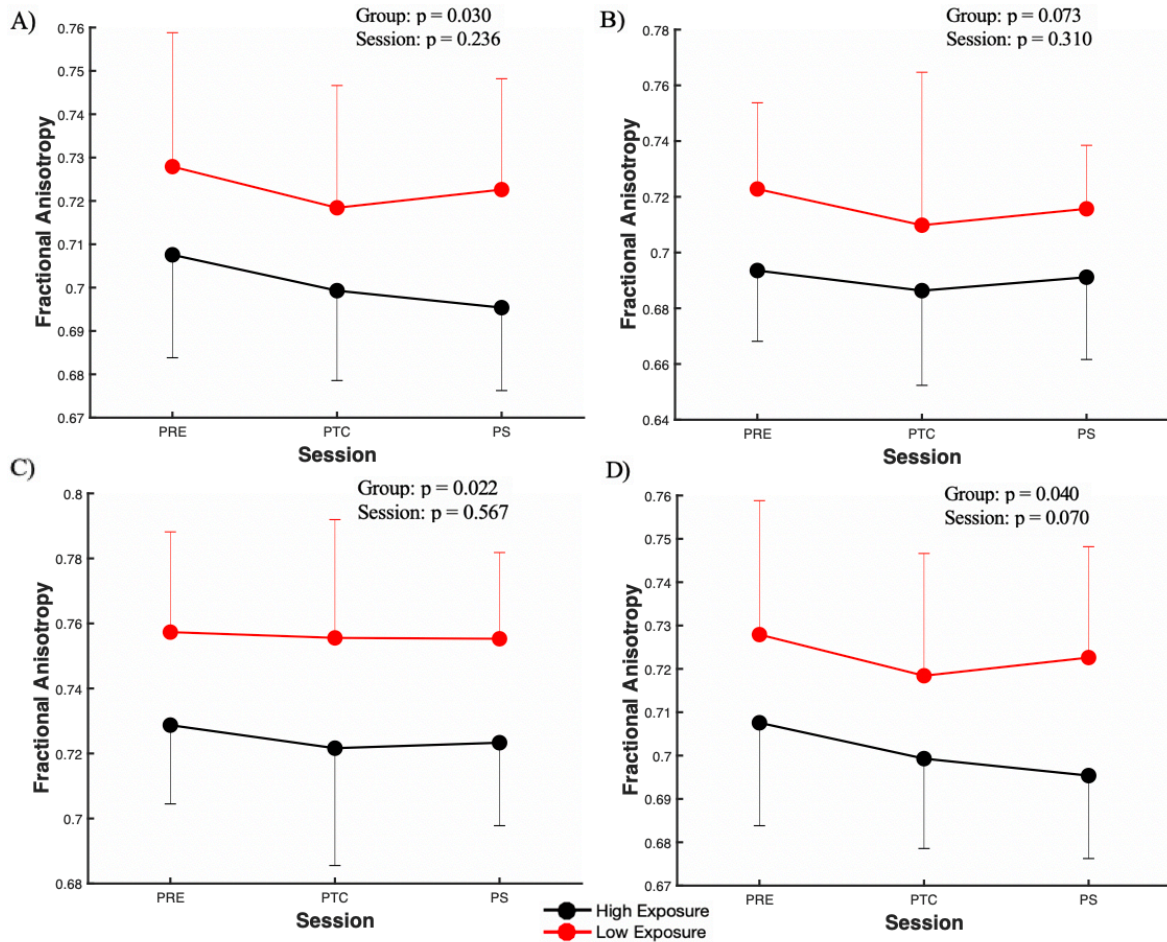
**Table 2.** Comparing the impact measurements of the two exposure groups

	High Exposure	Low Exposure	p-value
Impacts	503 (150.3)	229 (71.2)	<0.001*
Cumulative LA (g)	17031 (5413)	7527 (2837)	<0.001*
LA / Impact (g)	33.8 (3.4)	32.3 (5.2)	0.472
Cumulative RR (°/sec)	297397 (117851)	116406 (41761)	<0.001*
RR / Impact (°/sec)	580.7 (90.2)	502.8 (89.6)	0.069

All values are the mean value (standard deviation) per player, with the associated p-value comparing the two groups  
**Abbreviations** – LA: Linear acceleration. RR: Rotational resultant (rotational velocity). g: gForce

### 3.3 Along-tract analysis

To determine if any DTI changes occurred as a result of subconcussive impacts a two-way ANOVA was performed to analyze the differences between exposure groups and how the DTI metrics may have changed over time. The first tract analyzed was the CC, where there were no significant main effects (Group or Session) or interactions in the back section for any of the four DTI metrics. There was a significant main effect of Group for FA in the middle ( $F(1,18) = 6.3, p = 0.022$ ; Figure 5C) and front ( $F(1,18) = 4.9, p = 0.040$ ; Figure 5D) sections, as well as the whole tract average ( $F(1,18) = 5.6, p = 0.030$ ; Figure 5A). The HE group was found to have a lower FA compared to the LE group in all cases. There was no significant effect of Group when analyzing MD, AD or RD. There were no significant main effects of Session for FA, MD, AD or RD in any of the sections, as well as the whole tract average. There were also no significant interactions between Group and Session.

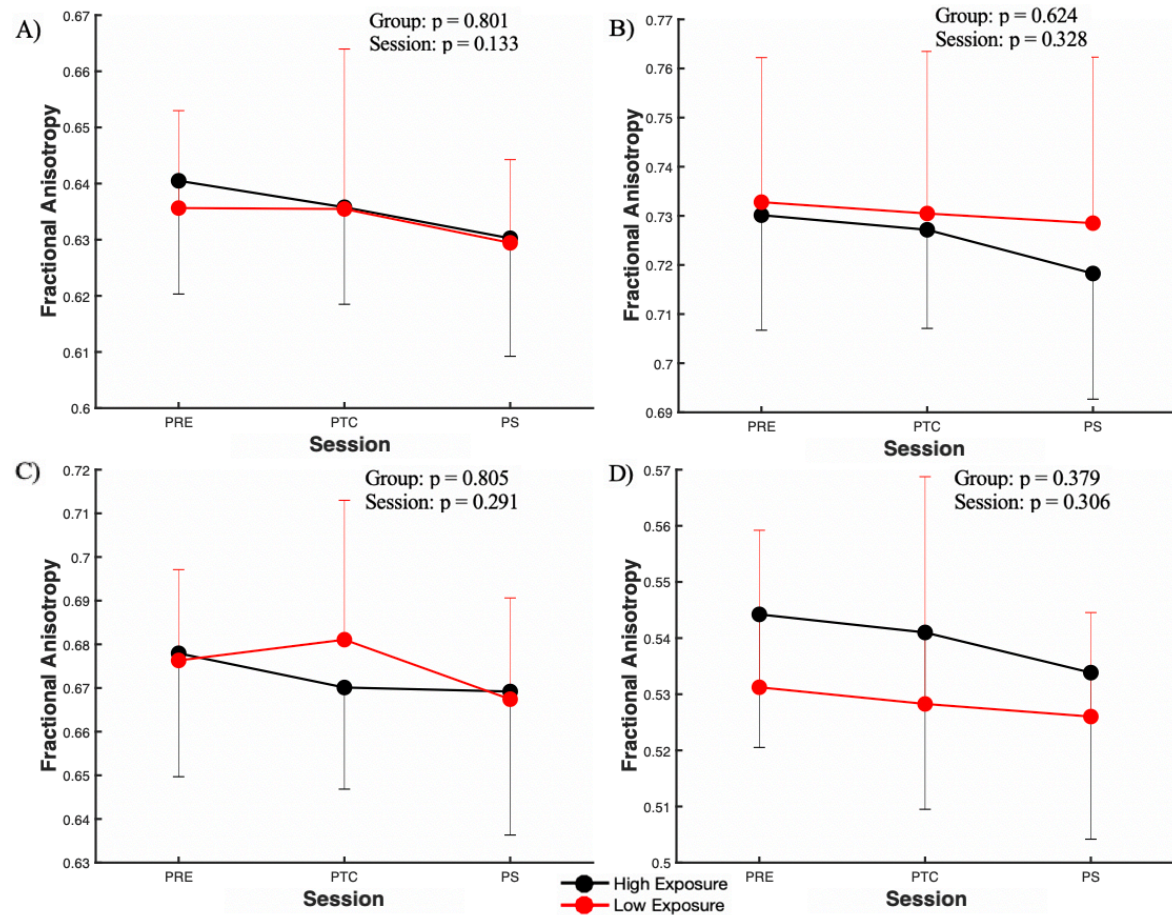


**Figure 5.** Corpus callosum average fractional anisotropy (FA; whole tract A, back B, middle C, front D) for both exposure groups. PRE = preseason, PTC = post-training camp, PS = postseason.

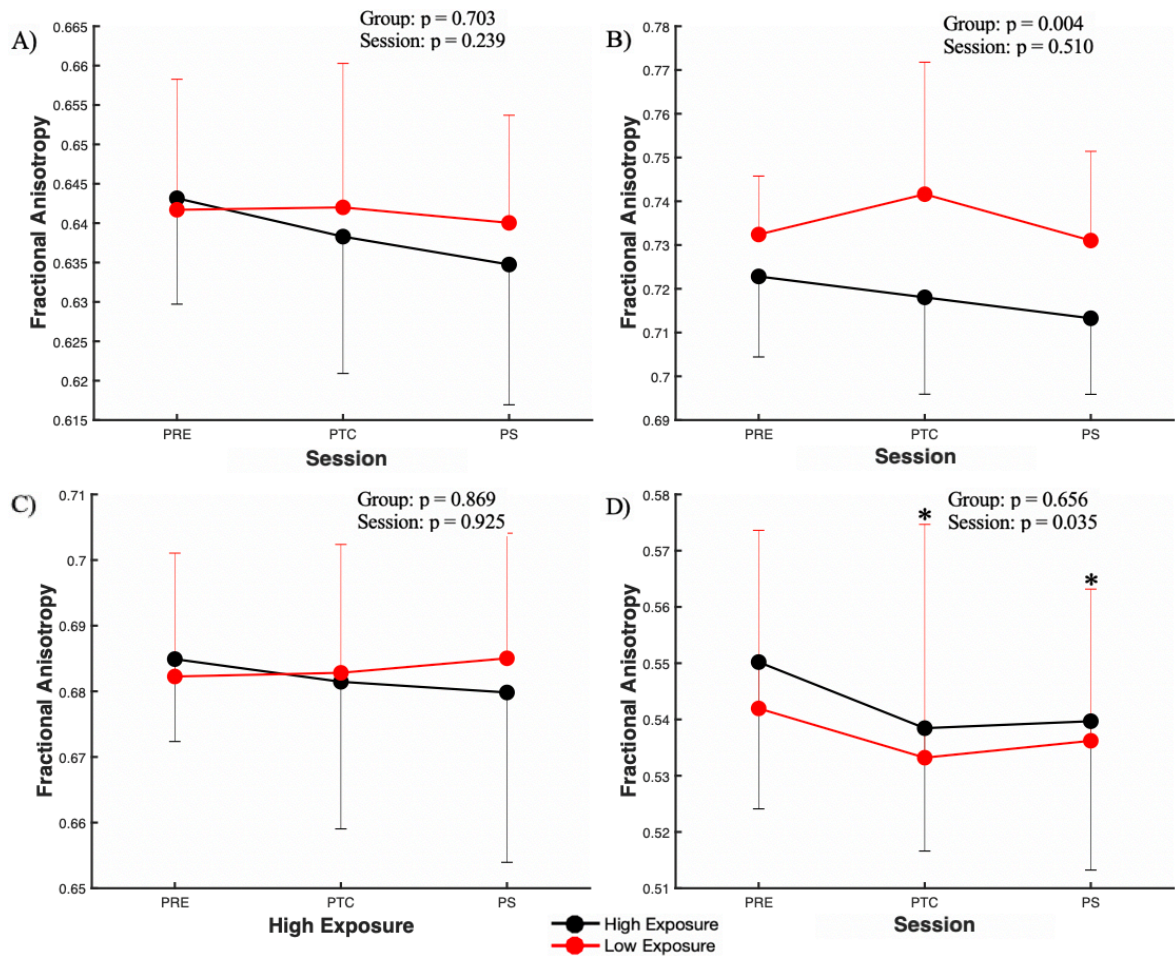
For the CST, there was a main effect of Group in the Bottom section of the left CST ( $F(1,18) = 10.7, p = 0.004$ ; Figure 7B), indicating that the HE group had significantly higher FA compared to the LE group. There was no effect of Group for FA in any of the other areas of the CST. There were no significant Group effects in MD, AD or RD. In the Top section of the left CST there was a main effect of Session ( $F(2,36) = 3.7, p = 0.035$ ), indicating a decreased FA over time (Figure 7D). The post-hoc analysis for this main effect revealed that there was a significant decrease in FA ( $p = 0.043$ ) from preseason to post-



training camp, as well as a significant decrease ( $p = 0.048$ ) from the preseason to post-season. Despite the change in FA (Figure 7D), there were no significant changes in MD, AD or RD over time in the CST. There were not any changes in the middle and bottom sections of the left CST over the season in any of the four DTI metrics. In the right CST there were no significant main effects in any section for the four DTI metrics. In both tracts and sections there were no significant interactions between Group and Session.



**Figure 6.** Right corticospinal tract average fractional anisotropy (FA; right tract A, bottom B, middle C, top D) for both exposure groups. PRE = preseason, PTC = post-training camp, PS = postseason. \*  $p < 0.05$  compared to PRE.



**Figure 7.** Left corticospinal tract average fractional anisotropy (FA; right tract A, bottom B, middle C, top D) for both exposure groups. PRE = preseason, PTC = post-training camp, PS = postseason. \*  $p < 0.05$  compared to PRE.

# Chapter 4

## Discussion and Conclusions

Recently, analysis of the effect that head impacts in contact sports have on neurological health has increased dramatically, with numerous sources questioning the short and long-term effects on the athletes (Hunter, Branch, & Lipton, 2019; Koerte et al., 2017; Talavage et al., 2014). Among this literature is a growing number of studies analyzing the structural and functional effects of head impacts on the brain with the use of various neuroimaging techniques (Bahrami et al., 2016; Chun et al., 2015; Lipton et al., 2013). Diffusion tensor imaging is a promising tool within this specific area of research since it is an indicator of microstructural changes within the white matter areas of the brain. While most research has placed an emphasis on the effects of mTBI (concussion) in contact sports, there has been a recent push to more critically analyze the effects of subconcussive head impacts to determine how they may alter the structure and function of the brain. While some players may never experience a concussion playing contact sports, they may sustain subconcussive head impacts, and it has been recently suggested that the effects of these impacts may accumulate over time with any changes or damage occurring as a result of the numerous, smaller impacts. This study focused specifically on the CST and CC, as they have both been shown to exhibit changes following repeated subconcussive impacts (Bazarian et al., 2012; Chamard, Lefebvre, Lassonde, & Theoret, 2016; Henry et al., 2011; McAllister et al., 2013). The tracts were analyzed as a whole unit, as well as broken down into segments to better understand if certain aspects of the tract are

more susceptible to damage. Prior to analysis it was hypothesized that both the CC and CST would experience changes in FA and MD, with a hypothesized decrease in FA and a corresponding increase in MD. However, it was thought that this effect would be magnified in the CC due to its structure and location as a result of the shearing forces exerted on the axons caused by head impacts. It was also hypothesized that the players who experience more impacts (high exposure players; HE) during a season would show greater changes in FA and MD compared to those players who had less impacts. .

#### **4.1 Impact Analysis**

The 20 players enrolled in this study sustained a median of total of 310 impacts over the course of one full football season. In a 2010 study by Crisco and colleagues, it was reported that the players in their sample experienced a median of 257 294 and 438 impacts per season for the three teams they tracked. The player who experienced the most impacts on each of these teams received 1022, 1413 and 1444 impacts over one season. No player in our sample was exposed to this level of RHI, with the maximum number of impacts sustained by any player being 801 impacts in one season. In a later study by Crisco et al. (2011) it was reported that the maximum (median) number of impacts sustained in a season was 2492 (240) impacts. While the maximum number of impacts sustained by one player is far higher than any player within our sample, the median number of impacts sustained by our players appears to be in line with previous literature. These studies reported that the seasons consisted of 76 practices and 15 games, while in Canadian University football the seasons consist of only 38 practices and 9 games. This drastic increase in time spent on the field would likely result in significantly more impacts, as seen

by one player experiencing over 2000 impacts in one season. This stands as the most likely reason for the discrepancies of maximum impacts between our study and previous literature. It is also possible that the data collection parameters played a role in any differences between studies. Crisco et al (2010, 2011) used a linear acceleration threshold of 10 g to record impacts, while our study only recorded impacts over 15 g. We chose a 15 g threshold to ensure that all recorded impacts were caused by contact, avoiding the potential collection of forces resulting from rapid changes of direction while moving. As a result of this choice we may have missed some impacts that would have otherwise been recorded in previous literature.

Each player was exposed to a wide variety of impact magnitudes regardless of their overall impact exposure. The purpose of this study was to examine the cumulative effect of repeated impacts; however, this may be confounded by certain players being repeatedly exposed to higher magnitude impacts that may be more injurious than their less forceful counterparts. To examine this, we performed a correlation to determine if there was a relationship between the impact forces and the number of total impacts received by each player. These tests showed that both the linear and rotational forces were directly correlated to the number of total impacts received. This is an important finding to help interpret the results of the DTI findings of this study as it confirms that any changes are occurring as a result of receiving more impacts, not necessarily receiving harder impacts.

## **4.2 Along-tract analysis**

When assessing the CC, no significant changes occurred in any segment over the season, however the HE group was found to have significantly lower FA regardless of time

compared to the LE group. Contrary to our hypothesis, the results of our analysis indicated that within our subject group the CST was more vulnerable to changes as a result of these impacts. It was found that the top section of the left tract had a significant decrease to FA from preseason to post-training camp as well as postseason, with no changes occurring in the middle and bottom sections. The right tract showed no such changes, with all sections remaining unchanged over the season.

While there are a few determining factors that affect diffusion within the brain's white matter, the most frequently discussed are demyelination (Sun et al., 2006), neuroinflammation (Tievsky et al., 1999) and axonal shearing (Werring et al., 1999). Prior research has hypothesized that demyelination presents as a large change in radial diffusivity, allowing water molecules to travel perpendicular to the tract, decreasing FA in the process (Sun et al., 2006). Neuroinflammation appears to present with significant increases in MD, as shown in multiple sclerosis patients (Tievsky et al., 1999). Axonal shearing often presents with a significant decrease in FA (Werring et al., 1999), which may be the basis for the decreased FA seen in our results. It is assumed that one or more of these factors will be playing a role in any observed changes that result from repeated head impacts. The shearing forces of the repeated head impacts could be causing diffuse axonal injury (DAI), coupled with demyelination of the axons, leading to a decrease in the directionality of diffusion within that area of the tract (a decrease in FA).

We hypothesized that the CC would experience changes in FA and MD as a result of repeated head impacts. This hypothesis was based on previous literature suggesting that the CC may be sensitive to the shearing forces that occur with head impacts (Bahrami et al., 2016; Bazarian et al., 2012; Henry et al., 2011; McAllistar et al., 2013). Recently,

Bazarian et al. (2014) showed that DTI changes can occur in the absence of changes to clinical outcome measures. Recent studies have provided evidence that the microstructural changes occurring may be a result of repeated, minor axonal injuries (Zhang et al., 2006). Zhang et al. (2006) reported that boxers experienced decreased FA along the CC when compared against controls, indicating that receiving numerous subconcussive head impacts would result in microstructural alterations. The results of this study align with previous literature in arguing that the CC is a susceptible area of the brain to damages caused by RHI. While the DTI metrics were not found to change over the course of the season, the lower FA found in the HE group may be representative of their entire football careers, not just a result of this current season. It is possible that the players did not receive enough impacts in this season to significantly change diffusion, however the players' exposure levels are most likely representative of their careers. This increased exposure over a long period of time has led to a lower FA in the CC.

In this population of football players, FA in the top section of the CST decreased, while the rest of the tract remained unchanged over the course of the season. While not specific to the top section, Henry et al. (2011) supported the idea that the CST is a vulnerable tract when it comes to head injury. In their study they found an increase in FA after SRC. This may be due to the top section's close proximity to the surface of the brain, or with the increasing complexity of connections and terminations as it approached the cortex. As FA was found to decrease over time, it appears as though this area of the CST is more prone to axonal shearing than the lower regions. It remains unclear why the Left tract was more affected than the Right tract, however this may be a result of receiving repeated impacts to one side of the head. These microstructural changes were found to

occur after only one month of football activities (training camp) and remained after one month of no exposure following the season (postseason), which aligns with the findings of Bazarian et al. (2014).

The results of this study were influenced by the heterogeneity of the team and the exposure groups. The overall FA and MD values indicated that no changes occurred in any section of the CC, however it was found that some players had a decrease in these values, while some had an increase over the season. There were found to be a number of players with an increased MD value by the post-season scan, however this was offset by a subset of players with a decreased MD. This set of players removed the chance of significant changes to MD values. It is possible that these players experienced different interactions in their brain as a result of their sustained impacts, leading to different alterations in their microstructure. It is not entirely uncommon to see subconcussive impacts or mTBI lead to increased FA and a corresponding decrease in MD (Bazarian et al., 2014; Churchill et al., 2017). Churchill and colleagues (2017) suggested that a decrease in FA and increase in MD is more indicative of more serious injuries, relating it closely to mTBI. They hypothesized that an increase in FA and a decrease in MD may be the result of a more minor, reversible injury resulting from the repeated head impacts. This was also supported by Henry et al. (2011), who hypothesized that their observed increase in FA in the CST was a result of cytotoxic edema and localized inflammation. They claimed that these are the results of a milder injury when compared to TBI. With this, it is possible that our entire group was affected by the impacts they received; however, they were all affected to different degrees leading to vastly different results. This leads to an overall lack of change when assessing the team's mean.



We hypothesized that increasing the frequency of impacts to the head would increase the observed changes. These changes would be a result of a cumulative effect, wherein each individual impact may not be overtly injurious, however when repeated they would impart repeated, minor axonal injuries to areas of the brain under stress. This was done with the hopes of isolating the cumulative effect as best as possible, without allowing other factors to play a role in why certain players experience different changes. The results of our study indicate that the HE did not experience greater diffusion changes compared to the LE group over the course of the season. A 2019 study by Champagne et al. sought to examine how FA changes in the CC as a result of RHI. In contrast to our results, their findings indicate that changes were occurred in their high exposure players, while no such changes occurred in the low exposure group. In their high exposure group FA was found to decrease when comparing preseason to post-training camp and post season. While similar in some respects, the methodology used by Champagne et al. differed from the current study in how it assessed a player's exposure. The exposure groups were formed based on their average number of impacts per session, as opposed to the total number of impacts received over the season. This may have altered the exposure groups in a way that better separated the players into groups that reflect their risk of injury. While the two groups did not experience different changes over time (no significant interaction between group and session) in either tract, there was a significant difference between the groups (regardless of session) in a number of tract segments.

### 4.3 Future directions and study limitations

Several suggestions can be made to improve future research endeavours in this area. The first improvement that should be made is a more accurate way of filtering improper head impacts received by the accelerometers. While the researchers removed any erroneous impacts from the results, there remains a large amount of human error, possibly inflating impact numbers for all players. Notes were always taken when an improper impact occurred, however there was not always a researcher present if none were able to attend. This was a rare occurrence over the two seasons in which players were followed, however these occurrences were often for games, where the majority of impacts occur. Also, when researchers were present, it was still possible that impacts were missed as a result of watching many players across a large field. The simplest way to improve this impact accuracy would be to have more researchers present to detect any impacts that require deletion. Ideally it would be possible in the future to perform these deletions automatically as a result of an algorithm that may be able to isolate erroneous impacts.

Another possible limitation surrounding the collection of head impact data was the lower threshold of 15g impacts. This threshold was used as a method of filtering out non-impact related events, as running and changing directions can cause relatively large “impacts” on these sensors.

The third limitation of the study is the isolation of only two white matter tracts. It may be beneficial to perform a similar study on numerous tracts throughout the brain, as previous studies have shown that various other tracts are also susceptible to damage caused by repeated head impacts as well. It would also be interesting to perform the same along-

tract analysis on various other tracts to determine if any changes found are localized to specific regions or the tract as a whole.

Another possible limitation is the relatively small sample size of players in this study. It was difficult to increase our sample size for this research as players were frequently injured (head related or otherwise). For the purpose of this research it was vital that players participated for the entire duration of the season (or reasonably close to its entirety) to reduce variability among players. For example, if an injured player was included, it could be possible that they were highly exposed during the time that they played, but with missing time their overall impact tally would be low, leading to them being labelled a LE player. With this, the number of players available from our original sample was reduced.

These results point to a need to increase player safety in the reduction of head impacts. It would also be beneficial to expand the horizons of this research by including more white matter tracts, creating a more diverse representation of the changes occurring as a result of these impacts. It may also prove beneficial to expand the impact analysis to better determine the players at a higher risk of injury. It is possible that by differentiating players who were exposed to higher magnitude impacts, players of different positions, or players exposed to higher rotational impacts may be at a higher risk. In future we could expand on the idea of exposure, possibly finding closer links between the observed changes and why they are occurring. One more possible direction would be to expand this research to sports outside of football, as this specific type DTI research has not been performed. It would be possible to perform this same along-tract analysis in sports such as hockey, rugby and boxing among others, which may lead to similar findings.

## **4.4 Conclusions**

The results of this research study indicate that over the course of one full season of Canadian collegiate football, players will be subject to numerous head impacts, resulting in microstructural changes in the corticospinal tract. Our results indicate that the CST was susceptible to damage caused by repeated head impacts. More specifically, the top section of the CST was found to be the most susceptible to microstructural changes. The HE group was found to have significantly lower FA values compared to the LE group in the CC and parts of the CST, which may be a result of exposure over their careers. Further, the group-based analysis showed that DTI changes were not linked to the number of head impacts received in one season, indicating that the number of impacts received by this group may not be enough to cause significant damage. The changes found in this study indicate that despite no outward symptoms of injury (SRC), “silent” changes are occurring within the brain’s microstructures as a result of receiving numerous subconcussive impacts during a season of football.

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