4-2020

Serum-based biomarkers and magnetic resonance imaging following mild traumatic brain injury in collegiate athletes post return-to-play

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SERUM-BASED BIOMARKERS AND MAGNETIC RESONANCE IMAGING FOLLOWING MILD TRAUMATIC BRAIN INJURY IN COLLEGIATE ATHLETES POST RETURN-TO-PLAY

By

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THESIS

Submitted to

Northern Michigan University

In partial fulfillment of the requirements

For the degree of

MASTERS OF SCIENCE IN PSYCHOLOGICAL SCIENCE

Office of Graduate Education and Research

April 2020

SIGNATURE APPROVAL FORM

Serum-based biomarkers and magnetic resonance imaging following mild traumatic brain injury in collegiate athletes post return-to-play

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ABSTRACT

SERUM-BASED BIOMARKERS AND MAGNETIC RESONANCE IMAGING FOLLOWING MILD TRAUMATIC BRAIN INJURY IN COLLEGIATE ATHLETES POST RETURN-TO-PLAY

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Recently there has been an increase in the use of MRI (Magnetic Resonance Imaging), to measure the effects of traumatic brain injury (TBI). Proteins such as BDNF, S100B, UCH-L1, and Tau have been found to have altered levels in blood serum after TBI. However, there is limited knowledge about the relationship between serum-based and MRI-based biomarkers in concussed athletes post return-to-play. This study aimed to bridge this gap by collecting serum samples from 42 participants across two groups. The first group (n = 21) consisted of recently cleared to return-to-play collegiate athletes after experiencing a sports-related concussion. The second group (n = 21) was collegiate athlete controls matched on age, sex, and sport. Structural and functional MRI were used for analysis from a subgroup of 26 participants (13 concussed, 13 control). Blood samples were collected to assess the levels of BDNF, S100B, UCH-L1, and Tau. To the best of our knowledge, this is the first study to analyze protein levels in association with gray matter volume (GMV) and resting state functional connectivity (rsFC) following sports-related concussion. BDNF was the most effective protein at differentiating concussion from control. Significant differential relationships were found between BDNF, S100B, UCH-L1, and Tau with GMV and rsFC, suggesting that serum-based biomarkers may have important clinical implications in concussion diagnosis and treatment.

DEDICATION

This thesis is dedicated to my little sister, Calista Susa. Her severe traumatic brain injury experience sparked a passion within me for neuroscience that I didn't know existed. Her emphasis on the need to help others and joy for life has instilled in me a drive to make the world a safer place. Without her I would not be the aspiring researcher that I am today.

Additionally, this thesis is also dedicated to anyone who has endured traumatic brain injury or concussion(s) in their lifetime or who currently suffer from complications following brain injury. It is the hope that this thesis can contribute in some way to the deeper understanding of brain injury, especially sports-related concussion.

ACKNOWLEDGEMENTS

The author wishes to thank her adviser and mentor, Dr. Joshua M. Carlson, who has been instrumental in her research journey over the past five years, he gave her a chance at research and has continually provided insight throughout this project; Dr. Marguerite T. Moore for imparting your passion and deep love within her for concussion research; Dr. Ashley M. Shayter for sharing your insight on proteins with her; Dr. Vincent Jeevar for being a support system and for wanting to see her work change concussion for the safety of others; Indiana University for believing in her and wanting to bring this research to new heights; friends and family for enduring the long weeks of data collection, analysis, and writing with her and supporting her to attain her full potential; and her fiancé, Michael, for always telling her to reach for the stars and supporting her wildest dreams. Additionally, the author would like to thank Northern Michigan University for the following funding sources; the 2016 Northern Michigan University PRIME Grant and 2016 Faculty Research Grants awarded to Dr. Carlson and Dr. Moore; the 2018 Spooner Grant and the 2019 Excellence in Education Grant awarded to TRS – without this funding this project would have not been possible.

This thesis follows the format prescribed by the Publication Manual of the American Psychological Association, 7th edition and the Department of Psychological Science.

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LIST OF ABBREVIATIONS

TBI	Traumatic brain injury
mTBI	mild traumatic brain injury/mild head injury
DASS	Depression Anxiety Stress Scales
MRI	Magnetic resonance imaging
GMV	Gray matter volume
FC	Functional connectivity
PET	Positron emission topography
BDNF	Brain-derived neurotrophic factor
UCH-L1	Ubiquitin c-terminal hydrolase L1
rsFC	Resting state functional connectivity
GCS	Glasgow Coma Scale
ImPACT	Immediate Post-Concussion Assessment and Cognitive Testing
PCSS	Post-Concussion Symptom Scale
CT	Computed Tomography
DTI	Diffusion tensor imaging
DWI	Diffusion weighted imaging
fMRI	Functional magnetic resonance imaging
ROI	Region of interest
SPM	Statistical Parametric Mapping
DMN	Default mode network
DAN	Dorsal attention network
FPC	Frontoparietal control network
NSE	Neuron-specific enolase
GFAP	Glial fibrillary acidic protein

ELISA	Enzyme-linked immunosorbent assay
RPFC	Right prefrontal cortex
LPFC	Left prefrontal cortex
SMA	Supplementary motor area
FDR	False discovery rate

Introduction

With over 1 million emergency room visits a year and 1.7 million new cases annually in the United States, traumatic brain injury (TBI) is widely prevalent (Wu et al., 2016). Of those visits, 70%-90% are for mild traumatic brain injury (mTBI), more commonly referred to as a concussion. Further, 40%-50% of those who experience a concussion are symptomatic three months after injury (Wu et al., 2016). Physical and cognitive impairments (migraine, difficulty balancing, lapses of attention, and memory deficits) are regularly experienced as an outcome of concussion. Additionally, concussion is linked to elevated symptoms of depression and anxiety (Moore et al., 2006). We assessed levels of depression, anxiety, and stress using the Depression Anxiety Stress Scales (DASS) at the post return-to-play time point following recently concussed collegiate athletes and age, sex, and sport matched controls.

Concussions happen most frequently in sports, falls, and vehicle accidents (Moore et al., 2006). It is still unclear what the underlying mechanisms of concussion are long term on the brain in regards to physical and mental health. These underlying mechanisms could be explained with brain imaging and blood-based biomarkers.

Despite the high prevalence of concussion, there are not many studies using neuroimaging techniques to assess the post-injury phase. MRI (magnetic resonance imaging) is a neuroimaging technique that is used to assess a variety of clinical and research needs including TBI (Damadian et al., 1974). Several different scans can be conducted in one single scanning session, allowing for a thorough assessment of the brain at certain time frames including at the onset of an injury or disease. Using MRI, to not only look at the onset, but also the period after a concussion to measure the prolonged effects of injury in combination with their relation to

proteins is important in neuroplasticity and could improve how post-injury recovery is evaluated (Wu et al., 2016).

One way this can be done is by assessing functional connectivity (FC) levels which is brain activity that is associated with regional brain activation during resting state or task-based scanning (Ptito et al., 2007). We evaluated resting state FC (rsFC) of a task negative MRI scan in the frontal region of the brain including the left and right dorsolateral prefrontal cortex due to its prevalence in concussion (Czerniak et al., 2015; Eierud et al., 2014). We also assessed gray matter volume (GMV) to evaluate structure changes in the brainstem, prefrontal cortex, and hippocampus (Brezova et al., 2014; Churchill et al., 2017; Dean et al., 2015).

We recently assessed brain derived neurotrophic factor (BDNF), a protein that plays a vital role in synaptic plasticity and neuronal survival with the current study's participants (Susa et al., 2019). Based on our findings of significantly elevated levels of BDNF in serum, a derived component of blood, in the concussed group in comparison to controls we chose to further evaluate other proteins in serum as potential biomarkers of concussion post return-to-play. Previous research has indicated that S100B, ubiquitin c-terminal hydrolase L1 (UCH-L1), and Tau in serum may compare with differences in rsFC and GMV.

The use of serum is more effective than measures of cerebral spinal fluid, saliva or urine samples. However, when we assessed BDNF in serum, we also assessed BDNF in saliva. Although there we did not find any significant group differences, a significant relationship was found between serum and salivary BDNF measures in the concussed group (Susa et al., 2019). Even though salivary samples could be evaluated in addition to the serum samples in this study based on our findings, for the sake of serum being more robust and reliable this thesis will only be assessing serum in S100B, UCH-L1, and Tau.

Several proteins exist as potential serum-based biomarkers in TBI, some in mTBI, but none have been specified in the 1 – 45 day post-concussive phase of sports-related concussions (Kawata et al., 2016). Recent research examining serum biomarkers in concussion have found elevated levels of many proteins, but overall have indicated mixed results especially when trying to assess correlations with MRI (Kulbe & Geddes, 2016; Zetterberg & Blennow, 2015). The frontal and occipital lobes are commonly affected in concussion and the potential deficits of these areas may correlate with the serum-based protein levels (Churchill et al., 2017; Eierud et al., 2014).

Research suggests that S100B is related to exercise, hence it may be specific to concussion because of the high prevalence in sports (Dang et al., 2014). Regarding Tau, previous research has seen a significant increase in levels when concussed athletes were compared to their own preseason measures, yet we do not know the level of Tau following a concussion when compared to matched controls (Randall et al., 2013). UCH-L1 is one of two proteins approved by the FDA to diagnose TBI by serum samples within 12 hours post-injury (Welch et al., 2018). Specifically in mTBI, UCH-L1 has shown increases in non-sports related concussion in comparison to healthy controls (Li et al., 2015). Recently, UCH-L1 has shown to have an initial decrease when compared to baseline prior to increasing following non-sports related concussion (Welch et al., 2018).

Based on these findings it is still unknown what the effects of these specific proteins are when evaluated within three months post return-to-play of recently concussed collegiate athletes. We can achieve multiple levels of analysis examining images of the brain compared to samples of blood. Correlating protein levels in serum with MRI may be utilized as a future marker of individual-based recovery post-concussion.

The present study's first aim was to establish serum-based biomarkers in S100B, UCH-L1, and Tau as an assessment of post return-to-play in athletes with a recent sports-related concussion. The second aim was to assess changes in the structural and functional brain networks of the concussed group compared to the control group. The third aim was to compare MRI findings to S100B, UCH-L1, and Tau levels collected through serum samples. We hypothesized that there would be (1) an effect of group for each protein such that higher levels of S100B and Tau as well as lower levels of UCH-L1 would be expressed in the concussed group in comparison to the control group, (2) differences in GMV and rsFC between groups, and lastly (3) alterations in serum-based protein levels would be linked to differences found in GMV and rsFC from the MRI scans between groups.

Literature Review

Traumatic Brain Injury

Prevalence. A TBI is defined by the World Health Organization (World Health Organization, 2006) as any injury to the brain resulting from an external force that leads to a spectrum of problems, including concussion, contusion, and/or diffuse axonal injuries. According to the Centers for Disease Control and Prevention there were 1.88 million emergency department visits, hospitalizations, and deaths because of TBI in the United States in 2006 (Centers for Disease Control and Prevention, 2019). By 2014, in only 8 years, that number increased by 53% to 2.87 million TBIs (Centers for Disease Control and Prevention, 2019). In the United States, an average of 155 people die each day from injuries including TBI, with the leading cause of TBI-related death varying by age (Centers for Disease Control and Prevention, 2019). The leading cause of TBI-related emergency room visits in 2014 overall was falls accounting for 48% of all injuries and 17% being struck against or by an object (Centers for

Disease Control and Prevention, 2019). TBI-related hospitalizations for all ages were led by 52% falls and 20% motor vehicle crashes (Centers for Disease Control and Prevention, 2019).

Diagnosis. TBI is characterized in one of three ways; mild, moderate, or severe and most commonly determined by the Glasgow Coma Scale (GCS) (Teasdale & Jennett, 1974). This tool detects eye movements, verbal responses, and motor responses immediately after an injury (Teasdale & Jennett, 1974). A GCS score can range from 3 to 15, the lower the score the more severe the condition (Mallya et al., 2015). A value less than or equal to 8 is considered severe, a score between 9 and 12 is moderate and anything 13 or above is mild (Jeter et al., 2013). About 80% of all TBIs are classified as mild (Bernstein, 1999; Mallya et al., 2015; Wu et al., 2016). With such a high prevalence rate, mTBI has been coined the term "the silent epidemic" (Tellier et al., 1999). About one quarter of those with a mTBI are hospitalized each year, 35% are treated in the emergency room and released, 14% received some sort of outpatient treatment and 25% received no medical care (*Report to Congress on mild traumatic brain injury in the United States: Steps to prevent a serious public health problem*, 2003).

Males are twice as likely to have a head injury occur compared to females (Langlois et al., 2006; Mallya et al., 2015; Moore et al., 2006). Other risk factors for mTBI include alcohol consumption, substance abuse, learning disability, marital discord, area of living, or a previous TBI (Weight, 1998). Studies have been conducted assessing the association between mental illnesses and TBI, previous reports have been made stating that as high as 68% of psychiatric inpatients had experienced a TBI (Burg et al., 1996). That statistic is important, because 40-50% of people that endure a mTBI have symptoms three months after injury, thus, there could be a potential link between psychiatric illness and concussion (Wu et al., 2016).

All things considered, there are a large number of symptoms that individuals endure following TBI including vision problems, anxiety, depression, and attention deficits among others. The symptoms of mTBI and TBI have been continuously studied with different types of neuroimaging over the years. Scientists have been trying to find what is causing these symptoms, whether it be changes in brain volume, cortical activity, or FC patterns, etc. (Churchill et al., 2017; Dean et al., 2015; Dettwiler et al., 2014; Ptito et al., 2007). These studies are assessing TBI at all levels from mild to severe. An emerging facet of TBI research is a recent focus on sports-related concussion due to an increase in the number of concussions being experienced and the safety in sports surrounding head injury.

History of Sports-related Concussion. Concussion is a word that was coined back in the 1660s for a certain type of head injury. The word evolved from the earliest work on head injuries over 2,000 years ago (McCrory & Berkovic, 2001). The meaning of concussion is largely clinical with many changes that have taken place in the definition throughout the past 150 years.

Back in the 19th century after the Civil War (1861-1865), American football saw a rise in popularity (Stone et al., 2014). Unfortunately, at this time there was no protective equipment for those who participated. Seasons were characterized by the number of deaths and serious injuries with the majority of these happening to the head (Stone et al., 2014). Eventually, in 1935, helmets were made mandatory at the high school level and then in 1939 at the college level, skull protection was finally making an appearance (Mueller & Blyth, 1982). In the 1960s the Congress of Neurological Surgeons defined concussion as follows, “a clinical syndrome characterized by immediate and transient impairment of neural function, such as alteration of consciousness, disturbance of vision, equilibrium, etc., due to mechanical forces” (Gurdjian & Voris, 1966).

After the largest number of head and neck injuries was reported in the 1960s for football players there were rule changes implemented at the college level in 1978 and the high school level at 1980 (Mueller & Blyth, 1982). The 1978 rule prohibited blocking or tackling to have any contact with the head and face (Stone et al., 2014). Since this rise in the number of injuries reported, neurologists in the past 50 years have become more involved in sports-related concussions as the most popular sports tend to be the ones with the most head injuries (Dunn et al., 2006).

In the early 1990's, Dr. James Kelly, a neurologist, devised a sports concussion classification system (Kelly et al., 1991). In 1997 this was modified, and the emergence of return-to-play recommendations were made. At this point medical professionals were also making a reference to second impact syndrome. When a concussion was first recognized the player was then removed from the game (Kelly & Rosenberg, 1997). If the player was completely asymptomatic at rest within 15 minutes, the physician and athletic trainers considered the athlete's return-to-play that very day. Notion of post-concussion syndrome began being taken more seriously around this time stating that it consisted of headache with exertion, dizziness, fatigue, irritability, impaired memory and concentration, and could last several weeks or longer (Guthkelch, 1980; Murphey & Simmons, 1959).

In 2001, the first international symposia on concussion in sport was held in Vienna, Austria. The original aims of this meeting were to provide information about possible improvements for the health and safety of athletes that may suffer a concussion in the following sports; American football, rugby, soccer, ice hockey, etc. (McCrory et al., 2005). A definition for concussion rose from the meeting; "sports concussion is defined as a complex

pathophysiological process affecting the brain, induced by traumatic biomechanical forces" (McCrory et al., 2005, p. 196).

At the fourth meeting in 2012, it can be concluded that although mild traumatic brain injury and concussion are terms often used interchangeably in the sporting context, a concussion is a subset of TBI (McCrory et al., 2013). It was at this meeting where it was also stated that 80-90% of concussions resolve in a 7 to 10 day period, although this may not be true of everyone (McCrory et al., 2013). Persistent symptoms lasting longer than 10 days are typically reported in 10-15% of concussions (McCrory et al., 2013). At this concussion symposia it was determined that there should be no clearing of athletes to return-to-play on the same day of injury.

In 2016 at the fifth and most recent Concussion in Sports Group meeting in Berlin, Germany, there was a consensus to replace the term "Post-Concussion Syndrome" with "Persistent Concussion Symptoms" which was described as "a constellation of non-specific post-traumatic symptoms that may be linked to co-existing and/or confounding factors" (McCrory et al., 2017, p. 5). It was decided that the term persistent symptoms should be used when symptoms are experienced more than 10-14 days following injury for an adult and more than four weeks following injury for a child (McCrory et al., 2017).

Concussion at the Cellular/Molecular Level. Any neurologic dysfunction resulting from a biochemical force is defined as a concussion (Giza & Difiori, 2011). A loss of consciousness may happen, but is not a concrete symptom to qualify as a concussion diagnosis (Giza & Difiori, 2011). The neuronal dysfunction that tends to occur with concussion can be changes in neurotransmission, ionic shifts, altered metabolism, or impaired connectivity (Giza & Hovda, 2001).

Concussion is not a macro structural brain injury, but rather a functional disturbance (Leddy et al., 2017). After a biomechanical injury to the brain there is an immediate disruption in the neuronal membranes, axonal stretching, and opening of the voltage gated potassium (K^+) channels (Giza & Difiori, 2011). This then leads to an increase in K^+ extracellularly (Katayama et al., 1990). Normally, the excessive K^+ found extracellularly is taken up by glial cells (Paulson & Newman, 1987). Usually the brain by this mechanism can overcome small disturbances, but in the case of an event such as brain trauma, this method of compensation is not enough (D'Ambrosio et al., 1999). The massive excitation that has occurred is followed by a wave of neuronal suppression, which can also be referred to as "spreading depression" (Giza & Hovda, 2001). An important distinction between classic spreading depression and post concussive K^+ fluxes is that after trauma has been endured, diffuse areas of the brain are effected all at once (Giza & Hovda, 2001).

Energy-requiring membrane pumps are then activated, triggering an increase in the use of glucose to restore the ionic homeostasis of the cells (Richards et al., 1987). This acceleration of glycolysis increases lactate production, this action is seen in both stroke and concussion injuries of animal models (Richards et al., 1987). The oxidative metabolism in the brain is also altered by trauma and can lead to reduced adenosine triphosphate production which will provide another stimulus to increase glycolysis, again increasing lactate production (Giza & Hovda, 2001). Elevated levels of lactate can induce acidosis, altered blood brain barrier permeability, cerebral edema, and membrane damage by neuronal dysfunction (Gardiner et al., 1982). In TBI specifically, the increased levels of lactate can leave neurons vulnerable to a secondary injury (Becker et al., 1988).

Within hours of injury there is an accumulation of calcium as well as reduced intracellular levels of magnesium that can persist for up to four days after injury (Vink et al., 1988). Positron emission topography (PET) scans have shown decreases in the cerebral glucose metabolism of the brain that can last for 2-4 weeks following injury (Bergsneider et al., 2000). This type of glucose metabolism was seen in patients on both ends of the GCS spectrum (Bergsneider et al., 2000). This kind of neurotransmitter alteration has also been found in the cholinergic, adrenergic, and glutamatergic systems (Miller et al., 1990). These changes may be what causes deficits in memory and cognition after a concussion.

All the changes that can happen when a brain undergoes a concussion are difficult to examine in human models, thereby much of the work that has been done uses animal models. However, PET scans have demonstrated the depressed glucose metabolism in patients, but unfortunately this was seen in all aspects of the GCS range (Giza & Hovda, 2001). The physiology of concussion needs to be translated better over to humans for a greater understanding of the injury and so it can have clinical applicability to help the many who suffer a concussion every year.

Time Course of Post-concussive Symptoms. As mentioned, lingering effects of concussion can remain for more than 10 days post-injury and sometimes even up to months later, the symptoms experienced are known as post-concussive symptoms, classified by the International Classification of Diseases, 10th edition (ICD-10) (World Health Organization, 1992). Some of the symptoms include headache, dizziness, fatigue, anxiety, and depression, most people recover from these symptoms 1-3 months following injury (Belanger et al., 2005), but 24%-40% of people with concussion have reported experiencing these three months post-injury, with 10%-20% of people reporting these symptoms more than a year later (Bernstein, 1999;

Iverson et al., 2007; Wu et al., 2016). Post-concussive symptoms are not limited to only concussion, but can also be experienced after injury for any severity of TBI. Except, studying the symptomology is rather difficult because the nature of these symptoms goes beyond the specifics of TBI (Iverson et al., 2007) and long term follow-up is not completed or lost.

However, in a study conducted by Sigurdardottir and colleagues (2009) people diagnosed with mTBI reported post-concussive symptoms significantly more than moderate or severe at three months post injury. At one year following injury no significant differences were found in the presence of symptoms between groups (Sigurdardottir et al., 2009). It would have been beneficial for these researchers to include the MRI results that were collected in conjunction with the questionnaires at one-year post-injury. Inclusion of an MRI scan at three months post-injury when the discovery of mTBI participants reporting more symptoms than the moderate or severe participants would give a greater insight as to why the mTBI group might be experiencing more symptoms. A study conducted by Meares and colleagues (2008 & 2011) observed whether having a mTBI could predict if post-concussive symptoms would be experienced at three months after injury, but was unsuccessful (Meares et al., 2008; Meares et al., 2011). Researchers did not find that mTBI predicted post-concussion syndrome in the acute phase following injury. It is important to note that the control group used in this study was a sample of non-brain injured trauma controls. Therefore, measuring symptomology such as pain, anxiety, stress, and depression, all of which have been found to be commonly experienced after a concussion can also be experienced following any type of trauma. Future studies should use a non-injured control group, but also consider pre-existing comorbidities i.e. chronic pain, depression, or anxiety disorders.

One of the ways that concussion symptoms are assessed in sports is by the Immediate Post-Concussion Assessment and Cognitive Testing (ImPACT). The ImPACT test created by Lovell and colleagues (*ImPACT*, 2003), consists of three main sections; demographic data, neuropsychological tests, and the Post-Concussion Symptom Scale (PCSS) (Schatz et al., 2006). The 2.0 version of the ImPACT test has six neuropsychological tests assessing different areas of cognition including reaction time, processing speed, attention, and memory (Schatz et al., 2006). When combined these sections provide data used to assist in the assessment and recovery of concussions based on four key composite scores: verbal memory, visual memory, visuomotor speed, and reaction time. In this thesis we focused solely on the PCSS component of ImPACT.

Ideally, the ImPACT test would be completed at the start of every academic year or athletic season to be utilized as baseline. Baseline testing is important in sports, because when an athlete has a concussion the data collected during baseline is typically used to determine the individual's "normal" (non-injured levels). In doing so this then provides a more individualized treatment plan/recovery time. However, whether baseline ImPACT or the successive ImPACT tests following post-concussion, research has found that some athletes "sandbag" their scores in an effort to return to play sooner (Higgins et al., 2017). The term sandbag in this context refers to athletes purposely scoring low on their baseline testing so in the event of a concussion they will likely be able to return-to-play sooner. ImPACT's Impulse Control Composite Score detects some of these issues along with in module detection systems limiting the issue of "sandbagging", however it is important to educate athletes taking the tests on why they should do their best as well as individuals reading the results to red flag these cases and repeat testing. Despite this issue, ImPACT has been utilized by institutions at all levels ranging from high school to

professional level sports in order to better assess concussion on a case by case symptom basis as the best current test available.

Assessing symptoms during concussion as well as post-concussion is subjective, as they are self-reported without consistent scoring mechanisms (i.e. a dizziness score of 5 means the individual experiences dizziness in most daily activities and it limits several activities a day), making this a hard concept to study or measure reliability. It does not help that as an incredibly individual species, humans' bodies' reactions to the same kind of injury varies. Although, there has been a recent interest in correlating some of these behavioral changes with neuroimaging techniques like MRI especially in sports-related concussion.

Neuroimaging of Concussion. MRI is a neuroimaging technique that stemmed from nuclear magnetic resonance in the 1970s (Damadian et al., 1976), using radio waves, strong magnetic fields and gradients to create anatomical images of the brain (Damadian et al., 1974). MRI can detect diseased tissue from normal tissue through different magnetic strengths measured in teslas (T) ranging from 1.5T to 9T. The standard strength of an MRI scanner used for clinical purposes is 1.5T.

In the 21st century neuroimaging such as MRI has been more readily available for describing the acute and chronic effects following TBI. MRI specifically, has created an increase in diagnostic accuracy in identifying TBI due to the high signal and contrast to noise ratio, high spatial resolution, and the different tissue contrasts that can be generated (Suskauer & Huisman, 2009). For head injuries, MRI has been demonstrated to have a higher sensitivity in diffuse axonal injury in comparison to computed tomography (CT) scans (Lee et al., 2008).

T1 and T2 weighted sequences are frequently used in MRI studies for their spin-echo or fast spin-echo sequences (Suskauer & Huisman, 2009). Shorter repetition time of the radiofrequency pulse sequences produces T1 images where gray matter is darker than white matter. Longer repetition times of those radiofrequency pulse sequences produce T2 weighted images, appear brighter, with focus on the cerebral spinal fluid. Fluid attenuated inversion recovery is another type of sequencing that is also studied for structural measures. Not only is MRI capable of obtaining a spatial distribution of the entire body, but it also allows for various types of scans to be conducted including diffusion weighted imaging (DWI), diffusion tensor imaging, (DTI) and functional magnetic resonance imaging (fMRI).

Advanced MRI methods can be used to establish subtle post-injury abnormalities in brain metabolism, cerebral microstructure, and function (Churchill et al., 2017). In the last decade these specific types of potential brain abnormalities have been assessed following brain injury, including GMV, cerebral blood flow, rsFC, and cortical thickness (Brezova et al., 2014; Churchill et al., 2017; Czerniak et al., 2015; Dean et al., 2015; Meier et al., 2016; Rigon et al., 2017; Wu et al., 2016).

For the sake of this thesis, we used a T1 weighted scan to establish the potential alterations between groups in GMV. The composition of gray matter is in large glial cells, capillaries, and neuronal cell bodies in which GMV is the density volume of gray matter in certain areas of the brain or the brain as a whole (Eierud et al., 2014). In previous concussion literature, various findings have been made with GMV using T1 weighted scans (Brezova et al., 2014; Churchill et al., 2017; da Costa et al., 2016; Dean et al., 2015; Killgore et al., 2016; Singh & Killgore, 2016; Sussman et al., 2017; Terpstra et al., 2017).

Dean et al. (2015) discovered reduced gray matter in the precuneus, medial temporal and inferior parietal lobe in a small sample of people following concussion compared to non-head injured controls. An association was found between post-concussion symptom scores and lower GMV in the voxel-based morphometry analysis conducted by Dean et al., (2015). Sussman and colleagues (2017) also found reduced GMV following a single concussion in a male only sample of recently concussed athletes versus controls (Sussman et al., 2017). However, the researchers did not specify location of their findings. Despite not indicating specific locations of significance these researchers interestingly mentioned that they did not find significance in subcortical structures or the cerebellum (Sussman et al., 2017).

Da Costa et al. (2016) assessed mTBI in an older population in which they also discovered reduced GMV between experimental and control groups following injury (da Costa et al., 2016). Their experimental group consisted of 25 patients following a mTBI and 19 of those patients participated in a second check in. The researchers defined mTBI as manifestation of one of the following: alteration in mental state with a GCS score <13 after 30 minutes; amnesia time less than 24 hours; loss of consciousness less than 30 minutes; and focal neurological deficits based on the American Academy of Neurology criteria (da Costa et al., 2016, p. 2) The first time point was on average 64 days post-injury and the second time point was on average around 180 days following injury (da Costa et al., 2016). Roughly two months and six months had lapsed since injury. Researchers noted a positive correlation between cerebrovascular activity and sport concussion assessment tool (SCAT2) scores with GMV.

A study conducted in 2017 discovered that athletes with a prior history of concussion had lower volumes in their frontal cortex and cerebellum, however, they found higher volumes in the hippocampus and caudate (Churchill et al., 2017). Like Dean et al. (2015), Churchill and

colleagues (2017) also saw reduction of GMV in the middle temporal lobes. Qualifications for inclusion to the Churchill et al. (2017) study were number of previous concussions, time in months since last concussion, and recovery time for the most recent concussion. All of these participants were active athletes and none were assessed immediately following injury. Half of the participants were athletes with no documented history of previous concussion used as the control group. Although there were significant differences found between the athletes with a prior history of concussion and those without, the average time since injury was around two years and recovery time was about two and a half weeks. It is important to know these numbers to understand what these differences mean on a larger scale – the length of time showcases that even after a couple years there are still differences between injured and healthy brains.

Contrary to Churchill et al. (2017), two studies found decreases in hippocampus volume (Brezova et al., 2014; Terpstra et al., 2017). Brezova and colleagues (2014), evaluated GMV during the first year following moderate or severe TBI, not concussion. Despite the higher severity of TBI studied by Brezova et al. (2014), the differences found in the hippocampus were not significant until the 12-month time point following injury and only saw gradual changes up until that point. The patients who suffered post-traumatic amnesia longer than two weeks were more likely to have smaller hippocampi. They also noted a significant decrease in GMV of the brainstem between the early and three-month scans that they conducted. The more severe the injury the greater the difference in brainstem volume. The study conducted by Terpstra et al. (2017) found decreased hippocampal volume associated with higher symptoms of anxiety, based on the Beck Anxiety Inventory, following moderate to severe TBI. Researchers specifically assessed the right anterior hippocampus and discovered that anxiety symptomology could predict

hippocampal atrophy at five- and twelve-months following injury, but not based on whole brain volume (Terpstra et al., 2017).

Unlike previous TBI research which has found decreased GMV (Brezova et al., 2014; da Costa et al., 2016; Dean et al., 2015; Sussman et al., 2017; Terpstra et al., 2017), Killgore and colleagues (2016) discovered increased GMV correlated with time since injury (Killgore et al., 2016; Singh & Killgore, 2016). A total of 26 patients were included from one of five time points following mTBI: two weeks, one month, three months, six months, and one year (Killgore et al., 2016). These participants were divided into two equal experimental groups based on their time since injury. The first group (n=13) consisted of individuals who had experienced a mTBI in the previous 0 - 99 days and were classified as the post-acute group for analysis (Killgore et al., 2016). The second group (n = 13) consisted of individuals who had experienced a mTBI in the previous 100 - 367 days and were classified as the chronic group for analysis (Killgore et al., 2016). The control group (n = 12) was healthy individuals without a history of concussion or loss of consciousness. GMV significantly differed in the right fusiform gyrus and ventromedial prefrontal cortex/gyrus rectus between groups where chronic mTBI had the highest increase in GMV (Killgore et al., 2016; Singh & Killgore, 2016). This increase in GMV was only significant in chronic mTBI compared to post-acute mTBI and healthy controls in the ventromedial prefrontal cortex/gyrus rectus. However, in the fusiform gyrus, the healthy controls were found to have significantly higher GMV compared to post-acute mTBI and chronic mTBI displayed significantly higher GMV compared to the healthy controls (Killgore et al., 2016; Singh & Killgore, 2016).

Even with the amount of previous research conducted using T1-weighted scans to assess GMV following TBI and concussion there is uncertainty behind these results. The trouble with

these previous findings is the inconsistencies in when TBI is assessed following injury as not all time points have been adequately addressed. Our findings are an important addition to the field, especially with our experimental group within 1-45 days since return-to-play and an age, sex, and sport matched control group.

This thesis also utilized an fMRI scan to determine any changes in FC to the brainstem, prefrontal cortex, and the hippocampus. fMRI takes MRI one step further, enabling the location of function in the brain. The use of fMRI could provide insights into the function and pathophysiological aspects of injury (Ptito et al., 2007). fMRI scans can be both resting state (non-task based) and task based. In our study we only assessed rsFC, the measure of regional connectivity when the brain is at rest, to better understand the effect of concussion on the brain following post return-to-play in recently concussed collegiate athletes. Numerous studies have previously evaluated rsFC following TBI and concussion (Bharath et al., 2015; Czerniak et al., 2015; Eierud et al., 2014; McCrea et al., 2017; Meier et al., 2017; Militana et al., 2016; Shin et al., 2017; Slobounov et al., 2011; Zhu et al., 2015).

rsFC differences have been previously found between concussed and control athletes in the anterior cingulate cortex, left dorsolateral prefrontal cortex and the right dorsolateral prefrontal cortex seed regions (Czerniak et al., 2015). Czerniak et al. (2015) was a very small sample size of collegiate athletes, but gave an idea as to how concussed brains show a stronger connectivity when compared to age-matched controls. In a review by Shin et al. (2017) they discovered that fMRI gives an insight into specific measures by which concussion leads to alterations in brain function (Shin et al., 2017). This was brought on by the dysregulation in connectivity seen at both resting state and active state (task-based) measures (Shin et al., 2017).

Meier and colleagues (2017), conducted a study with recently concussed athletes at one day, an acute time point, as well as one week and one-month, subacute time points following injury to evaluate patterns of FC. Several areas had an increase at the subacute time point relative to the acute including the supplementary motor area (SMA), postcentral gyrus, paracentral lobule, lingual and fusiform gyri, the middle and superior temporal gyri (Meier et al., 2017). The middle and superior temporal gyri along with the superior medial frontal gyrus displayed a significant effect of time with FC in these levels decreased based on time since injury (Meier et al., 2017). The participants for this study were recruited from all sports from the department of athletics at a NCAA Division I school.

In 2015, Bharath and colleagues also evaluated rsFC longitudinally following mTBI. A total of 25 participants suffered from a non-sports-related mTBI and 21 participants were healthy controls (Bharath et al., 2015). The following time points were assessed post-injury: 36 hours, three months, and six months with region of interest (ROI) based connectivity. In whole brain group level analyses researchers discovered 33 ROI pairs between mTBI and healthy controls (Bharath et al., 2015). For the mTBI group during the recovery period 18 ROI pairs were discovered with most either from the three months to six-month time points or the 36 hours to six-month time points (Bharath et al., 2015). The group level comparisons between the three recovery time points and healthy controls revealed 15 ROIs. Most of the changes that occurred in the first three months were decreased connectivity and the changes that took place between three and six months were increased connectivity (Bharath et al., 2015). All of the significant differences in rsFC in this study took place predominantly in the frontal and parietal lobes including areas such as: the precuneus, superior parietal lobule, postcentral gyrus, and the bilateral frontal lobes (Bharath et al., 2015). The findings of Bharath et al.'s (2015) study give

insight into the effects of mTBI in general, but do not specifically address the ramification sports-related concussion has on the brain.

A sports-related concussion study conducted by Slobounov et al. (2011) assessed rsFC at on average ten days post-injury with 17 recently concussed collegiate athletes and 17 athlete controls without a history of concussion. The statistical parametric mapping 8 package (SPM8) and CONN, a functional connectivity toolbox were used to analyze the data with four ROI-based regions; the right dorsolateral prefrontal cortex, bilateral precuneus, bilateral primary visual cortex, and the bilateral hippocampus (Slobounov et al., 2011). Significant reductions were found in the primary visual cortex, hippocampus, and dorsolateral prefrontal cortex seeds, where the concussed group had weaker connectivity then the controls. However, the difference between groups in the precuneus did not reach significance (Slobounov et al., 2011).

Similarly, to Slobounov et al. (2011) on a smaller scale Militana and colleagues (2016) further evaluated rsFC within one week of a sports-related concussion. ROI-based analysis was also conducted to determine group differences, however, there were 18 different ROIs selected based on the default mode network (DMN), dorsal attention network (DAN), and the frontoparietal control network (FPC) (Militana et al., 2016). Increased connectivity was discovered in three regions of the DMN, the bilateral hippocampus, precuneus, and dorsolateral prefrontal cortex where the concussed group exhibited stronger connectivity compared to controls (Militana et al., 2016). As mentioned, there was a small sample size of only seven recently concussed athletes and 11 healthy controls which needs to be taken into consideration when evaluating these results.

Correspondingly, Zhu et al. (2015) also assessed rsFC in a small subset of concussed athletes (n = 8) compared to controls (n = 11) in the DMN. Researchers selected the following

eight nodes for analyses: bilateral posterior cingulate cortex, bilateral anterior cingulate cortex/medical frontal cortex, bilateral superior frontal gyri, and bilateral inferior parietal lobules/angular gyri (Zhu et al., 2015). All athletes displayed symptoms immediately post-injury and were assessed at one day, seven days, and 30 days following injury. There was a significant time and group interaction discovered in the overall DMN, bilateral hippocampus, and right posterior cingulate cortex where DMN exhibited higher connectivity on day one compared to day seven (Zhu et al., 2015). When compared to the controls, reduced FC in the DMN was significant on day seven in the bilateral hippocampus, bilateral superior frontal gyri, and right inferior parietal lobule/angular gyrus. Furthermore, no significant differences were found between the concussed athletes and controls in respect to day one and day 30 following injury (Zhu et al., 2015). The ImPACT results from this study need to be considered when addressing these FC results as on average athletes in this sample appeared to be recovered or performed better than on their baseline within about six days after obtaining a sports-related concussion. This is important as some of the athletes most likely still displayed symptoms at the day seven time point where as others may not have and was not accounted for in the FC analyses.

A recent review on advanced neuroimaging in sports-related concussion included eight studies that assessed rsFC and noted findings seemed to vary, however, these differences most likely existed based on the variations of methodology used (McCrea et al., 2017). McCrea and colleagues (2017) found that the DMN was most extensively studied, but has resulted in differing findings as some previous studies have found an increase in connectivity and others have found connectivity to decrease in the DMN following sports-related concussion. There have also been findings in regards to alterations in FC in areas pertaining to executive functions,

visual and motor networks, however, these too vary based on the methodology used, i.e. timeline of data collection, whether athletes are experiencing symptoms or they are asymptomatic, etc.

Additionally, Eierud et al. (2014) also addressed the current state of neuroimaging following mild traumatic brain injury in their combined review and meta-analysis. Unlike McCrea et al. (2017), this review also included studies utilizing structural MRI measures in which they discovered only two studies evaluated gray matter abnormalities, one used a T1 weighted scan and the other addressed these changes with a CT scan (Eierud et al., 2014). In response to publications utilizing fMRI, an anterior-to-posterior approach was suggested based on connectivity analysis findings of several studies. This approach was developed based on consistent discovery of reduced connectivity in the anterior portion of the brain and increased connectivity commonly located in the posterior regions. These regions of reduced activity were located in the inferior parietal lobes, insula, and cerebellum, where the mTBI group displayed less activity compared to controls, whereas controls had increased activity relative to mTBI participants in regions of the frontal lobe and angular gyrus (Eierud et al., 2014).

A majority of previous rsFC literature pertains to regions of the DMN, however, there are various findings in relation to the effects of concussion on the areas involved in this network. Our present study is imperative in generating a better understanding in rsFC of asymptomatic athletes following sports-related concussion at the post return-to-play timepoint as recent findings suggest there to be differences relative to controls. Further, we used ROI to voxel-based analysis to address FC in the bilateral hippocampus, brainstem, bilateral salience right prefrontal cortex (RPFC), and the bilateral frontoparietal left prefrontal cortex (LPFC) seeds.

The use of MRI in TBI research has granted a further understanding into the aftermath of injury on the brain and has led to various types of analysis including assessment of structural and

functional changes. The predominance of MRI compared to other neuroimaging methodology utilized in concussion research depicts the need to integrate with fluid biomarkers. As the inclusion of serum-based biomarkers in addition to current neuroimaging is vital in comprehending the effect of brain injury at a biological level. Specifically, the use of GMV and rsFC in conjunction with serum-based biomarkers is an area of concussion research that has not been explored in great detail. Although this is fairly new territory there is a need to incorporate protein levels with MRI in order to notably validate the use of blood or saliva samples to diagnose concussion.

Serum-Based Biomarkers

Background. Despite the high prevalence of sports-related concussion there are not many studies using protein-based biomarkers to assess the timeline of concussion, especially in regards to MRI-based biomarkers. "Biomarker" is a term broadly used to define a subgroup of medical signs; "objective indications of medical state observed from outside the patient – which can be measured accurately and reproducibly" (Strimbu & Tavel, 2010). Protein-based biomarkers are measures of protein levels in the body that can tell us about a certain type of disease and are typically assessed using serum, plasma, saliva, and cerebral spinal fluid. The use of serum, a derived component of blood, is more effective and easier to obtain than measures of cerebral spinal fluid samples. Recently this approach has seen an increase in use to study diseases and injuries like TBI. Using protein levels, to understand the brain physiologically in both healthy and injured brains is important in understanding the trajectory of injury.

Biomarkers for TBI/Concussion. A number of proteins exist as potential biomarkers of serum in TBI, some in mild TBI, but none have been specified in the 1-45 day post-concussive phase of sports-related concussions (Kawata et al., 2016). Proteins associated with the recovery

of brain function have been assessed before in TBI including; Alpha-synuclein, Neuron-specific enolase (NSE), Glial fibrillary acidic protein (GFAP) all of which have been reported to experience sudden increases after TBI (Kawata et al., 2016; Zetterberg & Blennow, 2015). Recently this approach has seen an increase in use to study diseases and injuries like TBI. Using protein levels, to understand the brain at a biological level in both healthy and injured brains is important, as is studying the time line of injury to gain a better picture. If this were done, post-concussion treatment could be greatly improved.

We recently assessed BDNF, a protein that plays a vital role in synaptic plasticity and neuronal survival with concussed and age, sex, and sport matched controls at the post return-to-play time point (Susa et al., 2019). Based on our findings of significantly elevated levels of serum-based BDNF in the concussed group in comparison to controls we chose to further evaluate other proteins in serum as potential biomarkers of concussion post return-to-play. This project is going to focus on four specific protein-based biomarkers to gain a better overall understanding on what their potential roles may be at an asymptomatic timepoint in conjunction with structural and functional MRI scans following sports-related concussion. The four proteins will be BDNF, Tau, S100B, and UCH-L1.

Research suggests that S100B may be specific to concussion, particularly sports-related due to the high prevalence because it is heavily related to exercise (Dang et al., 2014). A previous study conducted by Randall and colleagues (2013) found a significant increase in Tau levels when concussed athletes were compared to their own preseason measures, yet it is unknown what effect concussion may have on Tau after injury when compared to matched controls (Randall et al., 2013). Specifically in mTBI, UCH-L1 was found to increase following a non-sports related concussion when in comparison to healthy controls, but it is unknown what

the effects are following a sports-related concussion (Li et al., 2015). Based on these findings there is still a lot of unknown territory to be explored to discover what the effects of these specific proteins are when evaluated post return-to-play in a collegiate athlete recovering from a head injury. Tau and S100B have been frequently implicated in concussion, but UCH-L1, is a relatively newer protein being used as a possible biomarker for concussion even though it has been studied for severe TBI (Kawata et al., 2016; Kulbe & Geddes, 2016; Strathmann et al., 2014; Zetterberg & Blennow, 2015). It is unclear how UCH-L1 compares to BDNF, Tau or S100B in testing for concussive effects post return-to-play or the possible implications these proteins may have in relation to MRI measures.

Brain-derived Neurotrophic Factor. BDNF is a type of protein that is essential for the development and functioning of the central and peripheral nervous systems (Mandel, Ozdener, & Utermohlen, 2009). It is the most abundant neurotrophin in the central nervous system, however, BDNF has also been found in plasma and blood platelets (Rosenfeld et al., 1995; Yamamoto & Gurney, 1990). Furthermore, BDNF is composed of two forms, proBDNF and mature BDNF (mBDNF). The precursor molecule of BDNF that is first synthesized in the cells is known as proBDNF. ProBDNF plays an important role in dimerization and proper folding, it is involved in the targeting of mature BDNF. Majority of mBDNF is released by neurons in an activity-dependent manner or through constitutive secretion and binds to the tropomyosin-related kinase receptor (Chen et al., 2013; Massa et al., 2010; Mowla et al., 2001). Mature BDNF also binds to neurotrophin receptor p75 performing via interactions between these trans-membrane receptors (Chen et al., 2013; Massa et al., 2010). These actions can happen at once or separately, ultimately leading to either neuronal survival or death. Although proBDNF is necessary for mBDNF, these two forms can elicit their own distinct effects (Mandel et al., 2009). Cell

development and differentiation are promoted by mBNDF, whereas proBDNF brings about cellular apoptosis (Mandel et al., 2009).

BDNF communication following head injury appears to have a neuroprotective effect. Despite its size, BDNF can cross the blood brain barrier in both directions, from the periphery to the brain and the brain to the periphery (Ferris et al., 2007). This is possible via a high-capacity saturable transporter system (Pan et al., 1998). This means increased levels of BDNF can reflect either the brain levels or the periphery, but is hard to tell unless collecting cerebral spinal fluid in conjunction with serum or plasma samples. It is thought that high levels of BDNF exhibit active recovery following injury.

BDNF post-injury. The initial TBI of any severity can also be known as the primary injury. The effects that happen in a result of that primary injury are known as secondary injury. It seems that BDNF plays a major role in secondary injury following TBI, through changes in BDNF-induced gene expression of traumatized brain tissue, providing neuroprotection and restoration of connectivity (Kaplan et al., 2010). However, the majority of BDNF biomarker research conducted in regards to any form of TBI has been utilizing animal models (Kaplan et al., 2010; Massa et al., 2010). Due to the nature of these therapeutic studies, primarily those using spinal cord, it is difficult to translate and/or compare with human findings, therefore, leading to a lack of literature on sports-related concussion specifically. Animal studies are difficult to translate to humans, because they are not able to accurately translate pre-existing variables in humans such as the amount of time someone has played a sport, length and use of medication, or family history, etc.

In a small study (n = 14) of children with a severe TBI, plasma and cerebral spinal fluid were collected at 2 hours and 24 hours post-injury in comparison to controls (Chiaretti et al.,

2003). Although none of these TBIs were caused by sports, among the proteins they assessed, BDNF was significantly higher between the two time points following injury in both plasma and cerebral spinal fluid. Chiaretti et al (2003) findings suggest that BDNF may be a good indicator of injury severity.

As mentioned previously, we assessed BDNF in both serum and saliva in a sample of 30 collegiate athletes (table 1), half of which were recently cleared to return-to-play after sustaining a concussion and the other half were age, sex, and sport-matched controls (Susa et al., 2019). One of the additional measures we collected was behavioral data evaluating levels of depression, anxiety, stress using the DASS questionnaire. All the subscales and total scores were significantly elevated in the concussion group (table 1). We found significantly elevated levels of BDNF in serum between groups, see figure 1a (Susa et al., 2019). Furthermore, between groups saliva BDNF levels were not significantly different, but did exhibit a similar trend, see figure 1b (Susa et al., 2019). A correlation between serum and salivary-based BDNF levels did not exist, see figure 1c (Susa et al., 2019). Three outliers (2 concussion & 1 control) were excluded from all analyses involving saliva after observations of box and whisker plots indicated that were > three *SDs* above the respective group *M*.

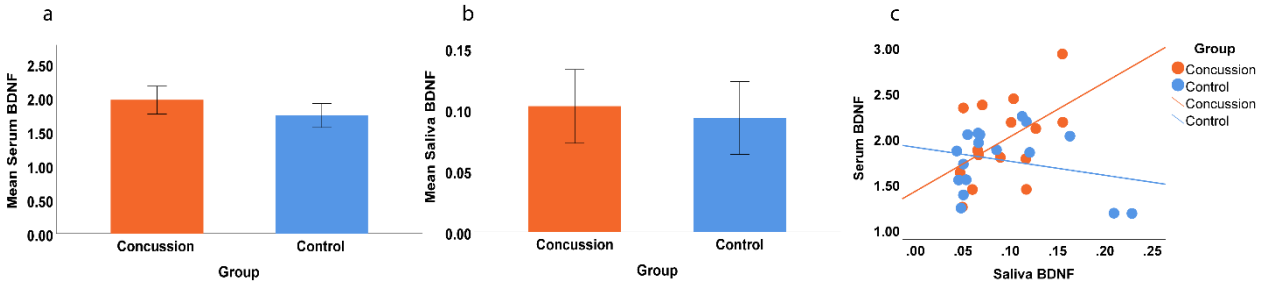

Figure 1. Serum and salivary BDNF. (a) Serum levels were significantly higher in the concussion group relative to the controls. (b) Salivary BDNF was also higher in the concussion group compared to the control group, but did not yield significance. (c) Serum and salivary BDNF levels were not correlated between groups. Reprinted from "Elevated levels of serum, but not salivary, brain-derived neurotrophic factor following mild traumatic brain injury in collegiate athletes post return-to-play," by TS. Susa, 2019, *Journal of Concussion, 3*, 4. Copyright [2020] by Dr. Joshua M. Carlson. Reprinted with permission.

Effects of exercise on BDNF. A 2011 study conducted by Bamac and colleagues (2011) assessed the effects of a repetitive heading exercise in professional male soccer players. They found significantly elevated levels of BDNF following the exercise compared to baseline measures (Bamac et al., 2011). These findings may be contradictory depending on what defines exercise as Bamac et al (2011) described the use of heading only to mimic sub-concussive blows to the head. Another study found salivary BDNF levels increased in non-athletes in response to an exercise program, but serum was not assessed nor were the participants concussed or recently concussed (McGeown et al., 2016).

It is thought that exercise might be a useful treatment for concussion due to the known increased effects with BDNF (Kaplan et al., 2010). However, this potential treatment approach is based on an animal study comparing rats with a TBI who had access to a running wheel and those who did not (Griesbach et al., 2002). The animals who were in the running wheel group

showed increase cognition and levels of BDNF following exercise compared to those who recovered without access to a running wheel.

S100B. S100B is a protein primarily expressed in astrocytes and belongs to a multi-genic family of calcium binding proteins, it is mainly found in astroglia and Schwann cell cytosol (Donato, 2003; Korfias et al., 2007). S100B partially gets its name from its solubility in a 100% saturated solution with ammonium sulphate (Michetti et al., 2012). The other portion comes from the term S100, used to describe a large gene family of mostly calcium-binding proteins (Michetti et al., 2012). Notably, S100B, is the beta-beta homodimer of the S100 protein family. The exact biological role of S100B is heavily debated. When secreted, it is thought to act on nearby cells and/or the signaling cell at physiological concentrations. However, at higher levels it is thought to elicit toxic effects (Michetti et al., 2012). Elevated levels in cerebral spinal fluid, serum, saliva, urine, and even amniotic fluid are considered biomarkers for certain medical circumstances, including concussion. High levels of S100B in these conditions grant an overall indication of cell damage.

S100B post-injury. The primary clinical role of S100B in concussion management has been to help identify intracranial hemorrhage on head CT scans, it is estimated that about 5% of concussion patients will experience this (Kiechle et al., 2014). Even though the specificity of S100B is low, the high sensitivity has led to several countries adopting the use of S100B as a clinical tool to screen for abnormal head CT (Bazarian et al., 2013; Unden & Romner, 2010). Yet, a significant overlap in S100B levels between controls and concussed athletes has dampened the enthusiasm towards using S100B to aid in the diagnosis of concussion. Based on comparisons between these groups, only very high S100B levels are considered abnormal – a rarity post-concussion.

One study found significantly elevated levels of S100B at 3 hours following sports-related concussion in comparison to baseline, but did not find differences at 2, 3, or 7 days post-injury (Kiechle et al., 2014). Another study assessed S100B within 6 hours following injury to determine how many individuals tested positive for 0.1 ng/ml S100B or higher. Of their 102 patients who had suffered a concussion, not limited to sports-related, 72.5% tested positive (Morochovic et al., 2009). Morochovic et al. (2009) suggest that 6 hours post-injury may be too long of a timeframe between injury and blood collection due to S100B's short half-life, rather using three hours similar to Kiechle and colleagues (2014) could increase the sensitivity of the measures.

Effects of exercise on S100B. Dang and colleagues (2014) suggest that S100B is related to exercise. Hence, it may be specific to concussion because of the high prevalence in sports (Dang et al., 2014). An issue is that S100B has been found to increase following head injury, but exercise has also been shown to have this effect on S100B, making it hard to determine what is causing this change (Hasselblatt et al., 2004; Stalnacke et al., 2003, 2004). A review completed in 2014 identified more than nine forms of physical activity – they noted a trend in which vigorous exercise might be the cause of increased S100B levels (Schulte et al., 2014). However, Schulte et al (2014) also mentioned levels greater than 0.1 ug/L without a concussion clearly stand out in data analysis. Some of those groups included professional or amateur soccer, ice hockey, trained swimmers, and runners, etc. Two previous studies evaluating professional athletes discovered higher than normal baseline measures of their participants prior to data collection (Michetti et al., 2012; Stalnacke et al., 2003). These higher levels are most likely due to the stressful conditions involved with high intensity physical training. Exercise influences S100B based on the intensity, but further research is needed to assess dual/multi-sport athletes,

and length of sport play, i.e. the amount of time someone has been playing hockey could influence their S100B levels more than someone who hasn't been playing for as long. However, depending on the study, the timing of sample collection varied from immediately before and after exercising to also include exercise programs taking blood samples on days one and ten training, not just following concussion.

The variability of the studies in the Schulte et al (2014) review make it hard to decipher what the actual effect exercise may have on S100B, especially in regards to sports-related concussion. These differences need to be further accounted for, but overall lead to the general consensus that exercise does affect S100B levels in the body. It is also unclear as to how long a protein such as S100B may take to reach normal levels following a period of no exercise similar to the return-to-play timeframe that a concussed athlete would endure. Since exercise is likely to play a large effect on S100B or proteins alike it could be possible that athletes will never fit a normal range of protein levels even following retirement compared to the general population due to the body's high exposure to rigorous training. These concepts should be considered when studying head injury, because exercise is a component that has the potential to affect more than just protein levels, but also mental health i.e., anxiety, stress, etc., in the entire active population rather than solely athletes. Knowing the effect of exercise would increase the likelihood of an individualized recovery plan for all people following head injury.

Ubiquitin C-Terminal Hydrolase L1. UCH-L1 is an abundant neuron-specific enzyme accounting for 1%-%5 of total soluble brain protein (Mondello et al., 2014). UCH-L1 is a protein involved in the augmentation and removal of ubiquitin metabolism-specific proteins (Tongaonkar et al., 2000). UCH-L1 has a vital role in the removal of oxidized, misfolded, or excessive proteins during normal and pathological conditions including TBI (Gong & Leznik,

2007). Under pathological conditions UCH-L1 is released into extracellular space as a consequence of cell destruction (Mondello et al., 2014). UCH-L1 can be measured in serum and cerebral spinal fluid directly proportional to injury severity (Mondello et al., 2014). Based on these characteristics UCH-L1 is a good candidate to be a biomarker for TBI.

UCH-L1 post-injury. Studies evaluating serum-based UCH-L1 following TBI remain uncertain and much has to do with the lack of research conducted utilizing UCH-L1. In part due to the recent discovery of UCH-L1 as a potential biomarker for TBI. A few studies have suggested that UCH-L1 is increased following concussion compared to controls, however, some previous findings have not found any differences in serum-based UCH-L1 levels (Diaz-Arrastia et al., 2014; Kou et al., 2013; Papa et al., 2012; Puvenna et al., 2014). Kobeissy et al (2006) assessed the subsequent effects of an induced TBI on a rat model described a two-fold increase UCH-L1 in the injured cortex two days post-injury (Kobeissy et al., 2006). Another study utilizing a rat model for TBI used a respective enzyme-linked immunosorbent assay (ELISA) to assess UCH-L1, they found significant levels of cerebral spinal fluid and serum UCH-L1 as early as two hours following injury (Liu et al., 2010). In regards to clinical research, a review found that UCH-L1 has shown increases in non-sports related concussion in comparison to healthy controls (Li et al., 2015). Previous research was able to detect significant elevations of UCH-L1 in human cerebral spinal fluid early after injury and found the levels to stay elevated for over a week following injury (Brophy et al., 2011; Papa et al., 2010).

One of the first studies assessing UCH-L1 conducted by Papa et al (2012) found a significant difference between the concussed group and uninjured controls, there was also a significant difference between the concussed and trauma patients (Papa et al., 2012). The trauma patients in this study were classified as those with non-head trauma used as a second control

group. If researchers were able to identify a traumatic intracranial lesion on a head CT scan in either the concussed or trauma groups, the UCH-L1 levels were significantly higher than those whose CT scan was negative (Papa et al., 2012). Furthermore, the concussed patients who had a negative CT scan exhibited significantly higher levels compared to the trauma patients who also had a negative CT scan (Papa et al., 2012). The patients in this study who had a neurological intervention also had substantially significant elevated levels of UCH-L1 compared to those who did not (Papa et al., 2012). This pattern suggests that even without a positive CT scan or a medical intervention it is still possible to have significantly higher levels of UCH-L1 following a concussion. UCH-L1 has not yet been implicated in clinical purposes (i.e. diagnosis, etc.) due to the unease surrounding its effectiveness in studies pertaining to diagnosis and treatment outcome.

Effects of exercise on UCH-L1. No research was found pertaining to protein-based UCH-L1 levels and exercise conducted in either humans or animals. However, an animal model study conducted recently in 2018 assessing mRNA levels of UCH-L1 randomly divided rats into an aerobic exercise and sedentary control groups (Liu et al., 2018). Over the course of 10 weeks maximum oxygen consumption (VO_{2max}) was gradually increased from 50%-55% to 65%-70% (Liu et al., 2018). Although they were assessing levels of mRNA a significant upregulation of UCH-L1 was discovered in the exercise group compared to the sedentary group after completing ten weeks. W. Liu and colleagues (2018) suggest that consistent aerobic exercise modulates proteins involved with striatum and mitochondrial development-related signaling transduction proteins. These results are promising, but more research is needed to clarify which mechanisms are causing this significant effect. A study like this also needs to be conducted using human subjects. Translational research is important to grant a better depiction of the effects exercise has

on UCH-L1 as a whole. UCH-L1 and exercise is a poorly studied area of research, more is needed to recognize the effects of exercise at a protein level.

Tau. In response to a concussion, axons are most susceptible to damage. Tau is an intracellular, microtubule-associated protein that is extremely abundant in axons and is involved with building axonal microtubule bundles (Teunissen et al., 2005). Although primarily abundant in axons, Tau can also be in cell bodies and dendrites. There are two molecular weights for Tau, low forms are found in the brain and high forms are typically found in the spinal cord and peripheral nervous system (Spittaels et al., 1999). Tau experiences several posttranslational modifications – phosphorylation being the most dominant (Caprelli et al., 2019). When a severe TBI takes place, Tau becomes hyperphosphorylated resulting in a release of Tau from microtubules (Caprelli et al., 2019). This eventually alters the affinity of Tau causing it to aggregate and form neurofibrillary tangles (Caprelli et al., 2019). Potentially leading to chronic traumatic encephalopathy following several TBIs. Tau is most known for its role in Alzheimer's, but the distribution is different than in TBI in which differences have been found in relation to various types including a single injury and repetitive head injury (Barrio et al., 2015). Tau can be measured in three different forms creating inconsistencies in the literature; cleaved-Tau (C- Tau), phosphorylated-Tau (P-Tau), and total-Tau (T-Tau).

Tau post-injury. In T-Tau, previous research has seen a significant increase in levels when concussed athletes were compared to their own preseason measures, yet we don't know what T-Tau levels are after a concussion when compared to matched controls (Randall et al., 2013). Similarly, a 2018 study conducted blood samples to assess levels of Tau to determine differences between pre-season and post-injury (Shahim et al., 2018). Researchers found plasma Tau to be higher one hour following injury in comparison to their pre-season measures. Tau was

the same result when compared to non-athletic controls, but not when compared to their gymnast control group (Shahim et al., 2018). Shahim and colleagues (2018) also noted Tau decreased 12 hours following injury, but saw an increase 7 days following injury, there after levels normalized. In comparison to the length of return-to-play, plasma Tau levels had no effect, however, this study did not indicate which type of Tau they were evaluating. (Shahim et al., 2018).

Kawata et al (2018) also evaluated plasma Tau as well as sub-concussive hits during practice in collegiate football players. Levels of Tau were independent of the number and severity of sub-concussive hits to the head (Kawata et al., 2018). Plasma levels experienced the highest increase after non-contact practice compared to the three contact practice time points. The levels surprisingly decreased over time following the non-contact practice (Kawata et al., 2018). Authors did not give any specific reasoning for these findings.

An animal study conducted by Gabbita and colleagues (2005) discovered increased levels of C-Tau in the hippocampus of TBI-induced rats, but did not find any significance in comparison to controls. Although there was no significant group difference, they did find C-Tau levels to increase in the hippocampus as the severity of injury increased (Gabbita et al., 2005). These researchers also evaluated cortical levels of C-Tau which also increased in response to an induced TBI (Gabbita et al., 2005).

Effects of exercise on Tau. Unsurprisingly, a majority of research on exercise and Tau relates to its role in Alzheimer's development and direction over time. Unlike Alzheimer's, the research that has been conducted on exercise is mostly studied with animal models and very little is assessed clinically. A large human study divided 103 participants into four subgroups; young sedentary, young athlete, older sedentary, and older athlete (Daniele et al., 2018). Age did not

have a significant effect on Tau platelet concentrations (Daniele et al., 2018). The population as a whole showed the athletes having significantly lower Tau levels in the platelet concentrations compared to the sedentary individuals (Daniele et al., 2018). As the only study on exercise and Tau this study is extremely difficult to replicate as the type of Tau measured is not specified nor is one type of fitness test a good indicator of someone's physical activity.

Biomarkers and Imaging. Even with the increase in protein-based biomarker research and a profound amount of studies utilizing different neuroimaging techniques following TBI, there are few studies assessing these measures together. This is particularly true in regards to sports-related concussion in humans as the time line following injury for both proteins and imaging is relatively unknown. Studies that have been conducted previously use both animal models and humans, however; they lack not only consistency, but also have many limitations such as differences in time of day, failing to control for medication, and extremely small sample sizes. These gaps in research make it difficult to gain a greater understanding of the potential relationship between protein-based biomarkers and neuroimaging (Di Battista et al., 2018; McMahon et al., 2015).

Di Battista and colleagues (2018) assessed the possible relationship between blood biomarkers and cerebral blood flow in a small sample of athletes. Samples were collected on average four days following injury. Recently concussed athletes exhibited greater differences in biomarker and MRI findings compared to both control groups; athletes with a history of concussion and athletes with no history of concussion (Di Battista et al., 2018). However, a negative relationship was found between global cerebral blood flow and T-Tau comparing concussed athletes and controls without a history of concussion. None of the other proteins

assessed, including S100B and BDNF showed any significant anti-modulation between groups (Di Battista et al., 2018).

Unlike Di Battista et al (2018), McMahon and colleagues (2015) assessed serum-based GFAP and its breakdown products within 24 hours of TBI, 83% were classified as a concussion. Researchers analyzed both initial CT scans and follow up (1-2 week) MRI scans in conjunction with serum levels of GFAP. Significantly higher GFAP was found in those with a CT-positive intracranial injury compared to those who had negative CTs (McMahon et al., 2015). However, neither MRI positive or negative patients displayed any significance in regards to GFAP levels, but levels were higher in those who were MRI-positive.

A portion of the large multi-cite TRACK-TBI study assessed the association between MRI abnormalities in civilian patients with CT-negative TBI and plasma GFAP concentrations (Yue et al., 2019). Blood samples were collected within 24 hours of injury and MRI scans were collected within 7-18 days following injury. Traumatic intracranial abnormality on a MRI and GFAP associations were dose dependent, 64% of patients in the highest GFAP concentration quintile had abnormal MRI findings (Yue et al., 2019). Yue and colleagues (2019) also found that the 43% of patients with negative CT scans had GFAP concentrations higher than the top percentile of the control group, of those patients 46% had an abnormal MRI. Even supposing these findings were from sports-related concussion, these results are vague and do not give direct insight as to the precise areas of these abnormalities. Knowing the exact areas of concussion-related injury would result in a more thorough analysis ultimately leading to a more personalized recovery.

Davies and colleagues (2019), collected 1-4 serum samples from semi-professional and professional athletes at different points post-injury. These samples were used to assess UCH-L1,

GFAP, NF-L, Tau, and miR-502, a serum microRNA following concussion. All participants were symptomatic at the time of testing. Even though the researchers found significantly higher levels miR-502 the farther the participants were from injury, they failed to assess the serum samples further with the MRI scans that were collected as a part of the study instead they chose to focus on the behavioral results (Davies et al., 2019). Several limitations existed, primarily the sample being almost entirely male athletes from the same sport. Many studies like this collect protein levels and neuroimaging, but never analyze both together.

There are more differences than similarities in TBI studies, specially sports-related concussion. In order to improve diagnosis and recovery of concussion we first need to have multi-modal studies including the use of proteins (or mRNA) and neuroimaging. Only studying one measurement or the other will not grant a full depiction of concussion as what is negative on a scan could be detrimental in terms of blood-based protein levels. Biomarker research needs to expand to include neuroimaging of both CT and MRI scans. This project aims to close the gap by assessing the differences between four specific serum-based protein biomarkers and MRI post return-to-play. The goal is to add to previous findings whilst continuing to strive for consistency in this realm of concussion research. To the best of our knowledge, we are the first to analyze proteins in conjunction with GMV and FC measures following sports-related concussion.

Specific Aims

The purpose of this thesis is to establish serum-based biomarkers for lingering post-concussive effects in BDNF, S100B, UCH-L1, and Tau as an assessment of post return-to-play in recently cleared concussed athletes. We aim to see changes in the structural and functional brain networks of the concussed group compared to the control group. We also aim to compare

MRI findings, both structure using gray matter volume and functional connectivity to the BDNF, S100B, UCH-L1, and Tau levels collected through serum samples.

Therefore, this thesis hypothesizes that each protein will have an effect of group. We expect that higher levels of S100B and Tau protein will be expressed in the concussion group in comparison to the control group similar to what we have previously found with BDNF. However, we expect the concussed group to express lower levels UCH-L1 compared to the control group. In addition, we hypothesize that there will be differences in the concussed and control groups in FC and GMV in the following areas: brainstem, prefrontal cortex, and hippocampus. Further, we also hypothesize that alterations in protein levels will be linked to differences found in FC and/or GMV between groups in the same areas as above. We expect to see these alterations based on the differences in protein levels between the concussed and control groups that have been known to exist. The proteins with the highest level of significance between groups are thought to exhibit FC and/or GMV changes.

Methods

Participants

Forty-two collegiate athletes between the ages of 18 and 22 from Northern Michigan University's athletic programs participated in this study. Half (n = 21) were recently cleared by an athletic trainer and/or physician to return-to-play after sustaining a concussion. The other half (n = 21) of the participants were controls without a history of concussion in the 36 months preceding the study. Controls were matched on age, sex, sport, and time of sample collection

(see Table 1). All participants gave informed consent and were monetarily compensated for their time and effort.

Procedure

Every participant gave a 5 ml. blood sample, completed the Depression Anxiety Stress Scales (DASS) Questionnaire. A subgroup of 30 participants underwent a one-hour MRI scan (15 concussed, 15 control). Participants in this study also completed the ImPACT test, three tasks using Near Infrared Spectroscopy, a device measuring hemoglobin levels over the prefrontal cortex, including a resting state task, and gave a 2 ml. saliva sample. However, the findings of those measures will not be assessed in this thesis. DASS and blood samples were done prior to the MRI. The MRI scan consists of ten different types of scans, including a calibration scan, a diffusion tensor imaging (DTI) scan, a diffusion weighted imaging (DWI) scan, two fMRI scans as well as a few types of structural scans. However, for this thesis we will be focused on; the structural T1 and resting state fMRI scans.

DASS

DASS consists of three self-report subscales designed to measure levels of depression, anxiety, and stress (Lovibond & Lovibond, 1995). Each scale consists of 14 statements on a 4-point rating scale ranging from 0-3, for a total of 42 items. Participants were asked to answer the statements based on the degree to which they experienced each statement over the past week. The total score for DASS ranges from 0-126. This questionnaire was used for further analysis between groups, because concussion is linked to elevated symptoms of anxiety and depression (Moore et al., 2006).

ImPACT

The ImPACT test is a widely used baseline assessment on neurocognitive performance to assist in medical clearance for sports-related concussion. One of the components is the PCSS, a 22 self-report item scale measuring concussion-related symptom. This scale was used to assess each participant's level of symptom severity. It has a 7-point scale ranging from 0-6.

Serum

Blood samples were collected in 5 ml. vacutainer serum separator tubes (Becton Dickinson, Franklin Lakes, NJ), by a trained phlebotomist in the Department of Clinical Laboratory Sciences. Following collection, the serum separator tubes were immediately inverted four times and allowed to clot for 30 minutes at room temperature. Serum was separated via centrifugation at 3,500 rpm for 10 minutes and 1ml aliquots will be transferred to sterile 2 ml. cryovials (Nalgene, Rochester, NY). Samples were stored at −80 °C until further use. After thawing, samples were centrifuged one more time to ensure all debris had been removed.

Saliva

Saliva was collected by the passive drool method, because it is known to detect higher levels of BDNF when compared to the salivette method. After pooling saliva in their mouth, participants used the saliva collection aid to force the saliva into a vial. At least 2.0 ml of saliva was collected from each participant. Samples were placed on ice immediately until further handling. All samples were centrifuged for 15 minutes at 4000 rpm to 4ºC. Consistent with serum sample, saliva samples were stored at -80ºC until analysis.

Protein Analysis

BDNF ELISA Assay

Both saliva and serum BDNF levels were analyzed using an enzyme-linked immunosorbent assay (ELISA) kit (CYT306, Millipore Sigma). Assay of saliva samples were not diluted while serum samples were diluted to a 1:32 ratio. Each assay was performed in duplicate. ELISA plate readings were performed using a microplate reader (Epoch2; BioTek Instruments, Inc., Winooski, VT, USA). Analysis was performed using Gen5 software (BioTek Instruments, Inc., Winooski, VT, USA). Standard curves were obtained for saliva (R^2 = .992) and serum (R^2 = .995).

S100B, UCH-L1, & Tau ELISA Assays

S100B levels were analyzed using a same day ELISA kit (EZHS100B-33K, Millipore Sigma). UCH-L1 and Tau levels were analyzed using an ELISA kit (RAB1766; RAB1085, Millipore Sigma). UCH-L1 serum samples were diluted a 1:2 ratio, Tau serum samples were diluted a 1:3 ratio, and S100B serum samples were not diluted. Every sample for each assay was performed in duplicate. ELISA plate readings were performed using a microplate reader (Epoch2; BioTek Instruments, Inc., Winooski, VT, USA). Analysis was performed using Gen5 software (BioTek Instruments, Inc., Winooski, VT, USA). A standard curve was obtained for each protein respectively; S100B (R^2 = .991)., UCH-L1 (R^2 = .945)., and Tau (R^2 = 1.0; degradation occurred by the time all the samples were assessed so re analysis is needed).

Magnetic Resonance Imaging

MRI Acquisition

A 1.5 Tesla General Electric MRI scanner located at the Upper Peninsula Health System Marquette Hospital will be utilized for all scanning purposes. The staff at the hospital conducted all of the scans and each scan should have lasted no longer than 45 minutes. Prior to beginning scanning participants were questioned about metal implants and claustrophobia to ensure safety of participants and quality of scan. Once ready, participants were instructed to relax, keep their eyes closed, and to not move other than breathing. This thesis is focusing primarily on two main types of scans; for structural purposes including GMV, the T1 weighted scan is being used and an fMRI scan is being used to assess FC in the brain. We examined a T1 weighted scan, (TR = 567.0 ms; TE = Min Full; flip angle = 45; 28 slices, FOV = 22.0 mm) and a resting state T2* fMRI scan with 240 volumes, (TR = 2500 ms; TE = 35.0; flip angle = 90; 150 volumes, matrix = 64 x 64; FOV = 22.0 mm; total scan time = 6.25 min).

MRI Preprocessing

Structural Data. Structural pre-processing measures was run through Statistical Parameter Mapping 12 (SPM12), an extension of MatLab (2019b version). Prior to beginning pre-processing the DICOM files from each MRI scan were first imported from CD disks burned off the MRI scanner from the staff at the hospital. Once these files were imported they were converted into NIfTI files specifically meant for anatomical and functional analysis, through a program called MRIcroGL. At this point the data was ready for pre-processing in SPM12. The following steps were done to pre-process the structural data in SPM12: (1) segmentation into six

different components; GMV, white matter, cerebral spinal fluid, skull, soft tissue, other; (2) unwarping; (3) normalization to MNI-152; and (4) smoothing with an 8mm smoothing kernel.

Segmentation is the process of segmenting the images into gray matter, white matter, and cerebral spinal fluid, for this project we are mainly focused on gray matter. Unwarping adjusts the images to correct for any distortions caused by the magnetic field of the scanner. This process attempts to return the image to the actual brain shape. Normalization is done to transform one brain to match shape with another, because people have different shaped brains allowing group analyses to be transformed into the same space. The normalization step in SPM utilizes the Montreal Neurological Institute 152 template. Smoothing is also known as spatial averaging; each voxel is replaced with a weighted average of the values in surrounding voxels. This step increases the signal to noise ratio and tends to reduce any random noise due to averaging.

Functional Data. The use of CONN, a FC toolbox, an extension of Matlab was utilized to examine FC of all participants (Whitfield-Gabrieli and Nieto-Castanon, 2012). Functional data was pre-processed with the following steps in CONN: (1) realignment and unwarp, (2) center, (3) slice time correction, (4) outlier detection, (5) functional segmentation, (6) functional normalization, (7) structural segmentation, (8) structural normalization, and (9) smoothing with an 8mm smoothing kernel.

Realignment is used to adjust the series of images in order to keep the brain always in the same orientation by aligning them to the center voxel (0,0,0). This needs to be done because people move their heads. Slice time correction adjusts the image to make it appear that all of the slices were acquired at the same time. Our scans were collected in slices meaning they were all acquired at different times making it important to use slice time correction. Outlier detection is to access any outlaying objects or artifacts within the scans.

Table 1. Comparison of Participant Groups Adapted from (Susa et al., 2019 & Carlson et al., 2020)

	Concussion	Control	Comparison
N	21	21	
Age	*M* = 19.67, *SD* = 1.39	*M* = 19.67, *SD* = 1.11	
Sex	Female = 8, Male = 13	Female = 8, Male = 13	
Days since RTP	*M* = 9.71, *SD* = ± 12.08	--	
Days since concussion	*M* = 26.82, *SD* = ± 13.10	--	
# of concussions	*M* = 1.81, *SD* = 0.98	*M* = 0.33, *SD* = 0.73	
PCSS T	*M* = 8.95, *SD* = 11.66 (n = 20)	*M* = 2.60, *SD* = 3.72 (n = 20)	*t*(38) = 2.32, *p* = 0.026
DASS D	*M* = 8.05, *SD* = 7.45	*M* = 2.90, *SD* = 5.06	*t*(40) = 2.62, *p* = .01
DASS A	*M* = 8.95, *SD* = 8.05	*M* = 2.62, *SD* = 2.91	*t*(40) = 3.39, *p* < .01
DASS S	*M* = 11.19, *SD* = 8.91	*M* = 4.48, *SD* = 5.06	*t*(40) = 3.00, *p* < .01
DASS T	*M* = 28.19, *SD* = 22.20	*M* = 10.00, *SD* = 10.70	*t*(40) = 3.38, *p* < .01
Intake prior to data collection	Nicotine = 2, OTC = 1, Caffeine = 5	Nicotine = 2, OTC = 5, Caffeine = 7	
Time of sample	*M* = 13.40, *SD* = 3.42	*M* = 13.15, *SD* = 2.64	*t*(40) = .265, *p* = .792
Sport	USA Wrestling = 2	USA Wrestling = 2	
	Football = 6	Football = 6	
	Diving = 2	Diving = 2	
	Soccer = 6	Soccer = 6	
	Club Hockey = 2	Club Hockey = 2	
	Club Volleyball = 1	Club Volleyball = 1	
	Swimming = 2	Swimming = 2	

RTP = return-to-play

Data Analysis

DASS Questionnaire

T-tests were used to assess the effect of group on each of the three DASS subscales; depression, anxiety, stress, and composite score (mTBI vs. control).

Proteins

Brain Derived Neurotrophic Factor. Of the 42 participants, 39 yielded data for the serum analysis, 36 for the saliva analysis, and 35 participants had both serum and saliva data.

Separate analyses of covariance (ANCOVA) were used to test the effect of concussion group on serum and salivary BDNF while controlling for negative affect (i.e., DASS) as symptoms of depression, anxiety, and stress have been previously found to be elevated following concussion. As an exploratory component additional analyses were conducted. Single-tailed Pearson correlations were used to test for a (positive) relationship between serum and salivary BDNF measures across the entire sample as well as separately for each group. Pearson correlations were also used to test for a relationship between BDNF levels and days since concussion and days since return-to-play.

S100B, UCH-L1, & Tau Analysis. Of the 42 participants, 34 yielded data for the S100B analysis, 36 for the UCH-L1 analysis, and 35 participants for Tau analysis. Separate ANCOVAs were used to test the effect of the concussion group on serum levels of S100B, UCH-L1, & Tau while controlling for negative affect (i.e., DASS). Pearson correlations were used to test for a relationship between serum levels and days since concussion and days since return-to-play in the concussion group.

MRI measures

Gray Matter Volume. Voxel based morphometry (VBM) analysis used a regression model with group and protein as potential predictors of whole brain GMV. The following covariates were included in the regression model; age, sex, DASS-T, and intracranial volume (ICV). Main effects and interactions were assessed at the .005 significance level.

Functional Connectivity. We used regression models with group and protein as predictors of FC based on hippocampus, brainstem, salience lateral prefrontal cortex, and

frontoparietal network seeds at the 0.001 significance level. For this thesis we utilized ROI-to-Voxel based FC.

Results

Return to play & days post-injury

There was no significant relationship between days since concussion and days since return-to-play in the concussion participants, $r = .30$, $p = .20$ (see **Table 1**).

DASS Questionnaire

The concussion group exhibited significantly higher levels of depression, anxiety, stress, and total DASS measures compared to the control group (see **Table 1**).

Hypothesis one: We expected each protein to have an effect of group.

BDNF Serum

As reported in Susa et al., (2019) there was an effect of group on serum BDNF levels, $F(1, 36) = 4.13$, $p < .05$, $\eta_p^2 = .10$, where concussion participants ($M = 2.0$ ng/ml, $SE = .099$) had greater levels of BDNF than controls ($M = 1.70$ ng/ml, $SE = .096$). The total DASS score was not related to serum BDNF levels, $F(1, 36) = 1.00$, $p = .32$, $\eta_p^2 = .03$. For concussion participants, the relationships between serum-based BDNF and time since return-to-play ($r = .19$, $p = .46$) as well as time since injury ($r = -.02$, $p = .95$) were not statistically significant.

BDNF Saliva

As reported in Susa et al., (2019), observations of box and whisker plots indicated three outliers (2 concussion & 1 control) that were > three *SDs* above the respective group *M*. These outliers were excluded from all analyses involving saliva. There was no effect of group on salivary BDNF levels, $F(1, 30) = .36$, $p = .55$, $\eta_p^2 = .01$. BDNF levels in concussion participants

(*M* = .11 ng/ml, *SE* = .02) did not differ from control participants (*M* = .09 ng/ml, *SE* = .02). The total DASS score was not related to salivary BDNF levels, $F(1, 30) = .16$, $p = .70$, $\eta_p^2 = .00$. In the concussion group, a strong positive correlation was found between salivary BDNF and time since return-to-play, $r = .69$, $p = .004$. However, no such correlation was found between salivary BDNF and time since injury, $r = .05$, $p = .87$.

S100B

There was not an effect of group on S100B levels, $F(1, 31) = .008$, $p = .93$, $\eta_p^2 = .00$. Concussion participants' S100B levels (*M* = .090 ng/ml, *SE* = .005) did not differ from controls (*M* = .090 ng/ml, *SE* = .005). The total DASS score was not related to S100B levels, $F(1, 31) = .016$, $p = .90$, $\eta_p^2 = .00$. For concussion participants, the relationships between S100B and time since return-to-play ($r = .22$, $p = .41$) as well as time since injury ($r = .03$, $p = .90$) were not statistically significant.

UCH-L1

There was not an effect of group on UCH-L1 levels, $F(1, 31) = 2.05$, $p = .16$, $\eta_p^2 = .06$. Concussion participants' UCH-L1 levels (*M* = .73 ng/ml, *SE* = .271) did not differ from controls (*M* = 1.32 ng/ml, *SE* = .271). The total DASS score was not related to UCH-L1 levels, $F(1, 33) = 1.14$, $p = .29$, $\eta_p^2 = .03$. For concussion participants, the relationships between UCH-L1 and time since return-to-play ($r = .35$, $p = .17$) as well as time since injury ($r = .32$, $p = .19$) were not statistically significant.

Tau

The effect of group on Tau levels did not reach significance, $F(1, 32) = 3.65$, $p = .07$, $\eta_p^2 = .10$, but there was a trend where concussion participants Tau levels (*M* = 1.39 ng/ml, *SE* =

.309) tended to be lower than controls (M = 2.30 ng/ml, SE = .320). The total DASS score was not related to Tau levels, $F(1, 32) = 1.94$, $p = .17$, $\eta_p^2 = .06$. For concussion participants, the relationship between Tau and time since return-to-play ($r = .39$, $p = .12$) was not statistically significant. However, the relationship between Tau and time since injury ($r = .58$, $p = .01$) was statistically significant. The more time that had elapsed since injury the higher the Tau levels (see **Figure 2**).

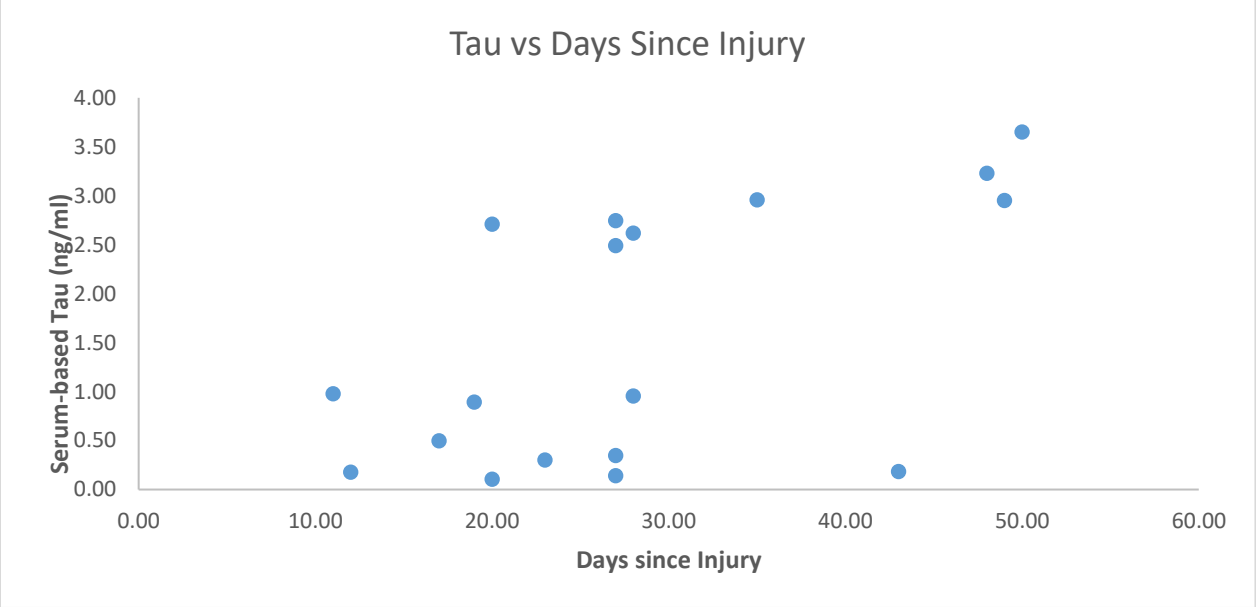

Figure 2. Tau and days since injury. The correlation between days since injury and serum Tau levels in the concussed group.

MRI Subset

Of the 30 participants that underwent an MRI scan, 26 were used for analysis (13 concussion and 13 control). A total of four were excluded from all analyses, two were excluded due to technical issues with their MRI scan (1 concussion and 1 control), the other two were excluded due to no blood data (1 concussion and 1 control; see **Table 2**). However, only 25 were included for the Tau analysis due to running out of sample for one of the participants (1 control).

Table 2. Comparison of Participant Groups (MRI Subset)			
	Concussion	**Control**	**Comparison**
N	13	13	
Age	*M* = 19.46, *SD* = 1.27	*M* = 19.46, *SD* = 0.88	
Sex	Female = 6, Male = 7	Female = 6, Male = 7	
DASS T	*M* = 35.92, *SD* = 24.14	*M* = 8.31, *SD* = 11.0	*t*(24) = 3.75, *p* = .001.
Intake prior to data collection	Nicotine = 1, OTC = 0, Caffeine = 3	Nicotine = 1, OTC = 3, Caffeine = 5	
Time of sample	*M* = 13.10, *SD* = 3.26	*M* = 12.81, *SD* = 2.34	*t*(24) = .259, *p* = .798
Sport	USA Wrestling = 1	USA Wrestling = 1	
	Football = 5	Football = 5	
	Diving = 2	Diving = 2	
	Soccer = 3	Soccer = 3	
	Club Hockey = 1	Club Hockey = 1	
	Swimming = 1	Swimming = 1	

Hypothesis two: There would be differences in FC and GMV between concussed and control groups in the following areas: brainstem, prefrontal cortex, and hippocampus.

Gray Matter Volume

There was no main effect of group on GMV at an FDR adjusted cluster level ($p < .05$) analysis, however a main effect of group on GMV was observed at the peak level uncorrected ($p = .001$). GMV was found to be greater in the concussion group relative to the control group in the superior frontal gyrus, superior temporal gyrus, post central gyrus, calcarine fissure and the surrounding cortex (see **Table 3**). The concussion group also exhibited lower GMV compared to the control group in the lingual gyrus and the calcarine fissure and surrounding cortex. (see **Table 3**).

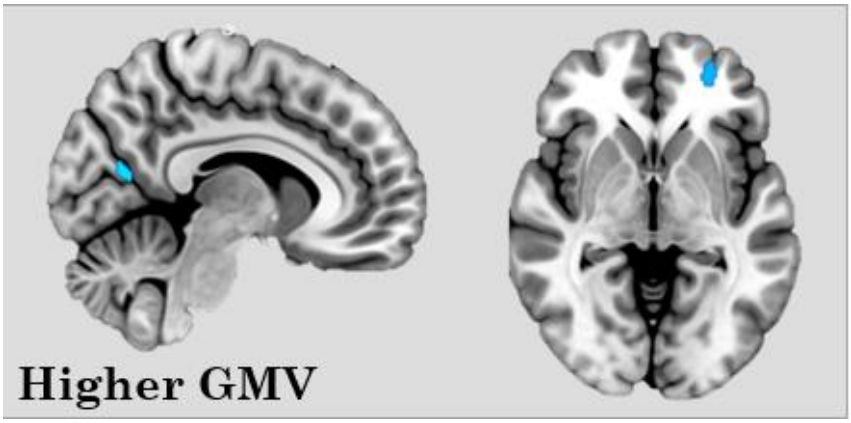

Figure 3. Group differences: higher GMV. Concussed participants exhibited significantly higher GMV in the calcarine fissure & surrounding cortex (left) and superior frontal gyrus (right) relative to the controls.

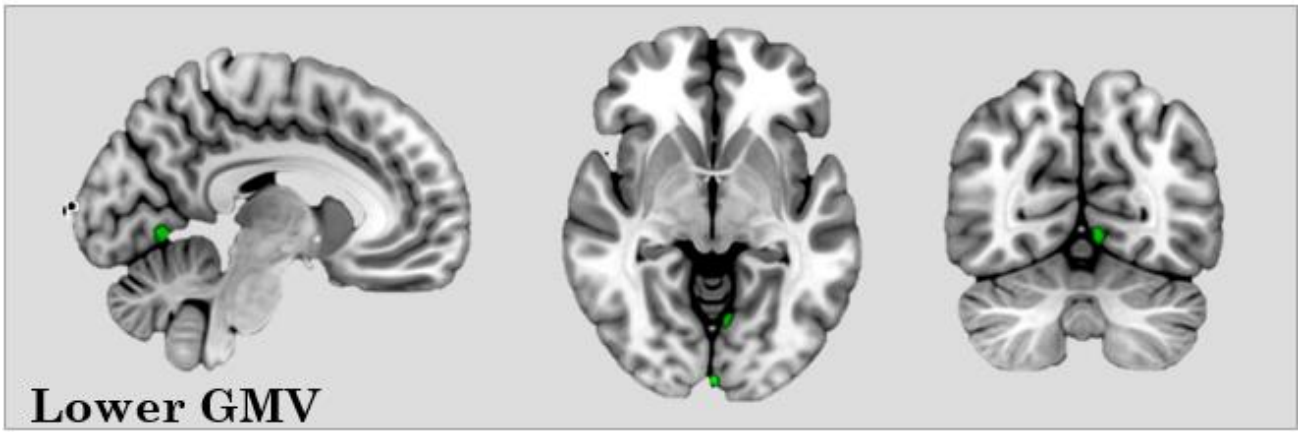

Figure 4. Group differences: lower GMV. Concussed participants displayed significantly lower GMV in the calcarine fissure & surrounding cortex (left and middle) and the lingual gyrus (middle and right) in comparison with the control group.

Table 3. Group Differences in GMV				
Concussion > Control				
Region	**Hemisphere**	**kE**	**t-value**	**Coordinates (xyz)**
Superior frontal gyrus/Dorsolateral	Right	96	5.20	23, 39, 39
Superior frontal gyrus/Orbital	Left	70	5.03	-27, 51, -3
Superior Temporal Gyrus	Left	38	4.56	-41, 6, -18
Postcentral Gyrus	Right	61	4.01	36, -26, 47
Calcarine Fissure and Surrounding Cortex	Right	43	3.79	6, -65, 17
Control > Concussion				
Region	**Hemisphere**	**kE**	**t-value**	**Coordinates (xyz)**
Lingual Gyrus		52	5.11	-6, -68, -3
Calcarine Fissure and Surrounding Cortex	Left	21	4.06	-2, -96, -6

All differences significant at p < .001 uncorrected

Functional Connectivity

There was no main effect of group on FC at an FDR adjusted cluster level ($p < .05$) analysis, however a main effect of group on FC was observed at the peak level uncorrected ($p = .001$) in each of the selected CONN-based seeds. Stronger connectivity was observed in the hippocampus between the middle temporal gyrus and inferior temporal gyrus in the concussion group. Weaker connectivity was observed between the hippocampus with the cerebellum and postcentral gyrus in the concussion group (see **Table 4**).

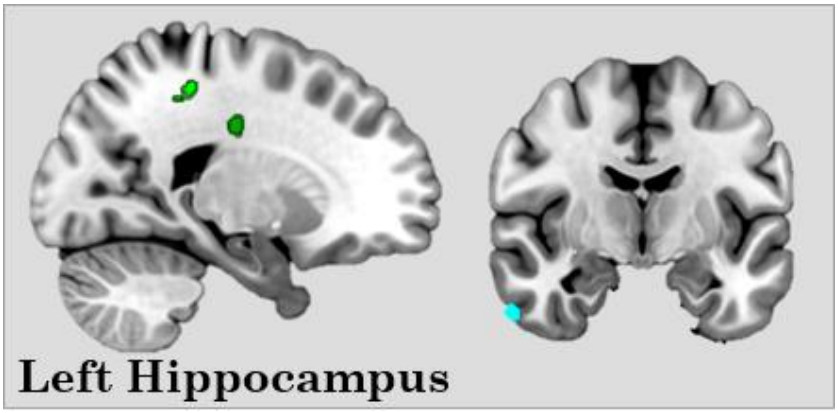

Figure 5. Group differences: left hippocampus FC. Weaker connectivity in the concussed group was observed between the left hippocampus seed with the postcentral gyrus and white matter (left). Stronger connectivity in the concussed group was observed between the left hippocampus seed and the inferior temporal gyrus (right).

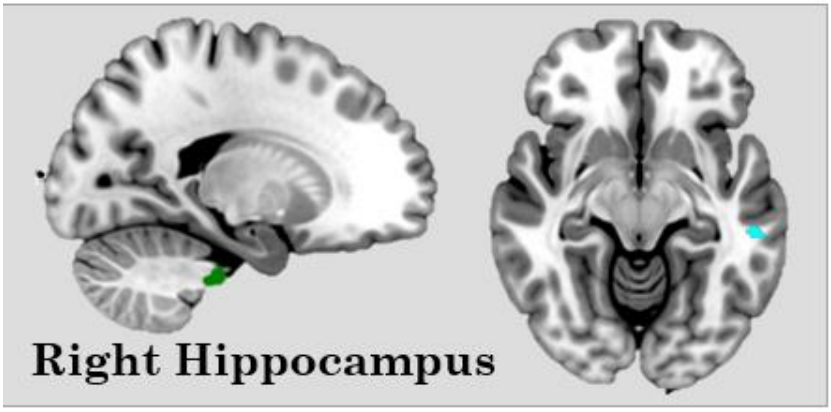

Figure 6. Group differences: right hippocampus FC. Weaker connectivity in the concussed group was observed between the right hippocampus seed and the cerebellum (left). Stronger connectivity in the concussed group was observed between the right hippocampus seed and the middle temporal gyrus (right).

Stronger connectivity in the concussion group was observed between the brainstem and the insula, middle frontal gyrus, middle cingulate and paracingulate gyri. No weaker connections between the brainstem and any region of the brain was observed (see **Table 4**).

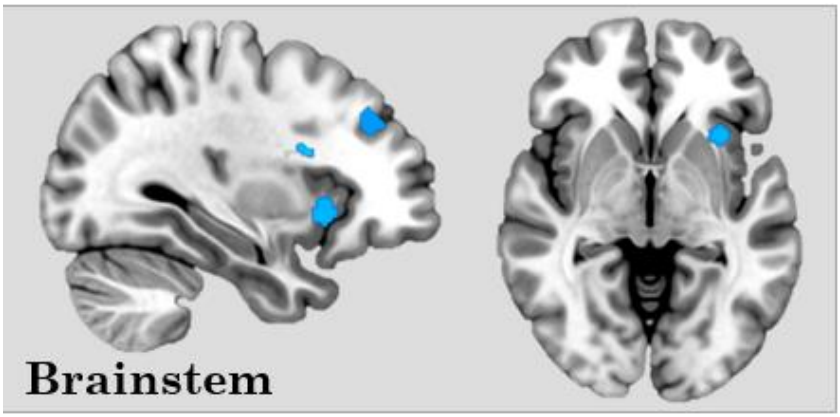

Figure 7. Group differences: brainstem FC. Stronger connectivity in the concussed group was observed between the brainstem and with the insula (left and right), middle cingulate cortex (left), and middle frontal gyrus (left).

Stronger connectivity was observed in the salience right prefrontal cortex (RPFC) between the brainstem, supplementary motor area (SMA), and cerebellum in the concussion

group. A weaker connection in the concussion group was observed in the salience RPFC between the inferior temporal gyrus, superior and middle frontal gyri, gyrus rectus, and the cerebellum (see **Table 4**).

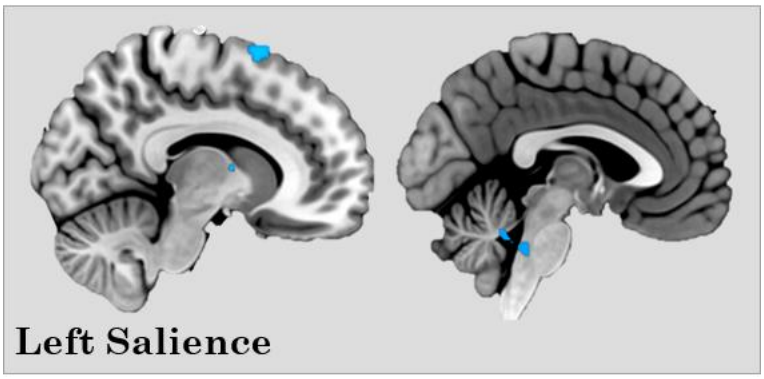

Figure 8. Group differences: left salience stronger FC. Stronger connectivity in the concussed group was observed between the left salience RPFC with the SMA (left), cerebellum (right), and brainstem (right).

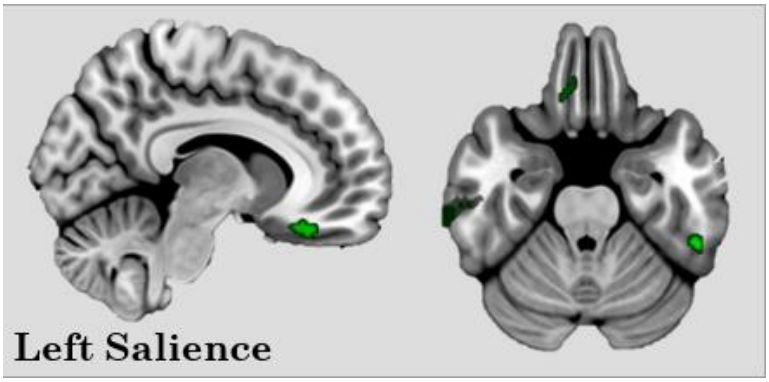

Figure 9. Group differences: left salience weaker FC. Weaker connectivity in the concussed group was observed between the left salience RPFC with the gyrus rectus/ventromedial prefrontal cortex (left and right) and the inferior temporal gyrus (right).

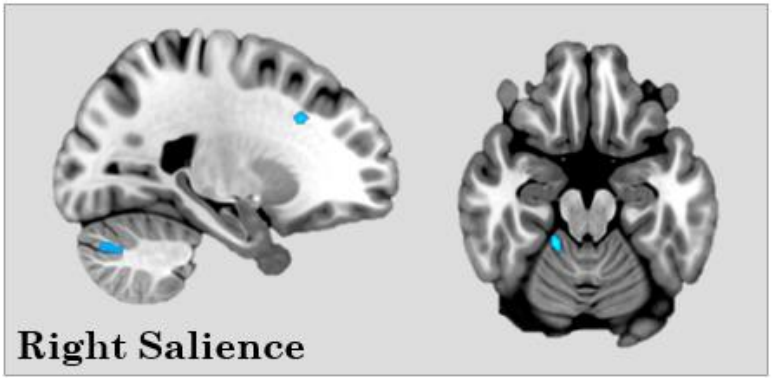

Figure 10. Group differences: right salience FC. Stronger connectivity in the concussed group was observed between the right salience RPFC with the cerebellum (left and right) and white matter (left).

Stronger connectivity in the concussion group was observed in the frontoparietal left prefrontal cortex (LPFC) between the superior temporal gyrus, lingual gyrus, precuneus, cerebellum, and the calcarine fissure and surrounding cortex. The concussion group exhibited weaker connections in the frontoparietal LPFC between the angular gyrus, middle and inferior frontal gyri (see **Table 4**).

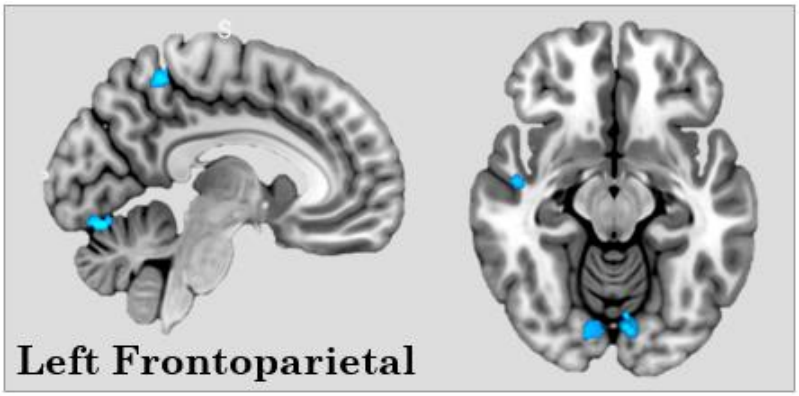

Figure 11. Group differences: left frontoparietal stronger FC. Stronger connectivity in the concussed group was observed between the left frontoparietal LPFC with the precuneus (left), superior temporal gyrus (right), calcarine fissure and surrounding cortex (left and right).

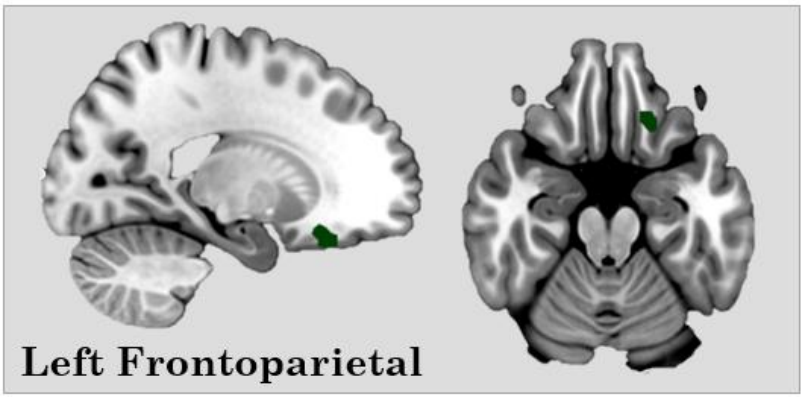

Figure 12. Group differences: left frontoparietal weaker FC. Weaker connectivity in the concussed group was observed between the left frontoparietal LPFC and the orbitofrontal cortex (left and right).

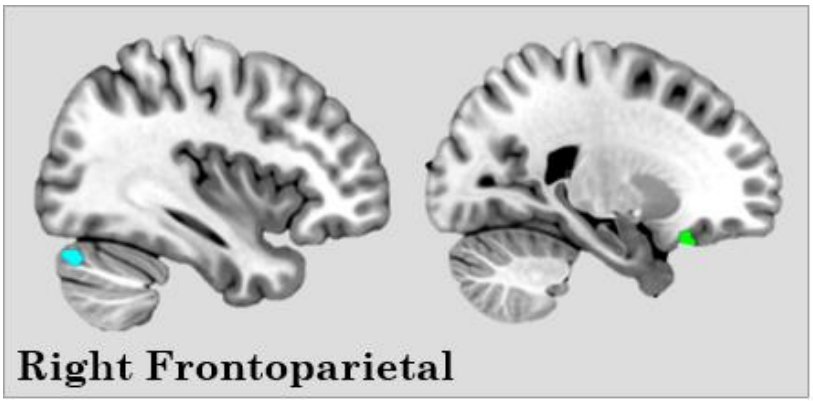

Figure 13. Group differences: right frontoparietal FC. Stronger connectivity in the concussed group was observed between the right frontoparietal LPFC and the cerebellum (left). Weaker connectivity in the concussed group was observed between the frontoparietal LPFC and the orbitofrontal cortex (right).

Table 4. Group Differences in FC					
Concussion > Control					
Seed	**Region**	**Hemisphere**	**kE**	**t-value**	**Coordinates (xyz)**
Hippocampus Right	Middle Temporal Gyrus	Left	29	4.29	-56, -30, -10
Hippocampus Left	Inferior Temporal Gyrus	Right	51	4.80	60, -10, -36
Brainstem	Insula	Left	65	5.94	-32, 16, -4
	Middle Frontal Gyrus	Left	101	4.76	-34, 36, 34
	Middle Frontal Gyrus	Right	39	4.67	32, 26, 36
	Median Cingulate & Paracingulate Gyri	Left	22	3.90	0, -26, 48
Salience RPFC Left	Medulla		52	4.80	0, -38, -34
	Supplementary Motor Area	Right	34	4.57	10, 14, 66
Salience RPFC Right	Cerebellum		53	4.68	-26, -78, -32
	Cerebellum		20	4.44	16, -42, -18
Frontoparietal LPFC Left	Superior Temporal Gyrus	Right	55	5.63	42, -28, -2
	Superior Temporal Gyrus	Left	44	4.92	-44, 22, -24
	Superior Temporal Gyrus	Right	33	4.63	48, -8, -10
	Cerebellum		27	4.10	-6, -78, -14
	Precuneus	Left	32	4.07	-6, -48, 58
	Lingual Gyrus	Right	48	4.04	6, -84, -10
	Calcarine Fissure & Surrounding Cortex	Left	67	3.87	2, -90, 8
Frontoparietal LPFC Right	Cerebellum		72	4.58	38, -78, -28
Control > Concussion					
Seed	**Region**	**Hemisphere**	**kE**	**t-value**	**Coordinates (xyz)**
Hippocampus Right	Cerebellum		99	5.12	-24, -30, -36
Hippocampus Left	Postcentral Gyrus		25	4.14	20, -36, 52
Salience RPFC Left	Inferior Temporal Gyrus	Right	122	5.98	68, -26, -28
	Superior Frontal Gyrus	Right	88	5.55	20, -12, 66
	Cerebellum		28	5.01	-46, -64, -50
	Cerebellum		94	4.70	46, -62, -44
	Gyrus Rectus	Right	46	4.65	8, 32, -22
	Middle Frontal Gyrus	Right	121	4.35	44, 54, -4
	Inferior Temporal Gyrus	Left	33	4.32	-54, -42, -26
Frontoparietal LPFC Left	Middle Frontal Gyrus	Right	54	5.49	48, 16, 52
	Inferior Frontal Gyrus	Left	71	5.13	-18, 28, -20
	Angular Gyrus	Right	61	4.19	60, -56, 34
Frontoparietal LPFC Right	Inferior Frontal Gyrus	Right	32	4.14	22, 24, -20

All differences significant at $p < .001$ uncorrected

Hypothesis three: Alterations in protein levels would be linked to differences in FC and/or GMV between groups in the same areas as above.

Gray Matter Volume

BDNF. No FDR adjusted ($p < .05$) cluster-level differential relationships exist between group for BDNF and GMV. However, relationships between group for BDNF and GMV do exist when assessed at peak level uncorrected ($p = .001$; see appendix **Table 6**).

S100B. A differential relationship was observed between groups for S100B and GMV at an FDR adjusted ($p < .05$) cluster level in the insula where the association between S100B and GMV was more positive in the concussed group relative to the control group. Group moderated the relationship between S100B and GMV in the cerebellum, supplementary motor area, superior parietal gyrus, paracentral lobule, and the postcentral gyrus where the concussion group was negative relative to the control group (see **Table 5**). More relationships between group for S100B and GMV exist at an uncorrected ($p = .001$) peak level (see appendix **Table 7**).

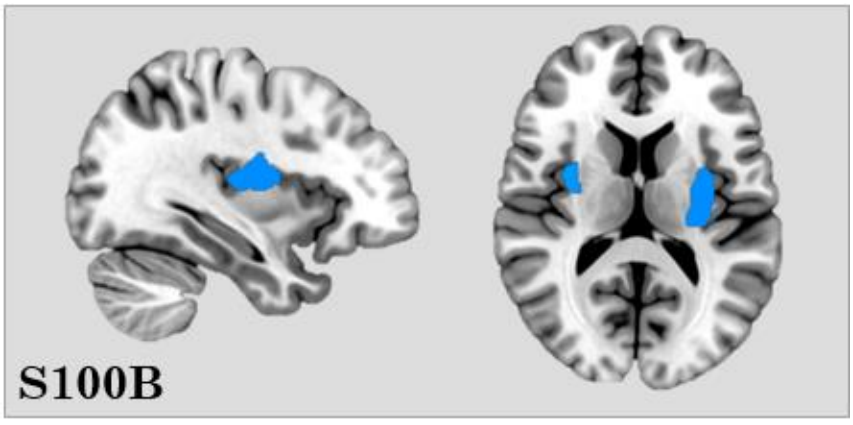

Figure 14. Positive association, S100B and GMV. A differential relationship was observed between S100B and GMV in the insula where the concussed group was more positive relative to control (left and right).

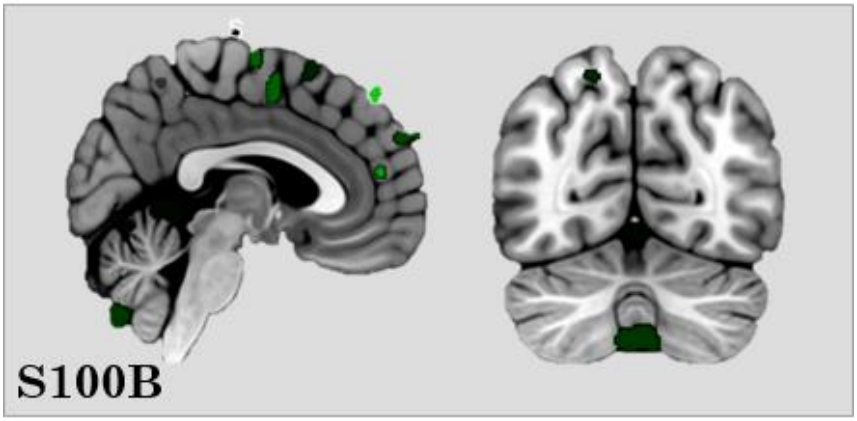

Figure 15. Negative association, S100B and GMV. A differential relationship was observed between S100B and GMV in the postcentral gyrus (left), SMA (left), medial frontal gyrus (left), paracentral lobule (left, superior parietal gyrus (left and right), and the cerebellum (left and right) where the concussed group was more negative relative to control.

UCH-L1. A differential relationship between group for UCH-L1 and GMV was observed at an FDR adjusted ($p < .05$) cluster level in the brainstem where concussion group was more positive. No other cluster level differential relationships exist between group for UCH-L1 and GMV (see **Table 5**). However, several relationships between group for UCH-L1 and GMV exist at an uncorrected ($p = .001$) peak level (see appendix **Table 8**).

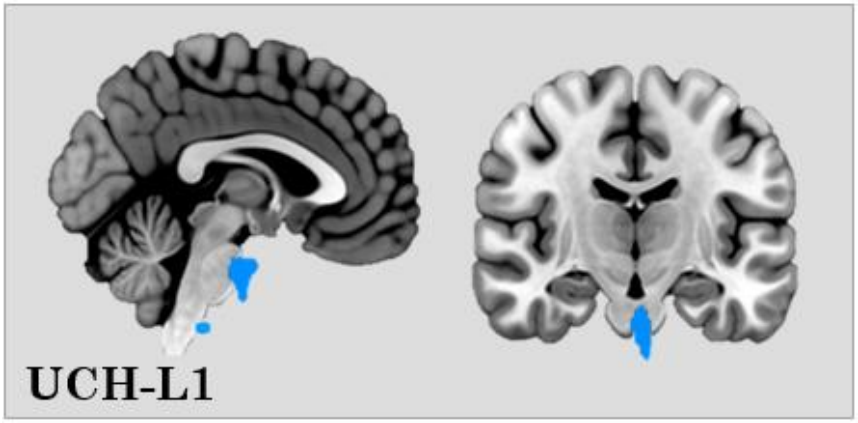

Figure 16. UCH-L1 and GMV. A differential relationship was observed between UCH-L1 and GMV in the brainstem where the concussed group was more positive relative to control (left and right).

Tau. No FDR adjusted ($p < .05$) cluster-level differential relationships exist between group for Tau and GMV. However, relationships between group for Tau and GMV do exist when assessed at an uncorrected ($p = .001$) peak level (see appendix **Table 9**).

Table 5. GMV Cluster Level					
Concussion > Control					
Protein	**Region**	**Hemisphere**	**kE**	**p-value**	**Coordinates (xyz)**
S100B	Insula		1011	0.000	-32, -8, 14
UCH-L1	Brainstem		417	0.001	-5, -18, -32
Control > Concussion					
Protein	**Region**	**Hemisphere**	**kE**	**p-value**	**Coordinates (xyz)**
S100B	Supplementary Motor Area	Left	428	0.020	-2, 18, 65
	Paracentral Lobule	Right	386	0.026	12, -27, 78
	Cerebellum		804	0.003	-3, -50, -3
	Postcentral Gyrus		501	0.016	-21, -30, 72
	Superior Parietal Gyrus	Right	366	0.027	26, -74, 57

Note. All differences significant at p < .05 corrected

Functional Connectivity

BDNF. FC between the left salience right prefrontal cortex seed with the cerebellum and the middle frontal gyrus was weaker in the concussion group relative to the control group at an adjusted ($p < .05$) cluster level (see **Table 10**). More relationships between group for BDNF and FC exist at an uncorrected ($p = .001$) peak level (See appendix **Table 11**).

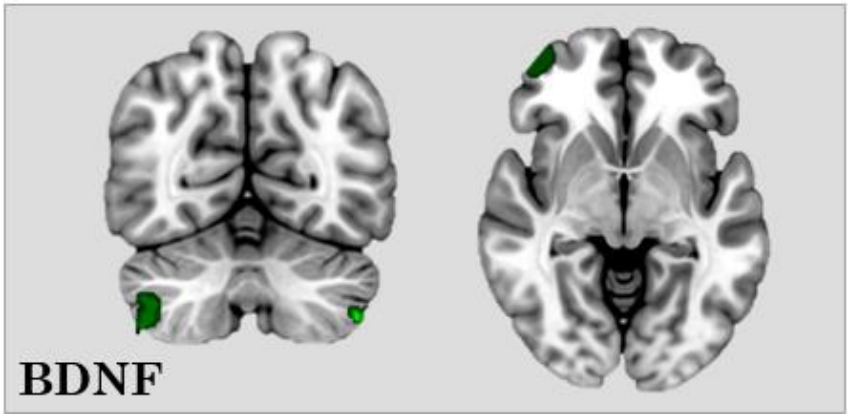

Figure 17. BDNF and FC. A differential relationship was observed between BDNF and FC in the left salience RPFC with the cerebellum (left) and the middle frontal gyrus (right) where the concussed group displayed weaker connectivity relative to control.

S100B. FC between the left salience right prefrontal cortex seed with the inferior temporal gyrus and the middle frontal gyrus was weaker in the concussion group relative to the control group at an adjusted ($p < .05$) cluster level (see **Table 10**). More relationships between group for S100B and FC exist at an uncorrected ($p = .001$) peak level (see appendix **Table 12**).

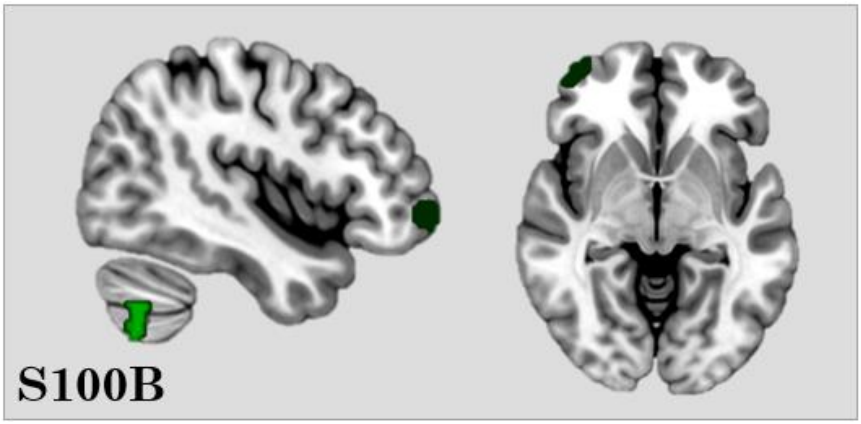

Figure 18. S100B and FC. A differential relationship was observed between S100B and FC in the left salience RPFC with the cerebellum (left) and the middle frontal gyrus (right) where the concussed group exhibited weaker connectivity relative to control.

UCH-L1. No adjusted ($p < .05$) cluster-level differential relationships exist between group for UCH-L1 and FC. However, relationships between group for UCH-L1 and FC do exist when assessed at an uncorrected ($p = .001$) peak level (see appendix **Table 13**).

Tau. Group moderated the relationship between Tau and FC in the superior frontal gyrus, middle frontal gyrus, and the medial superior frontal gyrus at an adjusted ($p < .05$) cluster level in the hippocampus seeds where the concussion group was stronger. FC in the right frontoparietal left prefrontal cortex with the cerebellum was also stronger in the concussion group at an adjusted ($p < .05$) cluster level (see **Table 10**). No negative differential relationships were observed between group for Tau and FC at an adjusted ($p < .05$) cluster level. More relationships between group for Tau and FC exist at an uncorrected ($p < .05$) peak level (see appendix **Table 14**).

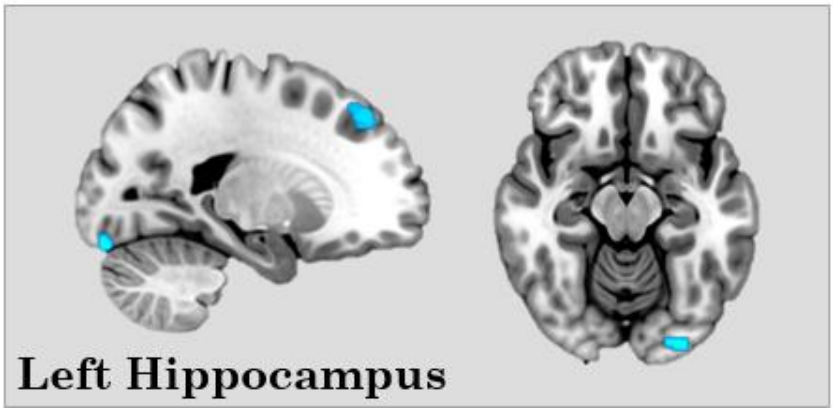

Figure 19. Tau and left hippocampus FC. A differential relationship was observed between Tau and FC in the left hippocampus with the middle frontal gyrus/middle superior frontal gyrus (left) and the visual cortex (left and right) where the concussed group displayed stronger connectivity relative to control.

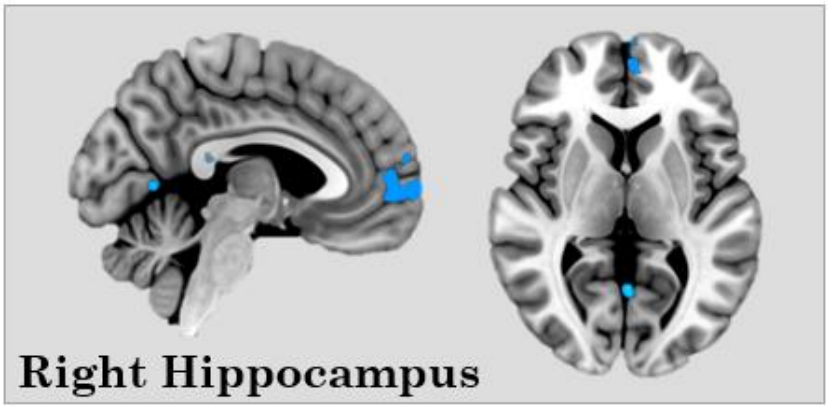

Figure 20. Tau and right hippocampus FC. A differential relationship was observed between Tau and FC in the right hippocampus with the medial frontal gyrus (left), lingual gyrus (left) and the superior frontal gyrus (right) where the concussed group displayed stronger connectivity relative to control.

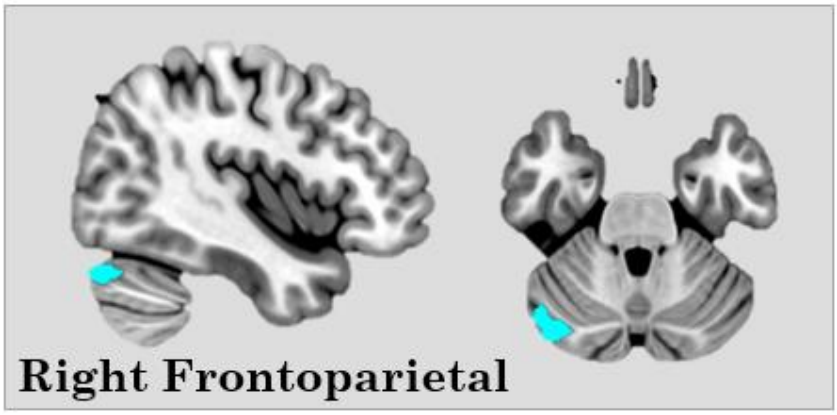

Figure 21. Tau and right frontoparietal FC. A differential relationship was observed between Tau and FC in the right frontoparietal LPFC with the cerebellum (left and right) where the concussed group displayed stronger connectivity relative to control.

Table 10. FC Cluster Level						
Concussion > Control						
Protein	**Seed**	**Region**	**Hemisphere**	**kE**	**p-value**	**Coordinates (xyz)**
Tau	Hippocampus Right	Superior Frontal Gyrus	Left	165	0.016	-12, 26, 52
		Medial Superior Frontal Gyrus	Left	273	0.003	-6, 66, 2
	Hippocampus Left	Middle Frontal Gyrus		154	0.029	26, 28, 32
		Medial Superior Frontal Gyrus	Left	204	0.017	-2, 54, 6
	Frontoparietal LPFC Right	Cerebellum		158	0.045	34, -78, -28
Control > Concussion						
Protein	**Seed**	**Region**	**Hemisphere**	**kE**	**p-value**	**Coordinates (xyz)**
BDNF	Salience RPFC Left	Cerebellum		131	0.036	44, -62, -42
		Middle Frontal Gyrus		146	0.036	42, 54, -8
S100B	Salience RPFC Left	Inferior Temporal Gyrus		158	0.024	64, -26, -28
		Middle Frontal Gyrus		175	0.024	38, 60, -6

All differences significant at $p < .05$ corrected

Discussion

Serum BDNF was elevated in collegiate athletes post return-to-play following a sports-related concussion relative to age, sex, and sport-matched controls. No significant difference was found between groups for the other proteins: S100B, UCH-L1 and Tau or salivary BDNF. Tau was significantly correlated with days since injury and salivary BDNF was significantly correlated with days since return-to-play, but no other proteins displayed significant correlations with either days since return-to-play or days since injury (see **Figure 2**). Overall, significant differences were only observed in BDNF and Tau, not S100B or UCH-L1.

GMV and FC were assessed at both peak and cluster level. No group differences were found at an FDR adjusted ($p < .05$) cluster level for GMV or FC. However, several main effect of group differences were found in GMV and FC at an uncorrected ($p = .001$) peak level (see **Tables 3 & 4**). Differential relationships were observed for S100B and UCH-L1 with GMV between groups at an FDR adjusted ($p < .05$) cluster level (see **Table 5**). Additional observations

of differential relationships were made for all proteins at an uncorrected (p = .001) peak level for GMV (see appendix **Tables 6-9**). BDNF, S100B, and Tau exhibited differential relationships at an FDR adjusted (p < .05) cluster level between groups and FC (see **Table 10**). Several additional differential relationships existed at an uncorrected (p = .001) peak level for all proteins between group and FC (see appendix **Tables 11-14**). In summary, it appears that concussion is associated with differences in brain structure and function (i.e. GMV and FC) as well as the association between these measures and proteins.

Hypothesis one: We expected each protein to have an effect of group.

An effect of group was displayed in serum-based BDNF where the concussion group was significantly elevated compared to controls. No other significant group differences were observed in BDNF, S100B, UCH-L1, and Tau. Even though the main effect of group was not significant in Tau, a trend was observed where the concussed participants tended to have lower levels of Tau compared to the age, sex, and sport-matched controls. Significant correlations were found between saliva-based BDNF and days since return-to-play as well as Tau and days since injury.

Interpretation of findings

Serum BDNF. Our finding of significantly elevated serum BDNF in concussion post return-to-play relative to control provides a better understanding of concussion at a biological level in the return-to-play timeframe. Previous TBI biomarker research has been primarily conducted in animals which showcase an initial reduction in BDNF immediately following injury before later increasing (Kaplan et al., 2010). This later increase in protein BDNF following TBI is thought to be a result of an increase in BDNF mRNA in the cortex (Griesbach

et al., 2002). One small study with humans found similar results of increased BDNF directly following severe TBI in children (Chiaretti et al., 2003).

BDNF is thought to play a major role in the reduction of secondary injury (Kaplan et al., 2010). Secondary injury is a combination of apoptotic, inflammatory, and ischemic processes following a direct primary TBI (Kaplan et al., 2010). Considering BNDF's role in neuroprotection and neurogenesis, previous research has suggested that the higher BDNF is following injury, the more secondary injury is likely to be reduced (Griesbach et al., 2002; Kaplan et al., 2010). Every sample for this project was collected after participants were medically cleared to return-to-play, therefore, our protein levels are likely to serve as part of the recovery or secondary injury phase. Thus, our finding of significantly elevated serum BDNF suggests that despite symptoms subsiding and being cleared to return-to-play following a sports-related concussion, athletes' brains are still healing.

Salivary BDNF. There was not a significant difference between groups for salivary BDNF, however, serum and salivary BDNF related were related in the concussed group. A strong significant correlation was found between saliva and days since return-to-play. This was a positive relationship implicating the longer an athlete had been cleared to return-to-play the higher their salivary BDNF. This null group effect indicates that even though there was not a significant difference in salivary BDNF between concussed and control, this may still be a way to assess recovery post-injury. In particular, the relationship between serum and salivary BDNF in the concussed group is promising as saliva may be another way to evaluate sports-related concussion. However, even with these novel findings salivary BDNF needs to studied at a larger scale and involve other proteins to determine its potential as a biomarker for concussion.

S100B. We did not find a significant difference between groups in S100B, rather concussion was nearly identical to the control. Although we expected to find significantly elevated measures of S100B in serum following injury, our finding that levels of serum S100B did not differ across groups aligns with previous research (Kiechle et al., 2014; Michetti et al., 2012; Morochovic et al., 2009). Elevated levels of S100B have been discovered following sports-related concussion, however, the increase in S100B was only within a couple hours following injury and did not persist 2-7 days after (Kiechle et al., 2014). Those findings correlate with S100B's suggested short half-life and the thought that extremely high levels are considered abnormal, but incredibly rare post-injury (Michetti et al., 2012; Morochovic et al., 2009). Our conclusion is that serum S100B is not a good measure for sports-related concussion at the post return-to-play time-point suggesting that it may be better suited for immediately following injury as previous research has found elevated levels at this time-point. More research is needed to further assess the relationship between concussion and serum S100B levels with a larger sample and at multiple time points to support this claim.

UCH-L1. Similar to S100B we did not find a significant difference between groups for UCH-L1, however, we observed a trend ($p = .16$) where concussion had reduced levels of serum UCH-L1 relative to the control. Even though we did not find a significant difference, we did expect to find lower levels of UCH-L1 in the concussed participants. A recent study conducted following sports-related concussion discovered a decrease of UCH-L1 directly after injury in comparison to baseline, but did not assess levels past 24 hours post-injury (Welch et al., 2018).

Serum UCH-L1 has also been found to be increased within four hours following non-sports related concussion compared to uninjured controls as well as non-head injury trauma patients (Papa et al., 2012). Additional research reported overall UCH-L1 increases immediately

following mild to severe TBI, however, this study did not assess alternative time-points other than immediately post-injury (Diaz-Arrastia et al., 2014; Li et al., 2015). Other research found there to be no significant difference in UCH-L1 levels between concussion and control within 24 hours of injury (Kou et al., 2013). Overall, the research is mixed regarding UCH-L1 as a possible biomarker for concussion, particularly due to the broad scope of previous findings pertaining to TBI, not specified to mTBI or concussion. Future research should focus on UCH-L1 with a larger sample at multiple time points following sports-related concussion rather than just immediately following injury to indicate what role it may play post-injury.

Tau. Although we did not find a significant effect of group on Tau our findings approached significance (p = .07), where levels of Tau were lower in concussion than control. This was surprising as we expected to find elevated levels of Tau in concussion relative to control. One study found an increase in plasma Tau following concussion immediately post-injury when compared to athletes' own preseason measures (Shahim et al., 2018).

Specifically, Shahim and colleagues (2018) discovered Tau increased within one hour of injury, decreased at 12 hours following injury, however, levels increased again at 7 days post-injury. Unfortunately, they did not indicate which form of Tau (A-Tau, C-Tau, or T-Tau) they had assessed making it difficult to distinguish the validity of their timeline (Shahim et al., 2018). Animal research has evaluated levels of C-Tau in the hippocampus following mTBI, but did not find differences between mTBI and control groups (Gabbita et al., 2005). Admittedly, our trend of decreased Tau post-concussion contradicts previous research. However, this trend level effect calls for more additional blood-based biomarker research at the serum level following sports-related concussion post return-to-play.

A significant positive correlation was discovered in our findings with Tau and days since injury. Tau was higher the more time had elapsed since day of injury, in other words as the number of days since injury increased, Tau increased too (see **Figure 2**). A recent study measuring plasma Tau after sports-related concussion evaluated levels with return-to-play, but not days since injury. No significant difference was found between length of return-to-play and Tau in their study (Shahim et al., 2018).To our knowledge, we are the first study to report a relationship between Tau assessed at a return-to-play time point and days since injury following sports-related concussion.

Summary of findings

Our primary finding was elevated serum-based BDNF post-concussion in recently cleared to return-to-play collegiate athletes relative to age, sex, and sport-matched controls. This is an important finding that implicates BDNF's significant role in neuronal recovery following concussion. Additionally, our study demonstrated that salivary BDNF is not an adequate measure of BDNF, although there was a significant correlation between saliva and days since return-to-play. We also found no differences in S100B at the return-to-play time-point in comparison to controls. Both UCH-L1 and Tau were not significant, but trended towards reduced levels in concussion relative to control. Despite not being significant, to our knowledge we are the first study to find lower Tau in concussion at the return-to-play time-point. To the best of our knowledge, this is also the first evidence for a positive correlation between Tau and days since injury.

Hypothesis two: There would be differences in FC and GMV between concussed and control groups in the following areas: brainstem, prefrontal cortex, and hippocampus.

Group differences were observed at an uncorrected (p = .001) peak level in GMV and FC, but not at an FDR adjusted (p < .05) cluster level. GMV differences were observed in the superior frontal gyrus, superior temporal gyrus, post central gyrus and calcarine fissure and surrounding cortex, where concussion exhibited greater GMV relative to control. Weaker GMV was also observed in concussion compared to control in the lingual gyrus, calcarine fissure and surrounding cortex.

Stronger FC was observed in the concussion group between the hippocampus and the middle and inferior temporal gyrus. Weaker FC was observed in the concussion group between the hippocampus and the post central gyrus. Stronger FC was observed between the brainstem and the insula, middle frontal gyrus, middle cingulate and paracingulate gyrus in the concussion group. Stronger FC was observed between the salience RPFC and the brainstem, SMA, and cerebellum in the concussion group. Weaker FC was observed in the concussion group between the salience RPFC and the gyrus rectus, cerebellum, inferior, middle, and superior frontal gyri. Stronger FC was observed between the frontoparietal LPFC and the superior temporal gyrus, lingual gyrus, precuneus, cerebellum, calcarine fissure and surrounding cortex in the concussion group. Weaker FC was observed between the frontoparietal LPFC and the angular gyrus, middle and inferior frontal gyrus in the concussion group. GMV and FC displayed significant group differences at an uncorrected peak level, but not an adjusted cluster level.

Interpretation of findings

Gray Matter Volume. Our findings of significant group differences in GMV following concussion post return-to-play with age, sex, and sport-matched controls adds to the growing body of literature outlining the effects of mTBI on the brain. Unlike previous studies that have found lower GMV in the frontal cortex and temporal lobe, we found significantly higher levels of GMV in the superior frontal gyrus, post central gyrus, and superior temporal gyrus between our groups (Churchill et al., 2017; da Costa et al., 2016; Dean et al., 2015). Churchill and colleagues (2017) discovered athletes with previous concussion history displayed lower volumes in the frontal cortex, middle temporal lobes, and cerebellum (Churchill et al., 2017). Dean et al (2015), saw a reduction in middle temporal lobes in their concussed participants compared to non-injured controls. Da Costa and colleagues (2016) as well as Sussman et al. (2017) also found decreased GMV in their study participants between injured and control groups, however, neither study went into detail on where this decrease was specifically located in the brain.

Interestingly, in our study concussion displayed higher and lower GMV in the calcarine fissure and surrounding cortex, an area of the brain that is vital to visual processing. The concussion group exhibited lower volume in the lingual gyrus, another area of the brain pertaining to vision. Even though we found higher and lower GMV differences between groups, we did not find any differences in the hippocampus, an area of the brain that has been studied extensively in concussion (Brezova et al., 2014; Terpstra et al., 2017).

Higher GMV in the superior frontal gyrus, postcentral gyrus, superior temporal gyrus, calcarine fissure & surrounding cortex may be linked to symptoms. These areas in the brain are important in a number of cognitive variables i.e. working memory, touch, sensations, auditory processing, and the vison. Whereas lower GMV discovered in the lingual gyrus, calcarine

fissure, and surrounding cortex are both areas primarily involved with vision. Our findings suggest these cognitive functions may be connected to possible symptomology following concussion and the differences in GMV could reflect these. Since all of our concussed athletes were previously cleared to return-to-play this could also be a potential factor in the differences we have discovered. That is, it could be possible that these differences in GMV exhibit that the brain is still healing similar to what our protein levels indicate. Future studies are needed to assess GMV differences between concussion and control with MRI at a symptom level, particularly vision i.e. eye tracking and qualitative measures such as visual motor speed via ImPACT. The role of vision in concussion is extremely important as many sports evolve around hand eye coordination.

Functional Connectivity. Consistent with previous research, we found significant differences in FC between concussion and control groups (Bharath et al., 2015; Czerniak et al., 2015; Dettwiler et al., 2014; Eierud et al., 2014; Meier et al., 2017; Militana et al., 2016; Shin et al., 2017; Slobounov et al., 2011; Zhu et al., 2015). Our findings indicate there are underlying functional differences between concussion and control even after concussed athletes have been medically cleared to return-to-play. Similar to previous research we also found stronger connectivity in recently concussed athletes relative to controls in the following brain regions: middle frontal gyrus, SMA, lingual gyrus, superior, middle, and inferior temporal gyri (Czerniak et al., 2015; Meier et al., 2017). Czerniak and colleagues (2015) also discovered high connectivity in the inferior frontal gyrus, precentral gyrus, the superior parietal lobule, and the paracentral gyrus. Meier et al. (2017) also found stronger connectivity in the postcentral gyrus, paracentral lobule, and fusiform gyrus. Although we did not find any group differences in those

additional regions we did find stronger connectivity in the insula, middle cingulate and paracingulate gyrus, brainstem, cerebellum, precuneus, calcarine fissure and surrounding cortex.

Unlike recent research, we discovered lower connectivity in the postcentral gyrus and the inferior frontal gyrus. In addition, we also discovered lower connectivity in concussion compared to control in the superior and middle frontal gyri, inferior temporal gyrus, gyrus rectus, angular gyrus, and cerebellum. Eierud et al. (2014) suggested an anterior-posterior approach when referring to fMRI results of numerous studies evaluating mTBI following injury. This approach is based on patterns of reduced connectivity in the anterior portion of the brain and increased connectivity in posterior areas of the brain (Eierud et al., 2014). Although our results do not mimic this view entirely, we do have some similarities in respect to anterior regions being reduced and posterior regions having increased connectivity. These differences with previous studies could be due to the seed-based regions we used in analysis which were the bilateral hippocampus, brainstem, salience RPFC, and frontoparietal LPFC. Czerniak et al. (2015), used a similar analysis plan as ours with the anterior cingulate cortex and bilateral dorsolateral prefrontal cortex as their seed regions. Slobounov and colleagues (2011) also analyzed their results in CONN like we did using the primary visual cortex, hippocampus, dorsolateral prefrontal cortex, and precuneus as ROI-based seeds.

Related to the GMV findings these differences in FC between groups could be lingering post-concussive abnormalities. It is likely that these differences exist due to the location of impact when the injury was experienced which then led to a duration of symptoms. However, we did not collect data on location, but this should be assessed in the future to determine if and how the area of injury and symptoms endured are linked. Another factor could be the length of injury as the number of symptomatic days and/or symptom severity could be correlated with

connectivity. Considering our findings relate to executive function, movement, coordination, and vision i.e. areas of the prefrontal cortex, cerebellum, temporal gyri these could be possible explanations for the changes in connectivity between concussion and control. The present study would need to address some of these factors to determine if they do result in connectivity differences. Moreover, future studies should acknowledge these possibilities and collect these data points from their athletes to better determine where these differences are stemming from in sports-related concussion.

Summary of findings

Gray Matter Volume. Of primary significance is our finding of higher GMV in concussion relative to control in the superior frontal gyrus, postcentral gyrus, superior temporal gyrus, calcarine fissure and surrounding cortex. Furthermore, our finding of lower GMV in the lingual gyrus, calcarine fissure and surrounding cortex in concussion compared to control. These GMV changes could possibly be reflective of the symptoms an individual (or group) experienced following injury indicating that the brain has not finished healing despite having been cleared to return-to-play.

Functional Connectivity. Of primary significance is our finding of additional higher connectivity regions in the brain following sports-related concussion whereas between groups concussion was higher relative to control in the insula, middle cingulate and paracingulate gyrus, brainstem, cerebellum, precuneus, calcarine fissure and surrounding cortex. Moreover, the discovery of lower connectivity in concussion compared to control in the superior, middle, and inferior frontal gyri, postcentral gyrus, inferior temporal gyrus, gyrus rectus, angular gyrus, and cerebellum. These FC differences could be the result of injury location, length of injury, or symptom severity illustrating underlying lingering post-concussive abnormalities.

Hypothesis three: Alterations in protein levels would be linked to differences in FC and/or GMV between groups in the same areas as above.

Differential relationships at an FDR adjusted ($p < .05$) cluster level were observed between groups in S100B and GMV as well as UCH-L1 and GMV (see **Table 5**). Additional differential relationships were observed between groups in all proteins at an uncorrected ($p = .001$) peak level in GMV (see **Appendix Tables 6-9**). Differential relationships in FC were observed in serum-based BDNF, S100B, and Tau protein between groups at an FDR adjusted ($p < .05$) cluster level (see **Table 10**). Additional observations of differential relationships at an uncorrected ($p = .001$) peak level were made for all proteins between group and FC (see **Appendix Tables 11-14**).

Interpretation of findings

Gray Matter Volume. Significant differential relationships between groups in proteins and GMV were observed in S100B and UCH-L1. Group moderated the relationship between S100B and GMV, where the association was more positive in the insula in concussion compared to control. Additionally, differential relationships existed between S100B and GMV in the SMA, paracentral lobule, postcentral gyrus, superior parietal gyrus, and cerebellum where the association was more negative in concussion compared to control. Group also moderated the relationship between UCH-L1 and GMV in the brainstem, where the association was more positive in concussion compared to control. There were no differential relationships between groups and GMV with BDNF or Tau. These are novel findings on the effects of sports-related concussion at the post return-to-play time-point. MRI has been analyzed with Glial Fibrillary Acidic Protein (GFAP) following mild-severe TBI in the civilian population to assess negative and positive findings in MRI scans (McMahon et al., 2015; Yue et al., 2019). As far we can tell

we are the first study to assess and find significance with serum-based biomarkers in conjunction with GMV following TBI, in particular sports-related concussion.

S100B is known for its role as a screening mechanism for abnormal head CT and elevated levels are thought to exhibit toxicity indicating cell damage (Bazarian et al., 2013; Michetti et al., 2012; Unden & Romner, 2010). We did not find a significant main effect of group in S100B, rather concussion and control had nearly identical levels. Based on the similar levels of S100B between groups it would seem the brain has entirely healed. However, differential associations between S100B and GMV were discovered in the insula, SMA, postcentral gyrus, paracentral lobule, superior parietal gyrus, and cerebellum. All things considered, S100B may give signs of recovery at a surface level, but biologically demonstrates a need for further healing hence the associations between S100B and GMV.

UCH-L1 is released as a result of cell destruction and has been measured in direct proportion to injury severity (Mondello et al., 2014). Like S100B, we did not find a significant difference between UCH-L1 levels and group, although concussion trended lower than control. Only one positive association was discovered between UCH-L1 and GMV in the brainstem which is an area involved with several body functions including wakefulness, breathing, and heartrate. This association between UCH-L1 and the brainstem may be related to recovery meaning the brain could be overcompensating for those functions as an outcome of injury. Ultimately portraying that the brain is still healing.

Functional Connectivity. Group moderated findings of differential relationships between proteins and FC in BDNF, S100B, and Tau at post return-to-play of recently concussed collegiate athletes and age, sex, sport-matched controls. These findings add to the recent research evaluating the possible roles of blood-biomarkers and functional connectivity following TBI.

Global neural connectivity (GConn) and cerebral blood flow (CBF) has been assessed previously with BDNF, S100B, and Tau (Di Battista et al., 2018). Negative associations between Gconn and Tau as well as CBF and Tau existed between healthy controls and acute concussion (1-7 days following injury) (Di Battista et al., 2018). Athletes with a history of concussion displayed a differential relationship between decreased Gconn and increased S100B levels, the higher S100B was the lower their Gconn compared to controls, however, this study did not find any differences with anything pertaining to BDNF (Di Battista et al., 2018).

Comparable to Thompson and colleagues (2016), we also found differences in FC of the default mode network, frontoparietal, sensory, auditory, and visual areas of the brain (Thompson et al., 2016). More specifically we also found negative relationships in FC between S100B and the default mode network (Thompson et al., 2016). However, we found that group moderated the relationship between FC and S100B in the middle frontal gyrus and inferior temporal gyrus. We discovered a similar group difference between FC and BDNF in the middle frontal gyrus and cerebellum in the salience RPFC where concussion was weaker compared to control. Moreover, FC in the hippocampus with the medial superior frontal gyrus and superior frontal gyrus was stronger in concussion relative to control. FC in the frontoparietal LPFC was also stronger with the cerebellum in concussion compared to control. Like, our GMV findings to the best of our knowledge we believe we are the first study to analyze and examine serum-based biomarkers and FC together following sports-related concussion.

As mentioned, S100B may be able to demonstrate further need for healing in the brain with GMV and is no different with FC. Negative differential associations were observed between S100B and FC in the middle frontal gyrus and inferior temporal gyrus. These findings point out

that the brain is likely not using these regions as efficiently as it could be due to concussion and could be the reason behind difficulties experienced with attention and perception.

BDNF is thought to have a role in active recovery following head injury. We found significantly elevated levels of serum BDNF in concussion relative to control implying that the brain is indeed still healing despite subsiding symptoms. Similar to S100B, negative associations were also observed between BDNF and FC in the middle frontal gyrus and cerebellum. The cerebellum has been implicated in concussion research as a common symptom is disruption of balance and coordination (Churchill et al., 2017). However, the cerebellum has recently been thought to have a role in anxiety (Moreno-Rius, 2018). Interestingly, BDNF has also been associated with stress-related factors including anxiety and attentional bias to threat (Aydemir et al., 2005; Carlson et al., 2014). Although we controlled for DASS it is likely due to the subjectivity of self-report questionnaires that the association we have discovered between the cerebellum and BDNF could be a result of anxiety, especially when all levels of depression, anxiety, and stress were significantly elevated in our concussion group compared to control (Carlson et al., 2020).

Tau is most known for its role in Alzheimer's, however, it is also a protein that has been found to display differences following TBI (Barrio et al., 2015). We did not find significance between groups, but did discover a trend where concussion Tau levels tended to be lower than controls. Unlike BDNF and S100B, we observed positive associations between Tau and FC in the superior and medial superior frontal gyrus and the cerebellum. These associations could potentially indicate the brain overcompensating in these areas due the brain needing more time to recover. This is plausible given the lower levels of Tau relative to controls, but also could be in

response to prolonged memory, balance, and/or anxiety symptoms experienced even after being cleared to return-to-play.

Summary of findings

Gray Matter Volume. Of great significance are our novel findings between groups with proteins and GMV. Significant differences were found between S100B and GMV as well as UCH-L1 and GMV at an adjusted ($p < .05$) clustered level. At an uncorrected ($p = .001$) peak level, all proteins and GMV displayed significance. As far as we know we are the first study to evaluate BDNF, S100B, UCH-L1, Tau, and GMV together at return-to-play post sports-related concussion.

Functional Connectivity. Of great significance are our novel findings between groups with proteins and FC in the salience RPFC, hippocampus, and frontoparietal LPFC seed regions. Group moderated the effect of FC with BDNF, S100B, and Tau at an adjusted ($p <.05$) cluster level. Comparable to our GMV results, all proteins and FC exhibited significance at an uncorrected ($p = .001$) peak level. To the best of our knowledge, we are the first study to assess serum-based biomarkers and rsFC together following at return-to-play following sports-related concussion in comparison with age, sex, and sport-matched controls.

Limitations and Future Directions

The present study has several limitations that need to be considered when drawing conclusions from our findings and designing future studies. First, we had a small sample size, future research should replicate our findings at a larger scale. G*Power analyses with our observed effect sizes determined that a total of 130 participants are needed for UCH-L1 and 72 participants are needed for Tau in order to reach significance based on the present findings.

Second, there was variability in the time of our sample collection, however, this was matched across groups so it is unlikely that this affected our results. Nonetheless future studies should be replicated with stricter timing guidelines for sample collection. Third, our study did not account for diurnal variation which has been previously related to BDNF and S100B, but not UCH-L1 and Tau (Morera-Fumero et al., 2017; Piccinni et al., 2008). To avoid any diurnal variation future research may need to base sample collection time on a participant's sleep-wake cycle rather than a set time of day. Fourth, we only assessed one return-to-play time point and had a lot of variability in the time course of recovery following sports-related concussion. Future studies should develop more than one set time point to better evaluate the effect of concussion on blood-based biomarkers and to avoid any wide variation during recovery. Fifth, our current Tau data is the result of a failed standard curve and will need to be reassessed before these results can be taken with serious consideration. Running ELISAs should take precautions and always remake the standard curve to avoid any poor outcomes. Sixth, we did not account for the effect of exercise on these protein levels and have been previously found to have an effect on BDNF, S100B, UCH-L1, and Tau (Bamac et al., 2011; Dang et al., 2014; Daniele et al., 2018; Liu et al., 2010; McGeown et al., 2016; Schulte et al., 2014).

Other proteins should potentially be studied to gain a better representation of the effects of sports-related concussion on blood-based biomarkers. Future studies should consider selecting proteins based on symptomology to possibly develop a more personalized method of treatment post-injury. These effects should also be studied long-term ideally to also study the effects of repetitive head injury and maybe grant insight into chronic traumatic encephalopathy. More studies should study blood and saliva concurrently to assess the possibility of saliva being a potential biomarker for concussion.

Clinical Implications

Due to the complexity and heterogeneity of the brain, the current approach of clinical examination and neuroimaging to diagnose concussion has being proven inadequate (Mondello et al., 2014). The use a blood biomarker is minimally invasive compared to neuroimaging, readily available (i.e. used in a hospital or on a sideline), and cost effective (Kawata et al., 2016). Various studies have already provided evidence of biomarkers and their ability to successfully diagnose concussion (Bazarian et al., 2013; Kawata et al., 2018; Kiechle et al., 2014; Kou et al., 2013; Li et al., 2015; Papa et al., 2012; Randall et al., 2013; Shahim et al., 2018; Susa et al., 2019; Unden & Romner, 2010). Furthermore, the addition of biomarkers to injury diagnosis does not fall on one single protein or measure, rather on multiple. Having more than one biomarker increases the likelihood of diagnosis accuracy as well as provide outcome prediction following injury. However, a major limitation is the inability to decide which biomarkers should be used and a complete lack of replication (Kawata et al., 2016; Kim et al., 2018; Mondello et al., 2014; Zetterberg et al., 2013). A clear set of guidelines illustrating time points and the type of biomarker i.e. serum, plasma, or saliva would lead to overall better replication and reproducibility for the development of new methodology in the assessment and treatment of concussion. With a consensus larger scale studies would ultimately provide the evidence and validation necessary in making this approach available for patients.

Practical Implications

Our findings of significant differences between BDNF, S100B, UCH-L1, and Tau with GMV and FC indicate that the brain is still actively recovering despite athletes having recently been cleared to return-to-play following sports-related concussion. The current approach to clear athletes and/or individuals from concussion is likely not a result of a healed injury, but rather

solely due to the absence of symptoms. Better medical practices need to be made surrounding concussion to reduce mental illness and save lives i.e. in particular the return-to-play protocol must be modified for athletes of all skill level.

The world of sports as we know it will need to change for concussion safety to take precedence. Every athlete, coach, and parent need to be educated on the signs and severity of concussion in order to make sports a safer place. Head injuries are not to be taken lightly and if someone must sit out an important game or race then this needs to be done. Without proper education on concussion, coaches, and parents lacking in education may be unknowingly putting the health and safety of their youth athletes at risk. It is not uncommon for risks to be taken with "star players" because of a failure to understand the potential damage associated with concussion. As shown in our research, even though symptoms may have subsided, the brain is far from fully-healed after athletes have been medically cleared to return-to-play. There is increased risk of further injury and the development of disorders later in life. While all risk of a concussion happening in sport cannot be removed, post-concussion care has substantial room for improvement in order to reduce the risks of serious, and potentially life-changing outcomes.

If we do not keep athletes safe starting at a young age then their safety will not be priority as they continue to move up to the high school, college, and professional level. Major sports leagues should be aware that in order for their athletes to play at their highest potential there needs to be stricter return-to-play regulations. Athletes must be given the proper amount of time to let their brain heal beyond the absence of symptoms.

Conclusion

To the best of our knowledge, this is the first study to analyze serum-based protein levels in conjunction with MRI measures following sports-related concussion. Findings of this study indicate protein-based biomarkers may have implications for targeting clinical assessment of sports-related concussion. Serum-based BDNF was the most effective measure to differentiate between concussion and control following return-to-play. Although S100B, UCH-L1, and Tau were not significant across groups, differential relationships were observed in GMV and FC of every protein. This study provides novel findings of the relationships between BDNF, S100B, UCH-L1, and Tau with whole brain GMV and hippocampus, brainstem, salience RPFC, and frontoparietal LPFC seed-based FC. Furthermore, these findings suggest that GMV and FC differences with BDNF, S100B, UCH-L1, and Tau may be linked to a number of factors including location of impact, duration of injury, symptom type and severity. Protein-based biomarkers may be useful in measuring the effects of concussion post return-to-play at a symptom level. Overall, the usage of blood biomarkers in partnership with MRI could lead to improved diagnosis and treatment of concussion.

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Appendix

Gray Matter Volume Additional Tables

Table 6. GMV BDNF				
Concussion > Control				
Region	**Hemisphere**	**kE**	**t-value**	**Coordinates (xyz)**
Middle Occipital Gyrus		20	5.64	-23, -90, 2
Cerebellum		89	4.71	-12, -77, -32
Cerebellum		25	4.29	15, -77, -35
Control > Concussion				
Region	**Hemisphere**	**kE**	**t-value**	**Coordinates (xyz)**
Insula Left	Left	65	5.88	-36, 11, -14
Inferior Temporal Gyrus	Right	24	5.62	60, -32, -19
Inferior Temporal Gyrus	Right	20	4.83	42, 3, -45
Superior Occipital Gyrus	Right	22	4.66	18, -87, 17

All differences significant at $p < .001$ uncorrected

Table 7. GMV S100B				
Concussion > Control				
Region	**Hemisphere**	**kE**	**t-value**	**Coordinates (xyz)**
Insula	Left	1011	7.22	-32, -8, 14
Supramarginal Gyrus	Right	60	6.06	59, -42, 33
Superior Frontal Gyrus	Left	29	5.14	-11, 60, -8
Superior Frontal Gyrus	Right	23	4.88	18, 60, 2
Superior Frontal Gyrus	Left	24	4.31	-14, 6, 51
Precentral Gyrus	Right	21	4.26	36, -11, 56
Supramarginal Gyrus	Left	43	4.14	-56, -35, 23
Insula	Left	20	3.99	-30, 18, 2
Insula	Left	31	3.93	-27, 20, -9
Control > Concussion				
Region	**Hemisphere**	**kE**	**t-value**	**Coordinates (xyz)**
Supplementary Motor Area	Left	124	6.20	0, -8, 66
Supplementary Motor Area	Left	148	5.90	0, 2, 53
Cuneus	Left	109	5.79	-5, -89, 33
Cerebellum		804	5.70	-3, -50, -3
Supplementary Motor Area	Left	428	5.46	-2, 18, 65
Inferior Frontal Gyrus	Left	56	5.45	-30, 33, -14
Superior Parietal Gyrus	Right	366	5.15	26, -74, 57
Precuneus	Right	30	5.13	6, -50, 57
Parahippocampal Gyrus		89	5.09	21, -26, -23
Paracentral Lobule		386	4.83	12, -27, 78
Postcentral Gyrus	Left	501	4.77	-21, -30, 72
Superior Frontal Gyrus	Left	56	4.68	0, 50, 15
Superior Temporal Gyrus	Right	23	4.53	23, 5, -20
Supramarginal Gyrus	Right	24	4.41	53, -17, 26
Paracentral Lobule	Left	39	4.15	-2, -33, 63
Superior Frontal Gyrus	Right	23	4.06	5, 47, 50

All differences significant at $p < .001$ uncorrected

Table 8. GMV UCH-L1				
Concussion > Control				
Region	**Hemisphere**	**kE**	**t-value**	**Coordinates (xyz)**
Brainstem		417	6.32	-5, -18, -32
Brainstem		170	4.67	3, -32, -59
Brainstem		25	4.29	11, -26, -21
Cerebellum		25	4.06	24, -41, -56
Superior Frontal Gyrus	Left	56	4.04	-30, -8, 68
Control > Concussion				
Region	**Hemisphere**	**kE**	**t-value**	**Coordinates (xyz)**
Precentral Gyrus	Left	169	4.55	-30, -6, 48
Calcarine Fissure and Surrounding Cortex	Left	33	4.34	2, -74, 14

All differences significant at $p < .001$ uncorrected

Table 9. GMV Tau				
Concussion > Control				
Region	**Hemisphere**	**kE**	**t-value**	**Coordinates (xyz)**
Rolandic Operculum		61	5.52	47, -3, 11
Precentral Gyrus	Left	41	5.12	-33, -15, 51
Brainstem		144	4.63	3, -33, -59
Middle Frontal Gyrus	Left	27	4.56	-41, 36, 27
Inferior Frontal Gyrus	Right	20	4.51	53, 14, 11
Control > Concussion				
Region	**Hemisphere**	**kE**	**t-value**	**Coordinates (xyz)**
Anterior Cingulate & Paracingulate Gyri	Right	177	5.56	11, 48, 11
Inferior Frontal Gyrus	Right	76	5.19	30, 23, -12
Fusiform Gyrus	Left	63	4.98	-41, -65, -18
Lenticular Nucleus Putamen	Right	21	4.62	26, 12, 3
Superior Frontal Gyrus	Left	21	4.33	-18, 27, 45
Superior Temporal Gyrus	Left	24	4.29	-50, 3, -9

All differences significant at p < .001 uncorrected

Functional Connectivity Additional Tables

Table 11. FC BDNF					
Concussion > Control					
Seed	**Region**	**Hemisphere**	**kE**	**t-value**	**Coordinates (xyz)**
Hippocampus Right	Inferior Temporal Gyrus	Right	25	4.08	58, -8, -36
Hippocampus Left	Inferior Temporal Gyrus	Right	40	4.74	60, -10, -36
	Superior Frontal Gyrus	Right	24	4.00	30, 62, 10
Brainstem	Insula	Left	75	5.94	-32, 16, -4
	Middle Frontal Gyrus	Right	51	5.07	30, 24, 34
	Middle Frontal Gyrus	Left	125	4.99	-34, 36, 34
	Median Cingulate & Paracingulate Gyri	Right	38	4.32	6, -32, 46
	Cerebellum		28	4.02	36, -50, -38
Salience RPFC Left	Brainstem		66	5.18	0, -38, -34
	Brainstem		31	5.09	-6, -38, -52
Salience RPFC Right	Middle Frontal Gyrus	Left	34	4.80	-22, 22, 34
	Cerebellum		61	4.79	-28, -78, -34
	Brainstem		25	4.26	12, -28, -42
Frontoparietal LPFC Left	Superior Temporal Gyrus	Right	50	5.69	42, -28, -2
	Superior Temporal Gyrus	Left	47	4.58	-44, 20, -22
Frontoparietal LPFC Right	Middle Temporal Gyrus	Left	28	4.73	-48, 14, -36
	Cerebellum		36	4.07	40, -80, -28
Control > Concussion					
Seed	**Region**	**Hemisphere**	**kE**	**t-value**	**Coordinates (xyz)**
Hippocampus Right	Cerebellum		25	4.89	-24, -30, -36
Hippocampus Left	Brainstem		91	5.04	-6, -22, -46
Salience RPFC Left	Inferior Temporal Gyrus	Right	143	5.95	68, -26, -28
	Superior Frontal Gyrus	Right	66	5.04	18, -12, 66
	Cerebellum		22	4.80	-46, -64, -50
	Gyrus Rectus	Right	40	4.51	8, 32, -22
	Cerebellum		71	4.35	42, -64, -44
	Middle Frontal Gyrus	Right	109	4.22	42, 52, -6
	Inferior Temporal Gyrus	Left	32	4.19	-48, -36, -28
Salience RPFC Right	Inferior Temporal Gyrus	Left	22	4.32	-44, -30, -26
	Middle Frontal Gyrus	Right	40	4.01	48, 46, 4
Frontoparietal LPFC Left	Middle Frontal Gyrus	Right	26	5.13	46, 14, 54
	Caudate Nucleus	Left	68	5.02	-6, 8, 8
	Inferior Frontal Gyrus	Left	56	4.81	-18, 28, -20
	Middle Occipital Gyrus	Left	66	4.25	-32, -62, 34
Frontoparietal LPFC Right	Inferior Frontal Gyrus	Right	25	4.87	22, 24, -20

All differences significant at $p < .001$ uncorrected

Table 12. FC S100B					
Concussion > Control					
Seed	**Region**	**Hemisphere**	**kE**	**t-value**	**Coordinates (xyz)**
Hippocampus Left	Inferior Temporal Gyrus	Right	44	4.98	60, -10, -36
	Superior Frontal Gyrus	Left	39	4.41	-10, 62, 20
	Lingual Gyrus	Left	33	4.12	-20, -92, -20
Brainstem	Insula	Left	96	6.69	-32, 16, -4
	Middle Frontal Gyrus	Left	189	5.28	-34, 36, 34
	Middle Frontal Gyrus	Right	50	5.02	32, 26, 36
	Supramarginal & Angular Gyri	Left	24	3.96	-44, -42, 42
	Middle Frontal Gyrus	Right	21	3.94	30, 46, 20
Salience RPFC Left	Brainstem		46	5.27	-6, -38, -52
	Brainstem		33	4.76	0, -36, -36
	Cerebellum		29	4.55	12, -44, -16
	Inferior Temporal Gyrus	Left	22	4.47	-36, 4, -40
Salience RPFC Right	Superior Temporal Gyrus	Left	27	5.22	-22, 20, 32
	Cerebellum		31	4.79	16, -42, -18
	Cerebellum		44	4.55	-24, -70, -36
Frontoparietal LPFC Left	Paracentral Lobule	Left	72	5.50	-8, -26, 56
	Superior Temporal Gyrus	Right	45	4.88	48, -8, -8
	Superior Temporal Gyrus	Left	31	4.70	-44, 22, -24
	Precuneus	Left	47	4.13	-6, -48, 56
	Precuneus	Right	22	4.10	8, -46, 58
	Lingual Gyrus	Right	55	3.92	10, -80, -12
	Calcarine Fissure & Surrounding Cortex	Left	23	3.74	2, -88, 12
Control > Concussion					
Seed	**Region**	**Hemisphere**	**kE**	**t-value**	**Coordinates (xyz)**
Hippocampus Right	Cerebellum		79	5.22	-24, -30, -36
	Inferior Frontal Gyrus	Right	20	3.94	34, 6, 30
Salience RPFC Left	Inferior Temporal Gyrus	Right	180	8.06	64, -26, -28
	Superior Frontal Gyrus	Right	101	5.62	20, -12, 66
	Cerebellum		26	5.33	-46, -64, -50
	Inferior Temporal Gyrus		48	4.97	-54, -42, -26
	Middle Frontal Gyrus	Right	181	4.79	38, 60, -6
	Cerebellum		80	4.40	46, -62, -42
Frontoparietal LPFC Left	Inferior Frontal Gyrus	Left	150	5.89	-18, 28, -20
	Middle Frontal Gyrus	Right	46	5.11	46, 16, 54
	Angular Gyrus	Right	76	4.53	58, -54, 34
	Inferior Frontal Gyrus	Right	23	4.41	52, 40, -16
	Olfactory Cortex	Left	49	4.39	6, 14, -10
	Middle Occipital Gyrus	Left	22	4.06	-34, -64, 36
Frontoparietal LPFC Right	Inferior Frontal Gyrus	Right	40	5.01	20, 22, -22
	Middle Frontal Gyrus	Left	30	3.93	-40, 44, 28

All differences significant at p < .001 uncorrected

Table 13. FC UCH-L1					
Concussion > Control					
Seed	**Region**	**Hemisphere**	**kE**	**t-value**	**Coordinates (xyz)**
Hippocampus Right	Corpus Callosum		37	5.65	10, -34, 20
	Postcentral Gyrus	Right	34	5.50	66, 2, 16
	Superior Frontal Gyrus	Left	68	4.98	-10, 22, 56
	Corpus Callosum		29	4.62	-8, -36, 18
	Lingual Gyrus	Left	25	4.32	-2, -62, 2
	Lingual Gyrus	Left	35	4.28	-28, -90, -20
Hippocampus Left	Middle Frontal Gyrus	Right	123	5.22	24, 28, 40
	Superior Frontal Gyrus	Left	90	4.81	-18, 32, 46
	Cerebellum		45	4.46	-4, -60, -16
	Precuneus	Right	27	4.40	8, -44, 16
	Cerebellum		46	4.36	-14, -60, -40
	Superior Frontal Gyrus	Right	134	4.15	6, 56, 6
	Precuneus	Left	37	3.90	-4, -54, 34
Brainstem	Inferior Frontal Gyrus	Left	20	4.34	-40, 28, -8
Salience RPFC Left	Cerebellum		36	5.59	18, -78, -44
	Superior Frontal Gyrus	Left	45	5.06	-14, 2, 76
Frontoparietal LPFC Left	Superior Temporal Gyrus	Right	28	5.16	48, -6, -8
	Insula	Right	38	4.32	44, 20, -10
	Calcarine Fissure & Surrounding Cortex	Left	27	4.18	0, -94, -4
	Cerebellum		39	3.82	0, -82, -16
Frontoparietal LPFC Right	Cerebellum		184	4.85	42, -80, -30
	Cerebellum		22	4.15	-4, -82, -22
Control > Concussion					
Seed	**Region**	**Hemisphere**	**kE**	**t-value**	**Coordinates (xyz)**
Salience RPFC Left	Lingual Gyrus	Left	22	4.49	-20, -80, -4
	Median Cingulate & Paracingulate Gyri	Left	65	4.21	0, -26, 36
	Inferior Occipital Gyrus	Right	32	4.13	36, -82, -16
	Middle Occipital Gyrus	Right	21	3.84	32, -86, 4
Frontoparietal LPFC Left	Middle Frontal Gyrus	Right	27	4.28	50, 18, 50
	Inferior Temporal Gyrus	Left	38	4.18	-52, -6, -28

All differences significant at p < .001 uncorrected

Table 14. FC Tau					
Concussion > Control					
Seed	**Region**	**Hemisphere**	**kE**	**t-value**	**Coordinates (xyz)**
Hippocampus Right	Superior Frontal Gyrus	Left	149	5.14	-16, 26, 52
	Corpus Callosum		23	4.90	-8, -36, 16
	Superior Frontal Gyrus	Left	235	4.80	-6, 66, 2
	Lingual Gyrus	Left	24	4.39	-2, -62, 4
	Superior Frontal Gyrus	Left	22	3.92	-16, 58, 14
Hippocampus Left	Middle Frontal Gyrus	Right	142	5.65	26, 28, 32
	Superior Frontal Gyrus	Left	186	4.92	-2, 54, 6
	Superior Frontal Gyrus	Left	109	4.78	-18, 36, 46
	Lingual Gyrus	Left	57	4.74	-22, -90, -18
Brainstem	Median Cingulate & Paracingulate Gyri	Right	67	4.52	4, -30, 46
	Superior Frontal Gyrus	Right	28	4.50	22, 44, 38
	Cerebellum		21	4.25	26, -60, -58
	Middle Frontal Gyrus	Right	28	4.07	32, 26, 38
	Inferior Temporal Gyrus	Right	23	4.01	54, -54, -12
Salience RPFC Right	Inferior Frontal Gyrus	Right	26	4.12	36, 18, -22
Frontoparietal LPFC Left	Middle Frontal Gyrus	Left	21	4.44	-30, 32, 52
Frontoparietal LPFC Right	Cerebellum		189	4.94	34, -78, -28
Control > Concussion					
Seed	**Region**	**Hemisphere**	**kE**	**t-value**	**Coordinates (xyz)**
Hippocampus Right	Cerebellum		25	5.03	-24, -30, -38
	Cerebellum		52	5.03	24, -30, -42
	Superior Temporal Gyrus	Right	20	4.63	50, -24, 4
	Cerebellum		21	4.44	-42, -40, -30
Salience RPFC Left	Cerebellum		42	5.46	-46, -64, -50
	Superior Frontal Gyrus	Right	30	5.13	18, -12, 66
	Inferior Temporal Gyrus	Right	22	4.22	68, -26, -28
	Median Cingulate & Paracingulate Gyri	Left	56	4.18	0, -26, 40
	Angular Gyrus	Right	21	3.93	36, -64, 50
	Middle Frontal Gyrus	Right	31	3.91	42, 52, -6

All differences significant at $p < .001$ uncorrected

MEMORANDUM

TO: Joshua Carlson
Department of Psychological Science

Maggy Moore
School of Health and Human Performance

Taylor Susa
Department of Psychological Science

DATE: September 4, 2019

FROM: Lisa Schade Eckert, Ph.D.
Dean of Graduate Education and Research

RE: Modification to HS15-680
Original IRB Approval Date: 8/19/2015
Expiration Date: 9/3/2020
Modification Approval Date: 9/4/2019
"Optical Imaging the Neural Correlates Post- Concussion"

Your modification for the project "Optical Imaging the Neural Correlates Post- Concussion" has been approved by the Northern Michigan University Institutional Review Board. Please include your proposal number (HS15-680) on all research materials and on any correspondence regarding this project.

Any additional personnel changes or revisions to your approved research plan must be approved by the IRB prior to implementation. Unless specified otherwise, all previous requirements included in your original approval notice remain in effect.

If you have any questions, please contact the IRB at hsrr@nmu.edu.