Long-term Effects of Concussions on Cerebral Autoregulatory Mechanisms in Retired Contact Sport Athletes

A Thesis
Submitted to the Faculty of Graduate Studies and Research
In partial Fulfillment of the Requirements
For the Degree of

Master of Science
in
Kinesiology and Health Studies
University of Regina

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Regina, Saskatchewan
June 2020

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Luke William Sirant, candidate for the degree of Master of Science in Kinesiology & Health Studies, has presented a thesis titled, *Long-term Effects of Concussions on Cerebral Autoregulatory Mechanisms in Retired Contact Sport Athletes*, in an oral examination held on May 20, 2020. The following committee members have found the thesis acceptable in form and content, and that the candidate demonstrated satisfactory knowledge of the subject material.

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Abstract

Increase in public awareness of the immediate effects of concussions has fueled researchers to better understand the injury from both a clinical and physiological standpoint in hopes to improve preventative and rehabilitation practices. However, there is very little information regarding the long-term change in the physiological systems in individuals with a history of previous concussions. Therefore, the purpose of this thesis was to explore how multiple concussions effected cerebral haemodynamics in the three major cerebral autoregulatory mechanisms in retired contact sport athletes. Specifically, three studies were conducted to focus on the different cerebral autoregulatory control mechanism. Study one (Chapter 3) focused on the neurovascular coupling mechanism (NVC), study two (Chapter 4) focused on cerebrovascular reactivity (CVR), and study three (Chapter 5) focused on dynamic cerebral autoregulation (dCA). 55 retired contact sport athletes, between the ages of 40-80 years of age (mean=59±8 yr), with a history of previous concussions were recruited with 29 non-contact athletes (mean =64±8 yr), with no history of concussion recruited as a control group. All participants underwent physiological testing using non-invasive near-infrared spectroscopy (NIRS). Each of the three protocols began with 5-minute of seated rest to determine physiological baselines. NVC was assessed using a 5-minute (20-second eyes closed:40-second eyes open x 5 repeats) object identification protocol (“Where’s Waldo”), CVR was assessed with a 5-minute hypercapnic challenge (20-second breath-hold:40-second normal breathing x 5 repeats), and dCA was assessed with a 5-minute squat-stand baroreflex maneuver (10-second squat:10-second stand; 0.05 Hz frequency). Results showed significant differences in all three mechanisms, primarily in the left prefrontal cortex between the
previously concussed group (mTBI) and the healthy controls (CTRL). Similar increases were observed in HbDiff ΔMAX and O₂Hb ΔMAX in the assessment of CVR and dCA but not seen in NVC. These findings provide evidence that there are autonomic nervous system dysregulation occurring in individuals with a history of multiple concussions due to evidence that cerebral autoregulation is impaired in all three autoregulatory mechanisms.

**Keywords:** Concussion, Aging, Cerebrovascular, Cerebral autoregulation, Prefrontal cortex, Cerebral haemodynamics
Acknowledgments

I would like to extend a sincere thank you to my supervisor Dr. Patrick Neary for his guidance, mentorship, and patience throughout the completion of this project. The lessons and experiences I have gained working with you has helped me develop into a better person both professionally and academically. I would like to thank my supervisory committee members, Dr. Darren Candow and Dr. Cameron Mang for their time and guidance for this project. I would also like to thank the external examiner Dr. Barry Willer, for the time and effort he put into reviewing this thesis.

I would like to sincerely thank the team at the University of Victoria that allowed us to use their facilities to collect data on participants: Dr. Catherine Gaul, Dr. Lynneth Stuart-Hill, Marisa Harrington, Kathleen Leahy, Cameron Bowers, Jake Bryan, and a special thank you to Dr. Steve Martin for acquiring funding from the Canadian Academy of Sport and Exercise Medicine (CASEM). Funding from CASEM is greatly acknowledged and appreciated to make this project possible.

I would like to acknowledge members of the exercise physiology lab at the University of Regina, specifically Jyotpal Singh, Ryan Dech, and Taylor Teckchandani for their efforts and contributions to the work conducted in the lab. A sincere thank you to all the participants for their time and dedication in support of this project.

Finally, I would like to thank my family for the support that they provided me to be able to pursue and complete this project.
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Chapter 1: Introduction

Concussions have become one of the most concerning areas in sports (and medicine) in the last few decades. Public awareness of the risks of concussions has increased with research linking concussions to neurodegenerative diseases and prominent athletes being sidelined for long periods of time or retiring due to issues with concussions. From 2002 to 2006, the number of annually reported new emergency room concussions had doubled (Langlois et al., 2006). More recent reports suggest that approximately 20% of adolescents in Canada and the United States had sustained a concussion in their lifetime (Ilie et al., 2013; Veliz et al., 2017). The number of sports related concussion has also increased, with a doubling of emergency visits in the United States for concussions in young athletes (Bakhos et al., 2010), and in Canada sports-related concussions account for over 54% of all pediatric concussions (Kazl & Torres, 2019). However, it is important to note that not all concussions occur in sporting environments, with epidemiological studies estimating that there is an approximately 1.7 million traumatic brain injuries occurring each year, with 1.6 to 3.8 million mild head injuries occurring as well in the United States alone (Daneshvar et al., 2011).

Unfortunately, the diagnosis and severity of concussions are often under-reported due to a lack of athlete and coach education, and pressure from outside sources to continue to participate in sport (Kroshus et al., 2015; McDonald et al., 2016; Meier et al., 2015; Williamson & Goodman, 2006).

The long-term consequences of concussions are starting to be brought to light with autopsies revealing the presence of chronic traumatic encephalopathy (CTE) in former professional football players (McKee et al., 2009; Mez et al., 2017; Omalu et al.,
It is believed that the constant, repetitive subconcussive head trauma that contact sport athletes experience greatly increases the risk for developing CTE, and has been suggested to lead to changes in behaviour, cognition, and mood (Daneshvar, Riley, et al., 2011; Guskiewicz et al., 2005; Montenigro et al., 2016). Further highlighting public awareness, a number of high profile athletes with severe CTE have committed suicide (Mez et al., 2017). Although these are high profile cases, it is important to note that the general rate of suicide is far less in current and former athlete populations than the general population (Baron et al., 2012) and suicide itself is a complex, multifactorial issue (Iverson, 2016). Evidence also suggests that a single moderate or severe traumatic brain injury can also induce pathological changes in the brain (Smith et al., 2019). With new research exploring both the short and long-term dangers of concussions, current medical treatment options have also been evolving. However, many retired athletes whether they be recreational or professional, did not have the same standard of care for concussions that is provided to athletes today (Caron et al., 2013). Athletes in the past might not have been aware of the seriousness of concussions or, in accordance to the culture of many sports, they might have dismissed or minimized symptoms to avoid showing perceived weakness (Caron et al., 2013). The current sport culture is still similar, and this may cause current athletes to hide or dismiss concussion symptoms despite the growing evidence of long-term problems that may occur.

It is unclear to what extent the physiological systems in the body are affected long-term by concussions. Therefore, there is a need to provide a more in-depth look into the changes in the physiological systems of the body while finding a non-invasive, objective way to measure readily available biomarkers. The purpose of this thesis was to
use near infrared spectroscopy (NIRS) to objectively measure the changes in cerebral haemodynamics of the three main cerebral autoregulatory mechanisms: neurovascular coupling, cerebrovascular reactivity, and dynamic cerebral autoregulation in subjects who have a history of multiple concussions during their lifetime and subjects who have not experienced concussions. Based on previous literature suggesting that concussions cause dysfunctions in the autonomic nervous system, it was hypothesised that there will be similar dysfunctions observed in retired contact sport athletes as seen in impairments to the three autoregulatory systems explored in this thesis.
Chapter 2: Review of the Literature

Concussion Overview

Concussion is classified under the general category of traumatic brain injuries which is defined as an external force causing an injury to the brain (Thurman et al., 1999). Traumatic brain injuries often involve obvious signs of trauma such as cerebral haemorrhaging and skull fractures that can be observed visually or through diagnostic imaging techniques, such as magnetic resonance imaging (MRI) and computer tomography (CT) scans. These injuries are typically caused by external forces that directly damage neurons, axons, and blood vessels in the area (McKee & Daneshvar, 2015). Mild traumatic brain injuries (mTBI) are the result of linear or rotational acceleration and deceleration forces causing the brain to elongate and deform, i.e., coup and contrecoup injury, stretching neurons (McKee & Daneshvar, 2015).

Defining the difference between mTBI and concussions is often difficult as both terms seem to be used interchangeably in clinical settings. However, there are some differences reported in the literature. While both terms are similar, mTBI is based on the Glasgow Coma Scale (GCS) that is used in TBI patients to measure their level of consciousness (Teasdale & Jennett, 1974) with mTBI being diagnosed with a GCS score of 13-15 and experiencing any loss of consciousness, any memory loss, altered mental state, and focal neurologic deficit (Sussman et al., 2018). Previous definitions for concussions have been vague with the most agreed upon definition coming from the 2017 Concussion in Sports Group proposal that define concussions as: “... a traumatic brain injury induced by biomechanical forces that can be the result of a blow to the head, neck, face or anywhere else on the body. Concussions are often seen with short-
lived neurological impairments that resolves spontaneously over time and acute
functional disturbances in the brain but no structural damage. There may or may not be
a loss of consciousness” (McCrory et al., 2017). While literature does not provide a
consistent definition for both mTBI and concussions, they are both similar in that their
definitions include neurologic dysfunctions following a head injury, with mTBI being
described as an acute diagnosis and concussions referring to the clinical signs and
symptoms following mTBI diagnosis (Sussman et al., 2018).

The Post-Concussion Symptom Scale (PCSS) is a list of common concussion
symptoms and was developed to provide medical professionals with a method to
measure recovery in patients suffering from a concussion (Chen et al., 2007). Patients
rank their symptoms on a Likert-like scale of 0 through 6, with scores of 0 representing
the absences of a particular symptom, and a score of 6, indicating that the specific
symptom is severe (King et al., 1995). Although PCSS reliability has come under
scrutiny as a way to measure recovery, it is still useful as an information gathering tool
to help determine which symptoms an individual might be experiencing with their
concussion (Lovell et al., 2006). Research has suggested that the PCSS is more valid if
used in conjunction with other testing protocols, such as ocular-motor assessment,
balance testing, and computer based concussion assessments (Alsalaheen et al., 2016).
The PCSS groups symptoms into two categories, with the first encompassing
physiological symptoms (headaches, dizziness, nausea, light or sound sensitivity), and
the second including psychological symptoms (anxiety, depression, changes in mood,
and irritability) (King et al., 1995). Symptoms and symptom severity can be different
with each consecutive concussion making it difficult to find a treatment strategy that can
work universally for all individuals diagnosed with concussions (Ellis et al., 2015). The recovery time can also vary for each subsequent concussion, and although it has been theorized that patients with a higher number of previous concussion experience longer recovery times, in many cases this has not been supported in the literature with recovery time being based on the individual (Leddy et al., 2016).

In contrast to traumatic brain injuries, concussions do not involve structural damage such as skull fractures or cerebral haemorrhaging, as all of the symptoms that are common in concussions only cause functional abnormalities in the patient (McCrory et al., 2017; Thurman et al., 1999). Additionally, concussions are not readily detectible with common imaging techniques such as CT scans and MRIs (Giza & Hovda, 2014). However, concussions will often accompany other injuries in instances of automotive accidents or high impact sporting plays. The disrupted processes which occur during a concussion usually resolve within days to weeks after the injury is sustained (Leddy et al., 2016). However, in a certain percentage of individuals the biological processes in the brain do not return to normal in that time frame, and the patient will still experience symptoms for months and even years following their injury (Barlow, 2016; Ryan & Warden, 2003). These individuals are categorized as having Post-Concussion Syndrome (PCS) with both psychological and physiological factors influencing the persistent symptoms for longer than three months (Boake et al., 2005; Broshek et al., 2015; Ellis et al., 2016; Ryan & Warden, 2003).

**Immediate physiological effects following a concussion.**

The potential short-term effects of concussions have been established, with reports of alterations in proper cellular function (Giza & Hovda, 2014) and disruptions to
physiological systems in the body, such as cerebral autoregulation (Bishop et al., 2017; Wright et al., 2018) and cardiac function (Bishop et al., 2018; Len & Neary, 2010; Neary et al., 2020). Immediately following a concussion, the brain is in a state of metabolic flux and an increased demand for fuel for the recover and repair process. Because of the importance of the brain as an organ, it is subject to extensive regulation and consists of various control centres that help regulate the rest of the body, and in concussions many of these control centres become disrupted. Dynamic cerebral autoregulation, cerebrovascular reactivity, and neurovascular coupling are all physiological mechanisms that are altered following concussion. These mechanisms in turn can alter cerebral blood flow, cerebral oxygenation, and baroreflex sensitivity. There are also alterations in general cell structure and function which can be used to explain the causes of dysfunctions that are observed during a concussion (Kurowski et al., 2017; Len & Neary, 2010).

**Cerebrovascular physiological dysfunctions in concussions**

In a concussed state, the brain experiences higher neural activity and experiences an increase in oxygen demand which is believed to be caused due to the recovery processes occurring in the brain as repairs to neural pathways are conducted (Len & Neary, 2010; Maxwell et al., 1997). Despite comprising only 2% of the total body weight of a human, the brain has a high energy demand and accounts for 20% of an individual’s total energy expenditure (Camandola & Mattson, 2017). Specifically in a healthy brain, there is a large demand for metabolic glucose as the brain has been shown to utilizes up to 25% of the body’s glucose stores to maintain proper brain function (Hyder & Rothman, 2017), and during a concussion the demand for glucose to provide energy
increases. However, it is interesting to note that while there is an increased demand for the delivery of oxygen and nutrients, younger individuals (<30 years) with a concussion have been shown to have decreases in their cerebral blood flow measures resulting in a reduction in the rate of oxygen being transported to the injured areas (Grindel, 2003; Len & Neary, 2010; Smirl et al., 2015; Wright et al., 2018). Cerebral blood flow is controlled through changes in cerebral perfusion pressure and cerebrovascular resistance (Golding et al., 1999). Cerebral perfusion pressure is the difference between arterial and venous pressure in the brain and combined with cerebrovascular resistance, both can be considered a function of cerebral blood flow (Ainslie & Duffin, 2009; Len & Neary, 2010; Willie et al., 2011). Controlling cerebral blood flow is a critical component of the regulatory system of the brain and changes to cerebral blood flow can influence static and dynamic cerebral autoregulation, cerebrovascular reactivity, and neurovascular coupling mechanisms (Ainslie & Duffin, 2009; Bishop et al., 2017; Bishop et al., 2018; Len & Neary, 2010; Willie et al., 2011).

The inability of concussed subjects to match cerebral blood flow and oxygen delivery to the increased oxygen demands in the brain indicates that there is dysfunction in the brain’s autoregulation ability. Cerebral autoregulation is the intrinsic ability of the brain to maintain constant cerebral blood flow in response to changing systemic blood pressures and stresses (Aaslid et al., 1989; Jünger et al., 1997; Strebel et al., 1997). Researchers have suggested that being unable to adapt to blood pressure changes may be one of the reasons for the onset and exacerbation of symptoms (Bishop et al., 2017; Kurowski et al., 2017; Len & Neary, 2010; Wright et al., 2018). Due to underlying cellular damage, the brain attempts to protect itself from changes in blood pressure even
with the increased demand for metabolites transported by blood (Len & Neary, 2010). If the brain is unable to control blood pressure in the brain, rapid changes to blood pressure results in changes to the normally stable cerebral perfusion pressure (Leddy et al., 2016). These changes are often associated with the headaches and migraines, which are common concussion symptoms (Leddy et al., 2016). This may explain why cerebral blood flow is reduced during the acute phase of a concussion as these rapid changes in perfusion pressure can be damaging.

Cerebrovascular reactivity is the brain’s ability to maintain a steady supply of oxygenated blood and control the partial pressure of carbon dioxide (PCO\(_2\)) to maintain cellular homeostasis. CO\(_2\) is a major modulator of cerebral blood flow with cerebral blood vessels dilating with increased levels of arterial CO\(_2\), and vessels constricting with decreased levels of arterial CO\(_2\) (Gardner et al., 2015). As the partial pressure of CO\(_2\) increases, cerebral blood flow will also increase to wash out any excess CO\(_2\) as it is being produced by brain cells (Ainslie & Duffin, 2009). If cerebral blood flow is decreased, the partial pressure of CO\(_2\) will steadily increase resulting in the body being unable to regulate central pH levels (Ainslie & Duffin, 2009). Research suggests that concussed individuals have an impaired cerebrovascular reactivity response and are unable to adequately reach the cerebral blood flow requirements that are needed to wash out the excess CO\(_2\) from brain tissue (Churchill et al., 2018; Len & Neary, 2010; Len et al., 2011, 2013; Mutch et al., 2014).

Reduction in cerebral blood flow also effects the brain’s neurovascular coupling mechanism that responds to instances of increased neural activity and metabolic demand by increasing cerebral blood flow to match these demands. Since cerebral blood flow is
decreased in individuals with concussions, the brain is unable to meet the elevated metabolic demands that are observed and this may contribute to the difficulty of concentrating on cognitive tasks, which is a commonly observed in concussion patients (Smirl et al., 2016). This uncoupling has been reported in animal studies, with trauma induced mice showing increases in glucose metabolism but a decrease in cerebral blood flow to those areas (Richards et al., 2001). Repetitive subconcussive head trauma is also feared to cause alteration in neurovascular coupling over time and these repetitive blows are often seen in contact sports such as ice-hockey, rugby and American football. However, some research suggests that subconcussive blows did not affect neurovascular coupling after a single sporting season for the tested, younger athletes (Wright et al., 2018). The mechanism in which neurovascular coupling occurs is unclear. Currently it is thought to be an interplay between ion content shifts, energy substrate change, interneurons, and astrocytes in brain tissue that contribute to pial artery resistance (Tan et al., 2014). More clinical studies are required to fully understand this relationship and view the changes that are occurring during a concussion.

The above mechanisms are sensitive to trauma (Jünger et al., 1997; Strebel et al., 1997), and when disrupted, there are changes to the cellular energetics and metabolism in the brain. These changes result in measures of cerebral oxygenation to also be disrupted during a concussion. For example, by using near infrared spectroscopy (NIRS) it is possible to measure different variables associated with the saturation of haemoglobin in the target tissues. NIRS is a non-invasive optical device that can monitor the relative changes in the haemodynamic properties. These variables include: a general tissue saturation index (TSI%; \( \frac{O_2\text{Hb}}{(O_2\text{Hb} + HHb)} \times 100 \)), oxygenated haemoglobin (\( O_2\text{Hb} \)),
deoxygenated haemoglobin (HHb), total haemoglobin (tHb; \(O_2\)Hb + HHb), and haemoglobin difference (HbDiff; \(O_2\)Hb - HHb). tHb has been suggested to be proportional to cerebral blood volume while HbDiff reflects an increase in arteriolar vasodilation and subsequent increases in local cerebral blood flow and cerebral blood volume, due to the activity of the neurovascular coupling mechanism (Ferrari & Quaresima, 2012). Haemoglobin saturation levels have been suggested to decrease immediately following a concussion and not resolve for up to seven days (Len & Neary, 2010). There is also a decrease in the variability of the cerebral oxygenation responses following acute concussion as assessed by standard deviation of the NIRS signal (Bishop & Neary, 2018). This continues to demonstrate that there is a mismatch of oxygen supply by the observed decreases in the levels of oxygen tissue saturation and cerebral blood flow suggesting that there is impairment to the autonomic functioning of the brain.

**Cardiovascular physiological dysfunctions in concussions**

The cerebral and cardiovascular systems are intrinsically linked due to the major role that the autonomic nervous system plays in controlling cardiovascular responses and although out of the scope of this thesis, it is important to mention these changes. There is evidence to suggest that the cardiovascular system, and the heart in particular, can become uncoupled from the autonomic nervous system due to the dysfunctions that occur with a concussion (Neary et al., 2020). This uncoupling may be part of the reason for the abnormal cerebrovascular responses observed in concussed athletes (Gall et al., 2004; Goldstein et al., 1998; Len & Neary, 2010). Heart rate variability (HRV) has been used as a biomarker for autonomous modulation on the cardiovascular system (Billman, Huikuri, Sacha, & Trimmel, 2015; Bishop et al., 2018), and has been studied in
individuals with traumatic brain injuries. HRV shows promise for use in concussion cases as both a potential rehabilitation intervention and marker for recovery (Conder & Conder, 2014; Papaioannou et al., 2008; Su et al., 2005) but is out of the scope of this thesis.

**Neurometabolic dysfunction in concussions.**

Immediately following a severe enough impact to result in a concussion, the biomechanical forces initiate changes at the cellular level influencing the neurons and neural connectivity within the brain (Dashnaw et al., 2012; Frattalone & Ling, 2013). There are consistent alterations that have been observed in both human and animal models falling under three general categories: alterations in membrane conductivity, alterations in neuron axon structure, changes in intracellular ion concentration, and alterations in glucose metabolism (Banks & Domínguez, 2019; Giza & Hovda, 2014; Maxwell et al., 1997; Petraglia et al., 2011). This is often referred to as the neurometabolic cascade (Giza & Hovda, 2014).

The forces experienced result in sublethal defects in the lipid membranes of neurons which results in ionic shifts in the membrane potential (Giza & Hovda, 2014; Petraglia et al., 2011). An influx of sodium ions into the cell is accompanied with an efflux of potassium out of the cell leading to depolarization of the cellular membrane causing a disruption of cellular homeostasis (Banks & Domínguez, 2019). Further leading to compromised cell stability, the neurotransmitter glutamate is released into the post-synaptic cleft, and when combined with the efflux of potassium out of the cell, causes increased activity of voltage regulated calcium channels leading to increased calcium uptake by neurons (Banks & Domínguez, 2019; Giza & Hovda, 2014). Calcium
acts as both a secondary messenger and as a regulator for neurotransmitter release in neurons, and glutamate is used in the brain as an excitatory neurotransmitter to transfer signals between neurons. Once an action potential reaches the end of presynaptic neuron, calcium is released into the synaptic cleft between the neurons, and the amount of calcium that is released determines how strong the neurotransmitter response will be (Simons, 1988). Excess calcium can be toxic to cellular structures resulting in damage to the microtubules and microfilaments that are critical for proper cellular function (Banks & Domínguez, 2019). Short-term relief from the calcium influx can occur through increased mitochondria uptake of excess calcium. However, this can lead to long-term issues such as mitochondrial dysfunction, increased levels of reactive oxygen species, and ultimately the inhibition of energy production in the neurons, which are already compromised in a concussed state (Banks & Domínguez, 2019; Giza & Hovda, 2014). The ionic shifts occurring have been suggested to be one of the underlying cellular causes of headaches and migraines experienced during a concussion (Giza & Hovda, 2014). These changes have also been related to symptoms of slowed cognition and impaired reaction time (Giza & Hovda, 2014).

The ionic shifts in the cell also cause alterations in the surrounding neurofilament and microtubular cytoskeleton and in turn these changes compromise proper cellular function (Giza & Hovda, 2014). Axonal transport is one of the areas that is effected (Petraglia et al., 2011). In healthy individuals, axonal transport is how neurons are able to transport organelles, proteins, lipids, and nutrients to and from the cell body through the axon (Petraglia et al., 2011). A series of neurofilaments and microtubules provide a structural framework outside of the neuron to support the neurons structurally and allow
the transportation of important nutrients. Disruption to this system can have a cascade effect that can lead to inadequate adenosine triphosphate (ATP) production due to mitochondrial damage, activation of inflammation cascades, and the generation of harmful reactive oxygen species (Giza & Hovda, 2014; Petraglia et al., 2011). All of these changes have been suggested to contribute to the onset and exacerbation of cognitive concussion symptoms such as slower cognition and slower reaction time (Giza & Hovda, 2014; Petraglia et al., 2011).

As mentioned above, cellular homeostasis is compromised in neurons due to the shifting ionic environment and influx of calcium into the cell. In an attempt to restore ionic and cellular homeostasis, cell membrane sodium potassium pumps increase their workload resulting in an increase in demand for cellular ATP, leading to an increased rate of glycolysis to make up for this demand (Giza & Hovda, 2014; Kim et al., 2018). The higher demand for substrates that can provide ATP results in the depletion of intercellular energy stores and when combined with the physiological decrease in cerebral blood flow leads to the mismatch between energy supply and demand as the blood is not able to provide the necessary nutrients (Giza & Hovda, 2014). The energy mismatch has been theorised to play a role in the vulnerability of the brain to repeated injuries (Giza & Hovda, 2014), especially if a second insult occurs before complete resolution of the initial injury. This vulnerability is thought not only to exist immediately following a concussion but is believed to be one of the reasons why individuals who have experienced previous concussions might be more susceptible to additional concussions in the future (Giza & Hovda, 2014). However, the research is split on this topic, and recent studies have suggested that previous research did not take into account
the different inherent lifestyle risks when comparing subjects who have had concussions to those who have not (Hamilton et al., 2011). In a study examining the risk of concussions in Cirque du Soleil, they found that the acrobats were more likely to have multiple concussions due to the increase in risk of collisions, but the risk of experiencing a second concussion was no greater than the risk of a first concussion (Shrier et al., 2019). The study also found that there was no increased risk of subsequent concussions if the proper concussion guidelines were followed, and the athletes had completely recovered from the previous concussion (Shrier et al., 2019). More research is required to determine whether it is actually the cellular vulnerability that does increase the risk of subsequent concussions, or if the perceived increase is in fact due to improper analysis of the inherent risk of sports and failure to follow proper recovery guidelines.

Inflammatory markers have been suggested as an avenue to explore the prognosis aspect of concussion management, however, most of the current research has focused on animal concussion models and on TBI (Giza & Hovda, 2014). One example of research that does focus on humans explores how serum levels of interleukin (IL)-6 and interleukin-1 receptor antagonist (IL-1RA) show potential to be used as markers for concussion diagnosis and as measures for athletes at risk for prolonged recovery from concussions (Nitta et al., 2019). In studies looking at mice models, it has been suggested that there is an increase in damaging oxidative species (Giza & Hovda, 2014), increase in the activation of microglia and astrocytes in brain tissue (Shultz et al., 2011), and increased levels of circulating pro-inflammatory cytokines, which are released in response to inflammation (Collins-Praino et al., 2018). Increases in inflammatory markers have been linked to symptoms of cognitive impairment, depression, and fatigue,
all common concussion symptoms (Felger & Lotrich, 2013; Harrington, 2012). Increased inflammation is suggested to disrupt the blood brain barrier resulting in autoantibodies gaining access to the central nervous system where they disrupt microglia, astrocytes, and neurons (Kim et al., 2018). However, conclusive evidence in human models has not been found and new techniques are required to properly assess the integrity of the blood brain barrier as currently only peripheral markers of blood brain barrier function have been reported to have been used (Kim et al., 2018).

It is also important to note that there has been little observed cell death or neuronal atrophy in studies looking into concussions. The majority of research in this area has utilized animal models, leading to some evidence suggesting that there is an inflammatory response in concussions and changes in ion concentrations can potentially trigger apoptosis in neurons which is also seen in traumatic brain injuries (Giza & Hovda, 2014; Raghupathi et al., 2002). While this is not considered to be a major concern in single concussion diagnosis, the worry is that chronic structural damage may occur after repeated concussions as functional impairments in animal models appear to be more severe after multiple subsequent concussions (Longhi et al., 2005; Petraglia et al., 2011; Prins et al., 2013). More human research is required to determine the actual effects that multiple concussions might have on cerebral structures.

**Long-term effects**

Although the short-term effects of concussions have been and continue to be studied, researchers have now begun to explore the long-term effects that single and multiple concussions can have on individuals as they age and progress through life. In individuals who have been medically cleared from single or multiple concussions, the
question arises whether there will still be lingering long-term effects from the result of the acute repeated trauma. Some studies examining the long-term effects of concussions have found that there are observable changes to the structure of the brain, with certain areas in the brain decreasing in volume, and changes in cognition and behaviour that is seen over time (Strain et al., 2015). Mental health issues, changes in cognition, and behavioral changes have been reported to arise later in life in many athletes who have experienced single and multiple concussions throughout their playing career. The overall research however is inconclusive, with other studies showing no differences due to concussions, or the influence that other factors have on cognitive and behavioural changes observed later in life (Esopenko et al., 2017; McMillan et al., 2017; Tarazi et al., 2018; Willer et al., 2018). There is also worry that the trauma experienced with a concussion can cause an increased risk of neurodegenerative diseases later in life.

Neuroimaging techniques have been used to observe structural changes in the brain of subjects with a history of concussions. Magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), and diffusion tensor imaging (DTI) are three popular techniques that have been used to observe any structural changes that may be present. Changes in behaviour, cognition, and brain structure, have been reported in previous research using subjects with a history of previous concussions but again findings are mixed and whether these changes are solely due to concussions is debated.

There is minimal research measuring the long-term changes in the physiological systems in the body that may occur due to past concussions. However, there are two studies that are important to note. The first study done by Sharma et al (2020) used fNIRS to examine the effects of multiple concussions on retired rugby players and
showed that haemoglobin indices (HbO₂, HHb, tHb) in the prefrontal cortex were different when compared to an aged-match control group without a history of concussion. The second study done by Lewis et al (2017) assessed corticomotor excitability and inhibition in retired rugby players with a history of concussions and found that there were higher degrees of cortical inhibition in the rugby players.

**Reported alterations in behaviour and cognition**

Numerous studies have explored how past concussion can affect behaviour and cognition. The majority of research has used clinical interviews, collection of demographic information, PCSS, and various questionnaires and cognitive tests (computerized and paper) to determine changes in cognition and behaviour (Guskiewicz et al., 2007; Kerr et al., 2014; Montenigro et al., 2016; Randolph et al., 2013; Stamm et al., 2015).

Behavioural changes are reported in previous concussed individuals later in life and depression has been reported in athletes with multiple previous concussions (Guskiewicz et al., 2005). Multiple studies have reported that former athletes with three or more concussion were also reported to be three times more likely to develop depression, with former high school and college football players reporting increased rates of depression and feelings of apathy affecting their behaviour and quality of life (Guskiewicz et al., 2005; Montenigro et al., 2016). Interviews with former professional ice hockey players who retired due to concussion symptoms, also suggested similar findings. Many retired athletes reported to occasionally still experience concussion symptoms up to fourteen years after retirement in addition to feeling more stressed, confused, and depressed (Caron et al., 2013). Although the findings of these studies do raise concern, it is important to note that when compared to the general population,
athletes have been reported to have lower levels of depression (Armstrong & Oomen-Early, 2009; Proctor & Boan-Lenzo, 2010). Whether a history of concussions is the direct link to increased levels of depression is difficult to determine as numerous factors such as, interpersonal conflict (Foster, 2011), financial difficulties (Richardson et al., 2013), substance abuse (Nock et al., 2010), poor general health (Conwell et al., 2010), and chronic pain (Hassett et al., 2014; Hooley et al., 2014; Tang & Crane, 2006) all have stronger links to depression than concussions. Therefore, more research is needed before any concrete link can be drawn between concussions early in life and behavioural changes later in life.

Questions also arise as to whether a history of concussions can influence cognitive changes in individuals. In studies of male athletes aged 17-22 with a history of three or more concussions there are reported significant decreases in verbal memory scores (Iverson et al., 2012). Although these athletes are still relatively young, it is suggested that the effects on verbal memory can continue as the athletes age (Iverson et al., 2012). In additional studies, self-reported measures of memory impairments, executive function, and prevalence of mild cognitive impairment diagnoses later in life were worse in retirees with multiple concussions than those with no history of concussion (Guskiewicz et al., 2005; Montenigro et al., 2016). Other research reports long-term deficits in the subjects executive functioning ability and information processing speed suggesting that these subjects have significant impairments to their attention and concentration abilities (Collins et al., 1999; Moser et al., 2005). However, not all researchers share similar findings and conclusions with the previously mentioned studies. Some researchers found no significant differences in self-reported cognitive
impairments and objective neuropsychological testing in former National Hockey League (NHL) and National Football League (NFL) compared to non-contact sport athletes (Willer et al., 2018) and no differences in mental health, social, or work function in former international rugby players when compared to age-matched controls (McMillan et al., 2017). Other studies found no differences on memory and executive function tests in former Canadian Football League players when compared to controls (Tarazi et al., 2018) and no differences in attention speed, verbal memory, visuospatial function, and response speed in former NHL players when compared to comparable age-match controls (Esopenko et al., 2017). The conflicting studies suggest that the cognitive impairments that are occurring later may not be entirely due to concussions and there could be other lifestyle and physiological factors that have a more prominent role in driving these impairments later in life. However, the impact that concussions can have on later life cognition and behaviour should not be dismissed and further investigation is required.

Additionally, research on various contact sports has explored the extent in which these changes occur. In one study looking at former international rugby players, many of the cognitive test did not show any difference between the control group and the rugby players. However, there was a significant decrease in verbal learning and dominant hand coordination tests in the rugby players (McMillan et al., 2017). Another study exploring youth rugby players during their high school years found that when compared to non-concussed controls rugby players had impaired performance in recall tests over the course of three years (Alexander et al., 2015). This study also found evidence to suggest that academically, the rugby players did not see improved scores over time like the
control group (Alexander et al., 2015). Although this study did not follow the youth rugby players past high school it provides insight into the changes to cognition that can already occur over a minimum amount of time. Additional studies found that retired rugby players with a history of concussion had increased levels of cortical inhibition (Lewis et al., 2017) and performed worse on tests of complex attention, executive functioning, and cognitive flexibility (Hume et al., 2017). Other research into retired rugby players investigated the self-report number of concussions sustained over their playing careers. Although the retired players who reported more concussions had no differences in neurocognitive tests when compared to less concussed groups, the concussed group with more concussions did report more symptoms of PCS and increased self-reported impairments of memory and cognition (Thornton et al., 2008).

Similar impairments are reported in boxers with up to 20% of professional boxers suffering from cognitive and behavioural changes (Förstl et al., 2010). Professional boxers with longer careers reported experiencing changes in motor skills (ataxia, Parkinson’s disease, and dysarthria), cognitive skills (cognitive slowing, memory disorders, and dementia) and behavioural changes (depression, irritability, aggression, and addiction) (McKee et al., 2009; Mendez, 1995; Rabadi & Jordan, 2001). Cerebral haemodynamic function is also suggested to be impaired in professional boxers, with boxers having impaired neurocognitive function (measured through psychometric tests), and lower dynamic cerebral autoregulation (determined through cerebral blood flow and mean arterial pressure) when compared to age and fitness matched controls (Bailey et al., 2013). Amateur boxers, however, did not show any significant neurological dysfunction.
with only decreases in fine motor control being reported in amateur boxers with longer careers (Butler, 1994; Di Virgilio et al., 2019).

European football or soccer players are also at risk for concussion due to the multiple subconcussive blows that players may experience during play. Heading the ball is a major concern for concussions, and studies suggest that the majority of concussions are due to the result of player collision (Barnes et al., 1998; Boden et al., 1998). Although heading may not be the predominant factor that leads to concussion, the long-term exposure to the subconcussive blows associated with heading has been reported to cause impairments. In professional Dutch soccer players there were impairments in memory, planning, and perception in players who recorded more headers over the course of a season (Matser et al., 1998). Additionally, amateur soccer players reported reduction in attention and memory scores when compared to controls (Matser et al., 1999). In studies utilizing cognitive test batteries, researchers reported controls had better scores of conceptual thinking, reaction time, concentration, and working memory (Downs & Abwender, 2002; Rutherford et al., 2009; Straume-Naesheim et al., 2009).

Finally, information regarding long-term changes in ice hockey players is sparse. The majority of research has focused on acute effects that concussions have on ice hockey players, although more reports are emerging that highlight the effect that multiple concussions can have on ice hockey players. These reports highlight the difficult decision that players have regarding the option to either return-to-play or retire from the sport (Cantu & Register-Mihalik, 2011). Five former National Hockey League players were interviewed and reported experience physical symptoms of headaches and visual impairments on top of increased feelings of depression, confusion, and anxiety.
(Caron et al., 2013). Other studies suggest that there are no differences in former professional hockey players during cognitive tests (Esopenko et al., 2017) and measures of mental health (Willer et al., 2018) when compared to control subjects.

**Concussions and the increased risk of neurodegenerative diseases**

There is also the worry that multiple concussions can lead to an increased risk of neurodegenerative diseases later in life by accelerating the progression of Alzheimer’s disease, Parkinson’s disease, and CTE (Daneshvar, Riley, et al., 2011). Autopsies of patients with CTE show presence of neurofibrillary fibers that are commonly seen in Alzheimer’s patients (Jellinger et al., 2001; Omalu et al., 2010). However, there is no consensus on whether concussions and head trauma can accelerate Alzheimer’s disease, with some research suggesting that symptoms that appear to be Alzheimer’s may be due to interference from other disease, namely CTE and dementia (Gavett et al., 2010).

It has been suggested that the behavioural changes due to concussions, namely depression, can precede the development of Alzheimer’s disease in a small number of cases (Guskiewicz et al., 2005), with reports linking to central nervous system trauma to Alzheimer’s and Parkinson’s disease (Gavett et al., 2010). Current research on the relationship is sparse but it is believed that the trauma towards the central nervous system results in an increase in the precursor compounds of the Lewy bodies that are seen in Parkinson patients (Daneshvar, Riley, et al., 2011; Gavett et al., 2010; Goldman et al., 2006).

Multiple concussions and repeated subconcussive blows have been suggested to be the primary risk factor for developing CTE. There are some controversies, however, as questions arise as to the number of concussions that lead to CTE, the question about the similar pathologies between CTE and other neurodegenerative diseases, the unknown
course of onset and progression of symptoms, and the interpretation of the cognitive changes that are associated with CTE (Asken et al., 2017; Smith et al., 2019). There are reported cases of CTE in American football, professional wrestlers, professional ice hockey players, soccer players, and boxers (Gavett et al., 2010). CTE is characterised by extensive tau neurofibrillary fibres and astrocyte tangles throughout the frontal and temporal cortices (Gavett et al., 2010) which is also commonly seen in other neurodegenerative diseases. The disease process is thought to start at the time of injury, however, symptoms of CTE do not tend to arise until later in life (Daneshvar, Riley, et al., 2011), although some athletes have displayed CTE as young as 21 years of age (McKee et al., 2009). CTE is characterized by decline of memory, decline in executive function, mood and behavioural changes, and eventually dementia (McKee et al., 2009; Mez et al., 2017; Montenigro et al., 2016; Randolph et al., 2013; Stamm et al., 2015).

There is evidence to suggest that there is a link between concussions and posttraumatic stress disorder (PTSD). The majority of evidence has explored how more severe TBI and PTSD are connected. It is theorized that there are several different mechanisms that can lead to the formation of PTSD in TBI and concussion patients. These mechanisms include: fear conditioning response, how our brains reconstruct our memory of the event, and distressing memories after the event (Bryant, 2011). While it has been argued that PTSD may not develop in sever TBI patients as they would be unable to form the traumatic memories due to loss of consciousness (Sbordone & Liter, 1995), more recent evidence suggests that PTSD can occur following a concussion (Gaylord et al., 2008; Hoge et al., 2008) and severe TBI (Bryant et al., 2000; McMillan, 1996). Researchers suggest that civilians with concussions were more likely to develop
PTSD (Richard A. Bryant et al., 2010; Fann et al., 2004), while 44% of military personal with a concussion, also screened positive for PTSD (Hoge et al., 2008; Jaffee & Meyer, 2009).

An issue arises with PCS as many of the symptoms related to PTSD and PCS are similar (Bryant, 2011). However, research has suggested that when combined concussions and PTSD can contribute to impairments in neuroanatomical structure and function beyond that of having only one condition. The presence of both a concussion and PTSD showed impairments in white mater integrity of the right cingulum bundle and impaired the encoding and retrieving information during processing speed tasks when compared to only the concussion group (Lopez et al., 2016). Therefore, it is important to provide the necessary treatment resources for both conditions as the both conditions can influence the other. One strategy in the literature is to reduce the arousal symptoms of PTSD and with this, many PCS symptoms can then be alleviated (Ponsford et al., 2002). Additionally, by reducing the negative appraisal of PCS symptoms, the patient can reduce the PTSD response in these situations (Bryant, 2011).

**Long-term structural and neurometabolic changes in the brain**

Various imaging techniques have been used to determine the extent of long-term structural changes that occur within individuals who have a concussion history. MRI, MRS, DTI, and NIRS have all been used in studies examining the changes in groups of PCS patients, previously concussed subjects, and subjects with no prior history. MRI, MRS, and DTI studies have focused on retired professional and retired university level American football players, boxers, soccer players, and ice hockey players.

MRI studies reported that in retired professional American football players there was an increase in the rate of cavum septum pellucidum (CSP) when compared to
healthy non-contact athlete controls (Koert, Hufschmidt, et al., 2015). The septum pellucidum is a thin membrane that separates the anterior horns of the left and right lateral ventricles of the brain. The presence of CSP indicates that there is a space between the septum pellucidum and this has been linked to various mental disorders while also seen in traumatic brain injuries (Zhang et al., 2003). Greater degrees of CSP has also been linked to decreased performance on memory tests and word pronunciation tests (Koert, Hufschmidt, et al., 2015). Other studies do suggest that in some former NFL players there was evidence of CSP and microbleeds in brain parenchyma. Although only a minority of subjects displayed these pathological characteristics, the majority of subjects tested did not show any evidence of chronic brain damage (Casson et al., 2014).

Additional studies have reported a significant cortical thinning of the anterior temporal lobe and orbital frontal cortex when comparing retired American football players and controls (Goswami et al., 2016). The normal functions of these regions play a role in semantic memory and decision-making, respectively (Bonner & Price, 2013; Rolls, 2004). Cortical thinning was also observed in retired professional soccer players in the temporal and occipital cortices and has been associated with lower cognitive performance (Koert et al., 2016). Older retired athletes with a history of at least one concussion in which they lost consciousness, were reported to have significantly smaller bilateral hippocampal volumes and decreased memory performance even when normal aging process were taken into account (Strain et al., 2015).

MRS differs from MRI as it can determine the composition of specific chemicals in brain tissue. Proton MRS (H MRS) has shown differences in neurochemical changes between aging, TBI, and mild cognitive impairments (Tremblay et al., 2013). Former
university level athletes between the ages of 51 and 75 with a history of concussions were shown to have abnormal enlargement of the lateral ventricles, cortical thinning in the frontal, temporal, and parietal lobes, and abnormal elevations in the neural metabolite myo-inositol (Tremblay et al., 2013). The alterations in brain structure correlated with decreases in episodic memory and verbal fluency (Tremblay et al., 2013).

Enlargement of lateral ventricles is consistent with findings in former athletes with verified CTE (McKee et al., 2009; Omalu et al., 2011) and it has been suggested to reflect white matter loss in the brain due to axonal injuries (Bendlin et al., 2008; Bigler, 2001). The abnormal elevations in myo-inositol was also observed in former soccer players with concussion history (Koerte, et al., 2015), and has been suggested to be a marker for increased glial activation (Manley et al., 2017). Increased glial activation corresponds to the initiation of neural repair processes (Lee & MacLean, 2015; Watkins et al., 2001) suggesting that neural damage has occurred in these individuals. Additional research studying former university level ice hockey and football players found that the metabolite glutamate is disproportionately reduced in individuals with a history of concussion as they age, and this decrease in glutamate was suggested to be correlated with the difficulties in motor learning (De Beaumont et al., 2013).

DTI was used to assess lifetime concussion history in amateur soccer players and boxers. DTI is capable of measuring fractional anisotropy which is a measure of the connectivity of different regions of the brain with lower connectivity being associated with poorer cognitive performance (Grieve et al., 2007). A study looking into the lifetime concussion history of amateur soccer players found that although there were no general changes of fractional anisotropy or overall cognitive performance, the players
with a high frequency of heading the ball had lower fractional anisotropy in temporoo-occipital white matter and this was associated with poorer memory scores (Lipton et al., 2013). Similar observations were seen in a study looking at amateur boxers, and while there was no difference in fractional anisotropy, both declarative memory and reaction time were reduced (Wilde et al., 2015).

Previous research has used NIRS to measure long-term functional changes in individuals with previous concussions. In adults with PCS there was observed to be changes in regional brain communications due to changes in cerebral oxygenation and functional connectivity (Hocke et al., 2018). This has been supported by previous research that suggested that in paediatric populations there was observed changes in functional coherence between right and left motor cortices (Urban et al., 2014). These changes were used as a measure of interhemispheric communication and the researchers found that there was differences between total haemoglobin and oxygenated haemoglobin between PCS patients and controls (Urban et al., 2014). More research is supporting the use of NIRS as a way to measure cerebrovascular health (Fabiani et al., 2014; Tan et al., 2017), and it has been suggested that NIRS can be used as a potential marker for recovery in concussion patients (Forcione et al., 2018; Hocke et al., 2018; Urban et al., 2014). The most recent paper on this topic was published by Sharma and colleagues (2020). Their study explored cerebral haemodynamic responses in retired contact sport athletes with a history of multiple concussions in the United Kingdom, specifically investigating the neurovascular coupling mechanism. They found that there was a significant reduction in HbO2, HHb, and HbDiff (Sharma et al., 2020). These results suggest that retired contact sport athletes, with a history of multiple concussions,
exhibit long-term changes in cerebral haemodynamics and in the brain’s ability to adapt cerebral metabolic demands (Sharma et al., 2020).

**Long-term effects on cardiovascular physiology**

Although out of the scope of this thesis, since there is an intrinsic link between the cerebrovascular and cardiovascular system, it is important to mention any long-term cardiovascular changes observed from multiple concussions. However, the literature on these potential changes is sparse. There are a small number of studies exploring the effects that TBI may have on cardiovascular physiology, but these focus on either the immediate effects of a TBI or within the first one to two years following a TBI. Dysautonomia is one aspect that has been reported in TBI patient and has been reported to last up to seven months post-injury (Baguley et al., 1999). The same group looked into HRV changes in TBI patients. HRV parameters were significantly different, and these differences were shown to lasted up to fourteen months post-injury in some patients (Baguley et al., 2006). The long-term effects that concussions can have on the cardiovascular system at this time are unknown. Research is needed to explore this relationship to determine if the cardiovascular system does suffer long-term impairments due to single or multiple concussions and the effects that constant subconcussive blows may have.

**Normal aging process**

It is important to keep in mind the natural aging process and how physiology changes as one ages when discussing the impairments that are occurring due to concussions. Aging results in normal changes that occur in both the structure of the brain, and the physiological processes that help to regulate control throughout the body.
There is a large body of research observing the changes in brain volume and structure that occur from normal aging processes. Grey matter and white matter are two important volumes of the brain that can be measured through neuroimaging techniques. Grey matter refers to the cellular body portion of neurons and play a role in proper brain function. White matter refers to the axons of neurons and these areas are responsible for propagating action potentials from grey matter. There is a consensus that grey matter tends to decrease with age with studies reporting that there were decreases in cortical grey matter, thalamus volume (Good et al., 2001; Sullivan et al., 2004), hippocampus volume, and cerebral cortex volume (Jernigan et al., 2001). However, there is still some question as to whether white matter decreases with age as research has been inconsistent. Several studies have reported that white matter is reduced in aging individuals (Guttmann et al., 1998; Jernigan et al., 2001), while other studies have reported no changes in white matter due to age (Good et al., 2001; Sullivan et al., 2004). It has been reported that white matter loss tends to occur only in individuals from seventy years onwards and that once white matter loss begins to occur, it is more rapid and exceeds the loss of grey matter (Courchesne et al., 2000; Jernigan et al., 2001). In healthy aging people, there has also been reported to be increases in cerebral spinal fluid spaces and ventricular spaces, especially in the lateral and inferior lateral ventricles. (Jernigan et al., 2001; Resnick et al., 2000; Walhovd et al., 2005).

There is evidence to suggest that some physiological processes in the body also tend to change as individuals age. There has been reported to be increases in systolic blood pressure, pulse pressure, and mean arterial pressure from ages thirty through eighty years while there is a decrease in diastolic blood pressure after fifty (Franklin et
al., 1997). These changes can be linked to age related increase in arterial stiffness and vascular inflammation, as a way to explain how pulse pressure increases while diastolic pressure decreases (Franklin et al., 1997; Wen & Wong, 2019). It has also been established that there are decreases in cardiac output as individuals age (Brandfonbrener et al., 1955).

Blood vessels and blood flow have been suggested to change with age. Research has found that in cerebral grey matter areas, there is 0.50% decrease per year in regional cerebral blood flow, cerebral blood volume, and oxygen utilization in brain tissue (Leenders et al., 1990). Researchers suggest that these changes in cerebral blood variables are the result of diminished neuron firing and decreased synaptic density because of the aging process (Leenders et al., 1990) as well as decreases in the rates of angiogenesis in cerebral tissue (Wen & Wong, 2019). Similarly, neurovascular coupling also tends to be affected by the aging process and has been explored in both human and mice models (Akif Topcuoglu et al., 2009; Balbi et al., 2015; Fabiani, Gordon, et al., 2014; Stefanova et al., 2013; Zaletel et al., 2005). There are many factors that contribute to the normal uncoupling in aging individuals including increases in oxidative stresses, endothelial dysfunction, and astrocyte dysfunction which are all observed in an aging individual (Sorond et al., 2013; Tarantini et al., 2017; Toth et al., 2013).

The cerebrovascular response in the brain also changes with age. The cerebrovascular response is part of the brains autonomic regulatory system and responds to increase in partial pressure of CO$_2$ by increasing cerebral blood flow to wash out the excess CO$_2$. In a study comparing young and elderly subjects, it was reported that the older subjects had a decreased cerebrovascular response in grey matter, but a larger and
slower response in the white matter (Galvin et al., 2010; Thomas et al., 2014). The authors suggest that this change is due to the changing vasculature in the brain, and CO₂ accumulating faster in white matter in older subjects.

**Summary**

The underlying pathophysiology of concussions are beginning to become clearer with research examining the effects on cellular systems within the body. While there is a large collection of evidence on the immediate dysfunctions following a concussion, the long-term effects of concussions on individuals are lacking in sufficient evidence and findings are mixed. Studies have begun to determine if long-term changes to the structure of the brain and the behavioral and cognitive changes can occur due to concussions. However, it is important to consider the physiological changes that are occurring in individuals with a history of concussions and currently there is the lack of non-invasive, easy to collect, objective measures that can provide insight on these systems. This thesis aims to begin to fill the gaps in the literature regarding the long-term effects of concussions on cerebral haemodynamics in the three, major cerebral autoregulatory mechanisms; i.e., neurovascular coupling, cerebrovascular reactivity and dynamic autoregulation.
Chapter 3: Long-term Effects of Multiple Concussions on Neurovascular Coupling in Retired Contact Sport Athletes

Abstract

Cerebral autoregulation has been reported to have long-term impairments following multiple concussions. This study aimed to investigate the long-term effects of multiple concussions on the neurovascular coupling mechanism by using NIRS to measure cerebral haemodynamics during a 5-minute object identification protocol ("Where’s Waldo") in 55 male retired contact sport athletes having had multiple concussions (mTBI), and 29 healthy retired athletes with no history of concussions (CTRL). Oxygenation parameters were recorded from the right and left prefrontal cortex during 5-minute seated rest and 5-minute “Where’s Waldo” protocol. NIRS variables were analysed by observing the change between the average of the maximal and minimal values (ΔMAX), Z-scores, and standard deviations. Independent t-tests were used to compare the mTBI to CTRL groups, and paired sample t-tests were used to compare right and left prefrontal cortices within both groups. This study showed that there were no significant differences in the right prefrontal cortex between the mTBI and CTRL groups. mTBI left prefrontal cortex deoxygenated haemoglobin ΔMAX (p= 0.031) and total haemoglobin ΔMAX (p= 0.044) were significantly lower than in the CTRL group. Within-group, right vs left prefrontal cortex differences showed significantly lower values in left HbDiff Z-scores (p= 0.019) in the mTBI group not seen in the CTRL group while the CTRL group showed significantly lower values in left HbDiff SD (p= 0.045) not observed in the mTBI group. This research suggests that there are potential long-term effects on neurovascular coupling in individuals who have a history of concussions.
Significantly lower measures in HHb and tHb in the mTBI group suggests an autonomic dysregulation in the brain’s ability to adapt cerebral blood flow to increased neuronal activity decades after experiencing multiple concussions.

**Introduction**

Concussion has become one of the most prevalent medical conditions over the last couple decades with increases in both public awareness and research observing changes in cognition and neurophysiology. Previous research reports that approximately 20% of North American adolescents have sustained at least one concussion in their lifetime (Ilie et al., 2013; Veliz et al., 2017), and it is estimated that 54% of paediatric concussion cases in Canada are sport-related (Kazl and Torres, 2019). In decades past, adolescent athletes might have been unaware of the seriousness of concussions, or just dismissed concussion symptoms to return-to-play and to avoid showing weakness, which was often an unwritten aspect of sport culture (Caron et al., 2013). Participating in contact sports greatly increases the risk of experiencing a concussion but current medical treatment and preventative options are improving. However, many retired athletes would not have received the same standard of care for a concussion that is available to today’s athletes (Caron et al., 2013).

The immediate effects of concussions on physiological systems are well known, with research suggesting dysfunctions in static and dynamic cerebral autoregulation, cerebrovascular reactivity and neurovascular coupling (Bishop et al., 2017; Bishop and Neary, 2018; Kurowski et al., 2017; Len et al., 2011; Sharma et al., 2020; Wright et al., 2018). However, the long-term effects that multiple concussions can have over the course of an individual’s life are just beginning to be understood. Sustaining multiple
concussions has been suggested to increase the risk of neurodegenerative diseases, negatively impact cognition, and cause changes in behaviour later in life (Daneshvar et al., 2011; Guskiewicz et al., 2005; McKee et al., 2009; Montenigro et al., 2016; Omalu et al., 2011). In studies of athletes with a history of three or more concussions, there were significant decreases in verbal memory scores (Iverson et al., 2012), increases in the rates of self-reported impairments in memory, executive function, and information processing speed (Collins et al., 1999; Guskiewicz et al., 2005; Montenigro et al., 2016; Moser et al., 2005). These impairments are seen in many sports with further evidence of cognitive impairments in retired boxers (McKee et al., 2009; Mendez, 1995; Rabadi and Jordan, 2001), football (soccer) players (Downs and Abwender, 2002; Matser et al., 1998; Rutherford et al., 2009; Straume-Naesheim et al., 2009), ice hockey players (Cantu and Register-Mihalik, 2011; Caron et al., 2013), American football players (Guskiewicz et al., 2005; McKee et al., 2009; Montenigro et al., 2016; B. I. Omalu et al., 2010), and rugby players (Alexander et al., 2015; Lewis et al., 2017; McMillan et al., 2017; Thornton et al., 2008).

While much of the relevant literature has focused on the reported cognitive changes that are occurring in these retired athletes, there is very limited research available that explores the long-term effects on physiological mechanisms. One mechanism that is disrupted immediately following a concussion, and has shown evidence of long-term impairment, is the neurovascular coupling (NVC) mechanism. The NVC mechanism provides the brain with the ability to adapt to increased neural activity and metabolic demand by increasing cerebral blood flow to supply oxygen and nutrients to meet this demand (Tan et al., 2014). In healthy individuals, increased neural
activity in the brain causes an increase in local oxygenated haemoglobin (O$_2$Hb) and a decrease in deoxygenated haemoglobin (HHb) leading to increases in cerebral blood flow and blood volume (Ferrari and Quaresima, 2012). It is known that NVC becomes uncoupled immediately following a concussion with cerebral blood flow being reduced in concussed patients (Smirl et al., 2015; Wright et al., 2018) resulting in unmet metabolic demands. This is believed to be one of the factors that contribute to concussion patients having difficulties concentrating on cognitive tasks (Smirl et al., 2016). New research suggests that a history of multiple concussions is linked to long-term impairments in NVC. Sharma and colleagues (2020) used functional near-infrared spectroscopy (NIRS) to measure cerebral oxygenation variables. They found that the measures of cerebral O$_2$Hb, HHb, and the difference between oxygenated and deoxygenated haemoglobin (HbDiff) was significantly lower in a group of elite retired rugby players, with at least three prior concussions, than an age matched control group (Sharma et al., 2020).

Near infrared spectroscopy (NIRS) is a non-invasive, optical imaging technique that can be used to monitor relative changes in haemodynamic properties (Ferrari & Quaresima, 2012). These variables include: a general tissue saturation index (TSI%; (O$_2$Hb/(O$_2$Hb + HHb) x 100), oxygenated haemoglobin (O$_2$Hb), deoxygenated haemoglobin (HHb), total haemoglobin (tHb; O$_2$Hb + HHb), and haemoglobin difference (HbDiff; O$_2$Hb - HHb). tHb has been suggested to be proportional to cerebral blood volume, while HbDiff reflects an increase in arteriolar vasodilation and subsequent increases in local cerebral blood flow and cerebral blood volume (Ferrari and Quaresima, 2012), due to the activity of the neurovascular coupling mechanism. Research supports
the use of NIRS as an accurate measure of cerebrovascular health (Fabiani, Low, et al., 2014; Tan et al., 2017), and as a valid tool to assess recovery in concussion patients (Bishop and Neary, 2018; Forcione et al., 2018; Hocke et al., 2018; Urban et al., 2014).

While the immediate acute effects of concussions on NVC have been studied (Smirl et al., 2016; Wright et al., 2018), there is currently a lack of research on whether NVC is affected long-term following multiple concussions. The purpose of this study was twofold. First, to explore the effects that a history of multiple concussions (mTBI) can have on NVC in retired contact sport athletes when compared to retired control athletes with no history of previous concussions by measuring prefrontal cortex oxygenation with NIRS. Second, to determine if there are any differences between right and left prefrontal cortex cerebral haemodynamics within both the mTBI group and control group. We hypothesized that there would be significantly lower cerebral oxygenation variables (O$_2$Hb, HHb, tHb, HbDiff) in the mTBI group when compared to CTRL group.

**Methodology**

Testing for this study was conducted at the University of Regina and the University of Victoria in Canada. Ethical approval from both institutional human research ethics boards was obtained prior to collection of data (REB#2017-032; REB#17-128). All procedures were conducted in accordance with the Declaration of Helsinki for the ethical testing of human subjects.

**Participants**

Male volunteer participants (n=84; 40-75 yrs) were recruited in Victoria, BC, Canada, and Regina, SK, Canada, from December 2018 to March 2020. Out of these 84
participants, 55 of them were retired contact sport athletes who had sustained 3 or more concussions in their playing careers (mTBI), and the remaining 29 were healthy controls with no history of concussions (CTRL). Both groups were briefed on the testing protocol and purpose before signing an informed consent form. To meet the inclusion criteria, participants in both groups were required to have had played sports in their youth. Additionally, participants did not have to still be participating in a contact sport but did have to maintain an active lifestyle up to the time of testing. The inclusion criteria was selected to control for any differences in fitness and athlete personality characteristics that might have occurred between the groups. Demographic information and physical characteristics were collected at the time of testing (see Table 1). There was a significant difference in the age of the groups (p=0.01), with the CTRL group (mean=64±8 yr) being older than the mTBI group (mean=59±8 yr). Height (p=0.26) and body mass (p=0.051) were not significantly different between the two groups, while previous number of concussions were significantly different (P<0.001). In addition to height and body mass, a brief medical history, including concussion history, was collected as well as details on sleep, meals, medication, caffeine and alcohol consumption, and exercise for the 24 hours prior to testing. Subjects also completed the Sport Concussion Assessment Tool (SCAT) symptom scale (McCrory et al., 2017) to assess if they were currently experiencing ongoing concussion symptoms. Exclusion criteria included any caffeine intake within 6 hours, exercise within 4 hours, and alcohol within 12 hours, and any active concussions.
Procedures

Participants were given a simple explanation on the equipment functionality and then were properly fitted with the equipment. For most participants, two PortaLite devices (Artinis Medical Systems, Einsteinweg, Netherlands) were used to monitor both the right and left prefrontal cortex hemispheres of the brain. For approximately 25% of the participants, the left side was monitored with an Oxymon NIRS device which has identical functionality as the PortaLite devices (Artinis Medical Systems, Einsteinweg, Netherlands). The PortaLite and Oxymon use near infrared light to assess cerebral oxygenation parameters. The probe on the devices use one receiver and 3 pairs of light emitting diodes (LED). The first pair of LEDs (760 and 843 nm) is located 30 mm from the receiver with the second pair (761 and 845 nm) located 35 mm from the receiver, and the third pair (762 and 848 nm) located 40 mm from the receiver. The probes were placed 1 cm above the participant’s eyebrows over the right and left prefrontal cortex on the lateral side of the supraorbital ridge to avoid the frontal sinus (Bishop and Neary, 2018). Both probes were covered by a headband to secure its position on the participant and to avoid external infrared light interfering with the signal. The devices were connected to separate Oxysoft 3.0.97.1 software using Bluetooth connection for data collection.

The study utilized a modified Neary Protocol (Neary et al., 2019) to assess changes in NVC. Participants were seated and asked to remain still for a 5-minute resting phase to establish resting physiology. Following the resting phase, an object identification protocol was conducted to stimulate an increase in NVC. Participants were seated 50 cm away from a 76 cm computer monitor. The protocol consists of 5 minutes
(5 cycles of 20 seconds eyes closed: 40 seconds eyes opened and searching) of “Where’s Waldo” to act as a complicated visual search paradigm that involves searching on a computer monitor for an object character of specific shape and colour (“Waldo”) that is hidden in a field of distractors of similar colour and shape. The protocol was started with eyes closed. “Where’s Waldo” has been used and validated in previous research to elicit a NVC response (Smirl et al., 2016). If the participant found “Waldo” within the 40 seconds of searching, then another novel picture was immediately presented, ensuring that the participant would continue searching until the entire 40 second segment was completed.

**Data Analysis**

All NIRS data for each participant were filtered using a low-pass filter to remove excess noise (Bishop and Neary, 2018) prior to being exported at 10 Hz into a custom-made Microsoft Excel spreadsheet template. Variables were analysed using the computed change between the average of the maximal and minimal values (ΔMAX) for each NIRS variable during the “Where’s Waldo” object identification protocol. Z-scores and standard deviations (SD) were also calculated and analysed. Statistical analysis was performed using IBM SPSS statistical software (IBM SPSS v.25, Chicago, IL) with tests for homogeneity of variance (Levene’s Test). Independent student t-tests were used to compare each NIRS variable between the two groups. Paired sample t-tests were used to compare within-group right and left prefrontal cortex differences. Statistical significance was set to p≤0.05.
Table 1. Participant Demographics and Physical Characteristics. Values are mean (standard deviation).

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Age (SD) (yrs)</th>
<th>Mean Height (SD) (cm)</th>
<th>Mean Body Mass (SD) (kg)</th>
<th>Mean number of previous concussions (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mTBI (n=55)</td>
<td>59 (8)*</td>
<td>177.4 (5.5)</td>
<td>91.0 (13.9)</td>
<td>4 (4)*</td>
</tr>
<tr>
<td>CTRL (n=29)</td>
<td>64 (8)*</td>
<td>175.8 (6.7)</td>
<td>84.6 (12.5)</td>
<td>0 (0)*</td>
</tr>
</tbody>
</table>

*p≤0.05 indicating significant differences

Results

Of the 55 previously concussed athletes, right-side data of 2 participants and left-side data of 9 participants was lost due to signal and equipment issues. Of the 29 control participants, the left-side data of 1 participant was lost. This resulted in the analysis of the right prefrontal cortex using 53 mTBI and 29 CTRL subjects, and the left analysis using 46 mTBI and 28 CTRL subjects. Right prefrontal cortex differences between the mTBI and CTRL groups are summarized in Table 2 and Figure 1. Left prefrontal cortex differences between the mTBI and CTRL groups are summarized in Table 3 and Figure 2. Within-group right vs. left prefrontal cortex differences are summarized in Table 4 and Figure 3 for the mTBI group, and Table 5 and Figure 4 for the CTRL group.

No between-group differences in any haemodynamic ∆MAX, Z-scores, or standard deviations were found in the right prefrontal cortex. Left prefrontal cortex measures showed a significantly lower HHb ∆MAX and tHb ∆MAX in the mTBI group when compared to the CTRL group.
Table 2. Right prefrontal cortex NIRS variables for individuals with multiple previous concussions (mTBI) and healthy control (CTRL) group. Values are mean (standard deviation).

<table>
<thead>
<tr>
<th>NIRS (µM)</th>
<th>mTBI n=53</th>
<th>CTRL n=29</th>
<th>Levene’s Test</th>
<th>(p≤0.05)</th>
<th>% DIFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>O$_2$Hb ∆MAX</td>
<td>2.77 (1.12)</td>
<td>2.21 (0.94)</td>
<td>0.92</td>
<td>0.679</td>
<td>25.34</td>
</tr>
<tr>
<td>O$_2$Hb Z#$^*$</td>
<td>4.09 (2.77)</td>
<td>4.19 (3.31)</td>
<td>0.22</td>
<td>0.887</td>
<td>-2.39</td>
</tr>
<tr>
<td>O$_2$Hb SD</td>
<td>0.72 (0.47)</td>
<td>0.90 (0.61)</td>
<td>0.52</td>
<td>0.158</td>
<td>-19.29</td>
</tr>
<tr>
<td>HHb ∆MAX</td>
<td>0.73 (0.47)</td>
<td>0.80 (0.52)</td>
<td>0.26</td>
<td>0.559</td>
<td>-8.30</td>
</tr>
<tr>
<td>HHb Z#$^*$</td>
<td>5.88 (3.53)</td>
<td>7.09 (5.03)</td>
<td>0.02</td>
<td>0.258</td>
<td>-17.07</td>
</tr>
<tr>
<td>HHb SD</td>
<td>0.24 (0.22)</td>
<td>0.23 (0.25)</td>
<td>0.78</td>
<td>0.806</td>
<td>5.63</td>
</tr>
<tr>
<td>tHb ∆MAX</td>
<td>2.70 (1.51)</td>
<td>2.72 (1.29)</td>
<td>0.98</td>
<td>0.954</td>
<td>-0.74</td>
</tr>
<tr>
<td>tHb Z#$^*$</td>
<td>5.12 (3.48)</td>
<td>5.53 (3.35)</td>
<td>0.08</td>
<td>0.633</td>
<td>-7.41</td>
</tr>
<tr>
<td>tHb SD</td>
<td>0.87 (0.66)</td>
<td>1.06 (0.86)</td>
<td>0.38</td>
<td>0.261</td>
<td>-18.40</td>
</tr>
<tr>
<td>HbDiff ∆MAX</td>
<td>2.07 (0.81)</td>
<td>1.93 (0.72)</td>
<td>0.93</td>
<td>0.463</td>
<td>7.25</td>
</tr>
<tr>
<td>HbDiff Z#$^*$</td>
<td>3.11 (2.25)</td>
<td>3.14 (2.84)</td>
<td>0.35</td>
<td>0.950</td>
<td>-0.96</td>
</tr>
<tr>
<td>HbDiff SD</td>
<td>0.65 (0.34)</td>
<td>0.77 (0.46)</td>
<td>0.26</td>
<td>0.183</td>
<td>-15.51</td>
</tr>
</tbody>
</table>

* p≤0.05 (If Levene’s test was significant, p values were used to reflect unequal variance). Percentage (%) DIFF is the difference between the groups (mTBI compared to CTRL). A negative value indicates that the variable was lower in the mTBI group. # Z-scores are unitless values.
Table 3. Left prefrontal cortex NIRS variables for the previously concussed (mTBI) and healthy control (CTRL) group. Values are mean (standard deviation).

<table>
<thead>
<tr>
<th>NIRS(µM)</th>
<th>mTBI n=46</th>
<th>CTRL n=28</th>
<th>Levene’s Test</th>
<th>(p≤0.05)</th>
<th>% DIFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₂Hb ∆MAX</td>
<td>2.08 (0.64)</td>
<td>2.71 (0.19)</td>
<td>0.00</td>
<td>0.095</td>
<td>-23.25</td>
</tr>
<tr>
<td>O₂Hb Z#</td>
<td>3.30 (1.99)</td>
<td>4.52 (4.11)</td>
<td>0.04</td>
<td>0.151</td>
<td>-26.99</td>
</tr>
<tr>
<td>O₂Hb SD</td>
<td>0.57 (0.35)</td>
<td>0.97 (1.08)</td>
<td>0.01</td>
<td>0.068</td>
<td>-40.93</td>
</tr>
<tr>
<td>HHb ∆MAX</td>
<td>0.78 (0.45)</td>
<td>1.33 (1.24)</td>
<td>0.00</td>
<td>0.031*</td>
<td>-41.13</td>
</tr>
<tr>
<td>HHb Z#</td>
<td>5.26 (2.56)</td>
<td>5.59 (3.65)</td>
<td>0.13</td>
<td>0.653</td>
<td>-5.90</td>
</tr>
<tr>
<td>HHb SD</td>
<td>0.22 (0.19)</td>
<td>0.47 (0.85)</td>
<td>0.01</td>
<td>0.142</td>
<td>-52.45</td>
</tr>
<tr>
<td>tHb ∆MAX</td>
<td>2.53 (0.95)</td>
<td>3.79 (3.11)</td>
<td>0.00</td>
<td>0.044*</td>
<td>-33.25</td>
</tr>
<tr>
<td>tHb Z#</td>
<td>4.61 (2.88)</td>
<td>5.45 (4.48)</td>
<td>0.07</td>
<td>0.328</td>
<td>-15.41</td>
</tr>
<tr>
<td>tHb SD</td>
<td>0.72 (0.49)</td>
<td>1.39 (1.88)</td>
<td>0.01</td>
<td>0.076</td>
<td>-48.13</td>
</tr>
<tr>
<td>HbDiff ∆MAX</td>
<td>1.85 (0.51)</td>
<td>1.88 (0.73)</td>
<td>0.07</td>
<td>0.826</td>
<td>-1.60</td>
</tr>
<tr>
<td>HbDiff Z#</td>
<td>2.15 (1.74)</td>
<td>3.18 (2.65)</td>
<td>0.04</td>
<td>0.075</td>
<td>-32.39</td>
</tr>
<tr>
<td>HbDiff SD</td>
<td>0.50 (0.29)</td>
<td>0.63 (0.38)</td>
<td>0.27</td>
<td>0.108</td>
<td>-20.16</td>
</tr>
</tbody>
</table>

* p≤0.05 (If Levene’s test was significant, p values were used to reflect unequal variance). Percentage (%) DIFF is the difference between the groups (mTBI compared to CTRL). A negative value indicates that the variable was lower in the mTBI group. # Z-scores are unitless values.

As provided in Tables 4 and 5, within-group differences between right and left prefrontal cortex showed both the mTBI and CTRL group had significantly higher left HHb ∆MAX and left tHb ∆MAX when compared to the right side. The mTBI group also showed significantly lower left HbDiff Z-scores compared to the right side which was not observed in the CTRL group. The CTRL group uniquely showed a significantly lower HbDiff SD in the left side compared to the right side.
Table 4. mTBI right vs left prefrontal cortex NIRS variables. Values are mean (standard deviation).

<table>
<thead>
<tr>
<th>NIRS (µM)</th>
<th>Right n=46</th>
<th>Left n=46</th>
<th>(p≤0.05)</th>
<th>% DIFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>O$_2$Hb ∆MAX</td>
<td>2.12 (0.75)</td>
<td>2.26 (0.09)</td>
<td>0.152</td>
<td>-6.19</td>
</tr>
<tr>
<td>O$_2$Hb Z$^#$</td>
<td>3.81 (2.30)</td>
<td>3.56 (2.22)</td>
<td>0.393</td>
<td>7.02</td>
</tr>
<tr>
<td>O$_2$Hb SD</td>
<td>0.66 (0.42)</td>
<td>0.65 (0.45)</td>
<td>0.924</td>
<td>0.77</td>
</tr>
<tr>
<td>HHb ∆MAX</td>
<td>0.65 (0.34)</td>
<td>0.85 (0.49)</td>
<td>0.001*</td>
<td>-24.09</td>
</tr>
<tr>
<td>HHb Z$^#$</td>
<td>5.59 (3.36)</td>
<td>5.56 (2.77)</td>
<td>0.930</td>
<td>0.54</td>
</tr>
<tr>
<td>HHb SD</td>
<td>0.20 (0.17)</td>
<td>0.26 (0.24)</td>
<td>0.108</td>
<td>-20.70</td>
</tr>
<tr>
<td>tHb ∆MAX</td>
<td>2.43 (0.99)</td>
<td>2.77 (1.24)</td>
<td>0.020*</td>
<td>-12.27</td>
</tr>
<tr>
<td>tHb Z$^#$</td>
<td>4.76 (2.98)</td>
<td>4.90 (3.03)</td>
<td>0.713</td>
<td>-2.86</td>
</tr>
<tr>
<td>tHb SD</td>
<td>0.75 (0.55)</td>
<td>0.83 (0.66)</td>
<td>0.355</td>
<td>-10.20</td>
</tr>
<tr>
<td>HbDiff ∆MAX</td>
<td>1.94 (0.61)</td>
<td>1.95 (0.61)</td>
<td>0.883</td>
<td>-0.51</td>
</tr>
<tr>
<td>HbDiff Z$^#$</td>
<td>2.90 (1.83)</td>
<td>2.34 (1.85)</td>
<td>0.019*</td>
<td>23.93</td>
</tr>
<tr>
<td>HbDiff SD</td>
<td>0.61 (0.32)</td>
<td>0.55 (0.31)</td>
<td>0.085</td>
<td>11.68</td>
</tr>
</tbody>
</table>

* *=p≤0.05. Percentage (%) DIFF is the difference between the two sides of the prefrontal cortex. A positive % difference score indicates that the right side was higher than the left side. $#=$Z-scores are unitless values.
Table 5. CTRL right vs. left prefrontal cortex NIRS variables. Values are mean (standard deviation).

<table>
<thead>
<tr>
<th>NIRS (µM)</th>
<th>Right n=28</th>
<th>Left n=28</th>
<th>(p≤0.05)</th>
<th>% DIFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>O$_2$Hb ∆MAX</td>
<td>2.15 (0.91)</td>
<td>2.71 (1.87)</td>
<td>0.098</td>
<td>-20.66</td>
</tr>
<tr>
<td>O$_2$Hb Z#</td>
<td>4.07 (3.31)</td>
<td>4.52 (4.11)</td>
<td>0.651</td>
<td>-9.96</td>
</tr>
<tr>
<td>O$_2$Hb SD</td>
<td>0.83 (0.50)</td>
<td>0.97 (1.08)</td>
<td>0.375</td>
<td>-14.64</td>
</tr>
<tr>
<td>HHb ∆MAX</td>
<td>0.76 (0.50)</td>
<td>1.33 (1.23)</td>
<td>0.009*</td>
<td>-42.78</td>
</tr>
<tr>
<td>HHb Z#</td>
<td>6.71 (0.47)</td>
<td>5.59 (3.65)</td>
<td>0.267</td>
<td>20.04</td>
</tr>
<tr>
<td>HHb SD</td>
<td>0.19 (0.15)</td>
<td>0.47 (0.85)</td>
<td>0.093</td>
<td>-58.85</td>
</tr>
<tr>
<td>tHb ∆MAX</td>
<td>2.64 (1.24)</td>
<td>3.79 (3.11)</td>
<td>0.043*</td>
<td>-30.34</td>
</tr>
<tr>
<td>tHb Z#</td>
<td>5.30 (4.25)</td>
<td>5.45 (4.48)</td>
<td>0.891</td>
<td>-2.75</td>
</tr>
<tr>
<td>tHb SD</td>
<td>0.94 (0.61)</td>
<td>1.39 (1.89)</td>
<td>0.168</td>
<td>-32.23</td>
</tr>
<tr>
<td>HbDiff ∆MAX</td>
<td>1.89 (0.69)</td>
<td>1.88 (0.73)</td>
<td>0.944</td>
<td>0.53</td>
</tr>
<tr>
<td>HbDiff Z#</td>
<td>3.08 (2.86)</td>
<td>3.18 (2.65)</td>
<td>0.873</td>
<td>-3.14</td>
</tr>
<tr>
<td>HbDiff SD</td>
<td>0.74 (0.44)</td>
<td>0.63 (0.38)</td>
<td>0.045*</td>
<td>16.67</td>
</tr>
</tbody>
</table>

*=p≤0.05. Percentage (%) DIFF is the difference between the two sides of the prefrontal cortex. A positive % difference score indicates that the right side was higher than the left side. #=Z-scores are unitless values.
Figure 1. Between-group right prefrontal cortex NIRS variables. Values are mean ± standard deviation (µM). Z-scores are unitless values.

Figure 2. Between-group left prefrontal cortex NIRS variables. Values are mean ± standard deviation (µM). *=p≤0.05. Z-scores are unitless values.
Figure 3. mTBI right vs. left prefrontal cortex NIRS variables. Values are mean ± standard deviation (µM). *=p≤0.05. Z-scores are unitless values.

Figure 4. CTRL right vs. left prefrontal cortex NIRS variables. Values are mean ± standard deviation (µM). *=p≤0.05. Z-scores are unitless values.
Discussion

This study explored the long-term effects that multiple concussions can have on NVC during an object identification protocol (‘Where’s Waldo’). This protocol has been used in previous research (Sharma et al., 2020; Smirl et al., 2016), and was shown to elicit a greater NVC response when compared to other methods (Smirl et al., 2016). To the authors’ knowledge, only the Sharma et al., (2020) research has used NIRS to explore the long-term effects of multiple concussions on NVC on retired contact sport athletes with a history of multiple concussions. The main findings of our study showed that there were significant differences in haemodynamic measures in the mTBI group compared to the CTRL group, and within-group right and left prefrontal cortex variance, suggesting that there are long-term effects on NVC in former contact sport athletes with a history of multiple concussions.

The prefrontal cortex plays a major role in the executive function, task focusing, and personality (Miller and Cohen, 2001). By using two probes, each monitoring one side of the prefrontal cortex, this study was able to explore differences in both prefrontal cortices to provide a clearer understanding of any potential effects on cerebral haemodynamics. While the mTBI participants did show significantly lower HHb ∆MAX and tHb ∆MAX in the left prefrontal cortex compared to the CTRL subjects during the object identification protocol, there were no significant group differences in any haemodynamic measures in the right prefrontal cortex. In addition to the significantly lower left HHb ∆MAX, the mTBI group also had a 23% lower left O₂Hb ∆MAX compared to the CTRLs, which although not statistically significant, suggests a trending physiological difference. If both O₂Hb and HHb are at lower levels in the mTBI group, it
would be expected that tHb $\Delta$MAX would also be lower, which was observed in this study. The tHb variable is measured by adding together the $O_2$Hb and HHb signals and has often been used as a surrogate measure for both cerebral blood volume and cerebral blood flow (Ferrari and Quaresima, 2012). If tHb was lower, it follows that the other signals would be affected as well. These findings support the research by Sharma et al. (2020) who also found that their previously concussed (retired) participants with multiple mTBI showed significantly lower responses in tHb in the left prefrontal cortex when comparing ex-rugby players to the healthy controls. Sharma et al. (2020) also found significant differences in all variables in the right prefrontal cortex and additional decreases in $O_2$Hb and HbDiff, which is contrary to the current study. The major difference between the current study and Sharma et al. (2020) is that they found HHb was increased in the left side of the prefrontal cortex in their mTBI group leading to the suggestion that $O_2$Hb was not being adequately delivered through the NVC mechanism. It is important to note that there are some major differences between these studies, as Sharma and colleagues used an 8-channel functional NIRS device that was able to explore different regions of the prefrontal cortex, while the current study was limited to a single channel and a single region on which to explore on each side. Another major difference between studies was that Sharma et al. (2020) used only ex-professional rugby players, while the mTBI participants in the current study were all non-professional former athletes, most with histories in contact sport.

NVC is thought to be regulated by astrocytes (Petzold and Murthy, 2011) and glial cells (Metea and Newman, 2006), and disruptions in the proper function of these cells may explain why there are potential long-term impairments in individuals with a
history of multiple concussions. Both cells are part of the neurovascular unit that acts as part of the blood brain barrier to respond to changes in cerebral homeostasis (Muoio et al., 2014). They do so by influencing blood vessel control (Attwell et al., 2010; Metea and Newman, 2006; Zonta et al., 2003), and act as mediators for cerebral blood flow control (Mathiisen et al., 2010; McCaslin et al., 2011; Xu et al., 2008). It is known that the biomechanical forces experienced during a concussion can have an impact on the neural networks in the brain which can lead to difficulties with cognition (Giza and Hovda, 2014). Although these impairments usually resolve over time, being exposed to repeated subconcussive forces or sustaining multiple concussions, often experienced by contact athletes, may lead to these systems being affected later into life. These long-term changes have been observed in retired boxers (Bailey et al., 2013; Di Virgilio et al., 2019), American football players (McKee et al., 2013; Omalu et al., 2010), military personnel (Mac Donald et al., 2011), and individuals with post-concussion syndrome (Di Virgilio et al., 2016; Pearce et al., 2019). Long-term impact to the neurovascular unit may be one reason why there are observed differences in measured HHb and tHb in the mTBI group of this study compared to CTRLs. It is possible that these impairments to the neurovascular unit, negatively impact the physiological mechanisms which controls NVC leading to the inability to respond as would normally happen in healthy individuals.

The inability to focus or concentrate immediately after a concussion is a common symptom that has been reported (Chen et al., 2007; King et al., 1995). Although concussion patients are usually able to overcome these impairments, many retired contact sport athletes observe that their ability to focus and concentrate decreases as they
age (Guskiewicz et al., 2005; Montenigro et al., 2016). Similar complaints were reported by the mTBI participants in this study and may be explained, in part, by impairments to the NVC mechanism.

Additionally, although not statistically significant, there were several measures in this study that showed a trend toward physiological differences. Notably, many of the left standard deviation (SD) variables had large percentage differences between the mTBI group and the CTRL group. Variance and randomness are associated with healthy physiological function in many physiological systems (Bishop et al., 2017; Lang et al., 2003; Pincus, 1991; Urban et al., 2014; Zweifel et al., 2008), therefore it is important to observe the response of NIRS SD values that occurred in the different groups. It has been suggested that disease or injury may suppress the normal healthy variance observed in a physiological system (Bishop and Neary, 2018; Pincus, 1991; Thayer et al., 2012), and this would support the idea that the trending differences in the SD values provide preliminary evidence that there are long-term dysfunctions to the NVC mechanism in individuals with a history of concussions.

Both the mTBI and CTRL groups demonstrated significant bilateral prefrontal cortex differences in HHb $\Delta$MAX and tHb $\Delta$MAX. Since both groups showed significant differences, it is assumed that there is a normal difference between right and left prefrontal cortices. Other studies have also shown similar differences between the right and left hemispheres during NVC activation in healthy subjects (Phillips et al., 2016; Sharma et al., 2020). As mentioned previously, variance in physiological processes is associated with healthy physiological function (Bishop et al., 2017; Lang et al., 2003; Pincus, 1991; Urban et al., 2014; Zweifel et al., 2008). While the CTRL group did show
significant differences in HbDiff SD between right and left prefrontal cortices, the mTBI group did not. The suppressed variance in HbDiff SD of the mTBI group indicates that this group is more likely to be in an impaired state. Due to equipment limitations, 25% of subject’s left prefrontal cortex measurements used the Oxymon NIRS device instead of the Portalite NIRS devices (both devices are made by the same manufacturer). Since both systems function in the same manner, the data gathered with one device would be consistent with the other.

It is important to note that the normal aging process does influence NVC in both human and mice models (Akif Topcuoglu et al., 2009; Balbi et al., 2015; Fabiani, Gordon, et al., 2014; Stefanova et al., 2013; Zaletel et al., 2005). There are many factors that contribute to an aging related normal uncoupling including increases in oxidative stresses, endothelial dysfunction, and astrocyte dysfunction (Sorond et al., 2013; Tarantini et al., 2017; Toth et al., 2013). There were significant differences between the ages of the participants in the two groups. However, since the CTRL group was older and still showing significant difference between cerebral oxygenation variables, it can be suggested that these differences are occurring regardless of any potential effect of aging.

There are several limitations to acknowledge in this study. Due to the age of the participants the differential path-length factor (DPF) calculation of the NIRS devices was not able to be specific for each participant. DPF calculations consist of values up until the age of 50 (Duncan et al., 1995; Kohl et al., 1998), and are used in the NIRS calculations to determine the thickness of the medium the light is travelling through. Since the majority of participants in this study were above the age of 50, the maximal DPF value possible was used for all participants. It is possible that using a DPF value
different than the participant’s actual age estimated DPF value led to an underestimation of the NIRS variables and thus the actual results could be of a greater magnitude than what was recorded. However, the DPF value was consistently applied to both groups. Signal consistency over the 5-minute object identification period was also a potential limitation for a number of participants. Participants were instructed on how to properly sit during the object identification protocol so as to optimize the NIRS signal by limiting as much head movement as possible. However, there were some participants who would move their head while performing their search which may have negatively impact the NIRS signal quality.

The data on concussions were self-reported by the participants and could have led to inaccuracies on the reported number of concussions experienced over the course of the participant’s life. This could have affected the eligibility of participants for this study. Additionally, inaccuracies in the disclosure of caffeine consumption and participation of exercise prior to testing were also potential limitations. Although all participants were asked to refrain from the consumption of caffeine 6 hours prior to conducting the test, and refrain from participating in strenuous physical activity 12 hours prior to testing, some participants may not have disclosed the amount or timing of caffeine consumption or exercise participation. These confounding variables could have potentially altered the cerebrovascular measures that were recorded throughout the test.

In summary, this study aimed to determine if there are long-term impairments to the NVC mechanism in retired contact sport athletes who have experienced multiple concussions in their youth and throughout their adulthood. The significant differences observed in the left prefrontal cortex for HHb ∆MAX and tHb ∆MAX in the mTBI
group compared to the CTRL group during the object identification protocol suggest that the mTBI NVC mechanism is impaired in its ability to adapt cerebral blood flow to increase neural demands. Additionally, a normal, healthy variance in HbDiff SD between hemispheres was not observed in the mTBI group, providing further evidence of an impaired NVC. Understanding the changes that occur in the brains of previously concussed retired contact sport athletes can better guide to develop novel post-concussion treatment options, and more effectively target impaired systems for intervention with the treatment options that are currently available. Importantly, these would not only be for the older athlete but could also guide treatment and preventative options for younger athletes to avoid any potential long-term impairments to the NVC mechanism.

Future research is warranted to determine the severity of cerebral haemodynamic impairments that occur on a long-term basis in retired athletes. This could be accomplished by conducting longitudinal studies to follow athletes from their competitive playing careers, into their recreational careers and beyond. A focus should also be made to determine the effects of multiple concussions on the physiology of female athletes as well as to determine if any long-term effects of concussions exist. Finally, additional studies exploring using an integrative physiology approach (e.g., cerebral blood flow response, blood pressure, electrocardiography, and expired gas analysis) are needed to fully assess the impact of former concussions, and particularly multiple concussions, on long-term performance of physiological systems. Providing greater insight into how these systems are affected over time could lead to the development of potential solutions for retired athletes who are currently experiencing
impairments and can better direct intervention strategies early in the concussion recovery process to minimize the effects that concussions can have long-term.

**Acknowledgements**

The authors would like to sincerely thank all the participants for their time and dedication in support of this data collection. Funding from the Canadian Academy of Sport and Exercise Medicine (CASEM) is greatly acknowledged. We would also like to thank Cameron Bowers, Jake Bryan, Marisa Harrington, and Kathleen Leahy for their assistant in this project.
References


https://doi.org/10.1016/j.neuroimage.2013.04.113

https://doi.org/10.1111/psyp.12288


https://doi.org/10.1227/01.NEU.0000175725.75780.DD


Iverson, G. L., Echemendia, R. J., LaMarre, A. K., Brooks, B. L., & Gaetz, M. B. (2012). *Possible Lingering Effects of Multiple Past Concussions* [Research article]. Rehabilitation Research and Practice. https://doi.org/10.1155/2012/316575


https://doi.org/10.1097/HTR.0000000000000238


Chapter 4: Long-term Effects of Multiple Concussions on Cerebrovascular Reactivity in Retired Contact Sport Athletes

Abstract

This study aimed to investigate the long-term effects of multiple concussions on the cerebrovascular reactivity mechanism by using NIRS to measure cerebral haemodynamics during a 5-minute hypercapnic challenge (20-second breath-hold, 40-second recovery breathing; 5 times) in 55 male retired contact sport athletes having had multiple concussions (mTBI), and 29 healthy retired athletes with no history of concussions (CTRL). Oxygenation parameters were recorded from the right and left prefrontal cortex during 5-minute seated rest and 5-minute hypercapnic challenge. NIRS variables were analysed by observing the change between the average of the maximal and minimal values (ΔMAX), Z-scores, and standard deviations. Independent t-tests were used to compare the mTBI to CTRL groups, and paired sample t-tests were used to test for bilateral differences in prefrontal cortex measures within each group. Right prefrontal cortex findings showed significantly higher HbDiff ΔMAX in the mTBI group compared with CTRL (p=0.045). Left prefrontal cortex measures included significantly higher O₂Hb ΔMAX (p=0.040), HHb Z-Scores (p=0.008), and HbDiff ΔMAX compared with CTRL (p=0.014). The mTBI group had significantly lower left HbDiff ΔMAX (p=0.048) and HbDiff Z-scores (p=0.002) compared with their right side. The CTRL group had significantly lower left HHb Z-scores (p=0.003) and left tHb Z-scores (p=0.042). These findings suggest that there are potential long-term effects on cerebrovascular reactivity in individuals who have a history of multiple concussions. Significantly higher values in HbDiff and O₂Hb in the mTBI group suggests there are...
differences in the brain’s ability to adapt cerebral blood flow in instances of hypercapnic stress compared to the CTRL group. Additionally, bilateral differences observed in HbDiff in the mTBI group may indicate the presence of long-term autonomic dysregulation from multiple previous concussion earlier in life or potentially related to ongoing subconcussive hits throughout a career of contact sport.

Introduction

The long-term effects that concussions might have on the physiological systems in the body is a concern, especially for contact sport athletes and the medical professionals who care for them. The prevalence of concussions in youth and athlete populations continues to rise, with approximately 20% of adolescents in Canada and the United States having sustained a concussion in their lifetime (Ilie et al., 2013; Veliz et al., 2017). In Canada alone, it is estimated that 54% of paediatric concussion cases are due to sports (Kazl & Torres, 2019), and concussions continue to occur throughout the lifetime of contact sport athletes. New guidelines and treatment options are constantly being developed to minimize the impact that concussions can have on individuals. However, many retired athletes would not have received the same standard of care for a concussion that is available to today’s athletes (Caron et al., 2013). Previously, due to the culture of the time in some sports, athletes returned from injury as soon as possible. This means that current older retired athletes, and their coaches, might have been unaware of the seriousness of concussions or dismissed concussion-like symptoms (Caron et al., 2013).

Concussions have been reported to cause alterations in many physiological processes in the human body. Research has shown that during the immediate aftermath
of a concussion there are physiological changes in cerebral autoregulatory function (Bishop et al., 2017; Bishop & Neary, 2018; Giza & Hovda, 2014; Len & Neary, 2010; Len et al., 2013). Since the autonomic nervous system controls many aspects of the cerebral autoregulation, any injury to the brain would most likely have a broad systemic effect on the whole body, not simply isolated to the brain (Len & Neary, 2011). If the control of blood vessel function is impaired, it would result in downstream impairments to physiological systems that depend on the regulation of blood flow to maintain homeostasis in the brain.

Cerebrovascular reactivity (CVR) is one mechanism of cerebral autoregulation that is reliant on blood flow regulation and has been reported to be impaired immediately following a concussion (Churchill et al., 2018; Len & Neary, 2011; Len et al., 2013). The partial pressure of arterial CO₂ (PaCO₂) acts as a major regulator of blood flow in the brain. In conditions where PaCO₂ exceeds normal levels a cerebrovascular reactivity response increases cerebral blood flow to remove excess the CO₂ from brain tissue and restore PaCO₂ to normal levels. In part, this is achieved through increases in heart rate (HR) and cardiac output resulting in the increase in blood flow to the brain (Ainslie & Duffin, 2009). The long-term effects of concussions on CVR are unknown at this time, however, recent evidence indicates that other mechanisms, such as neurovascular coupling and dynamic cerebral autoregulation, also experience long-term impairments in individuals with a history of multiple concussions (Bailey et al., 2013; Sharma et al., 2020).

Loading the brain with CO₂ for 20-seconds at a time has been shown to be an adequate duration to observe responses in cerebrovascular reactivity (Bailey et al., 2013;
Cummings et al., 2007; Liu et al., 2002; Low et al., 2008; Markus & Harrison, 1992; Niftrik et al., 2016), with supporting evidence from fMRI research (Mutch et al., 2014, 2018). Breath holding for 20-30 seconds results in an accumulation of PaCO₂, due to the restricted ability to wash out excess CO₂ through respiration concomitant with ongoing oxidative metabolism (Bishop & Neary, 2018; Kastrup et al., 1999; Len & Neary, 2010; Len et al., 2013; Peacock et al., 2016).

Near-infrared spectroscopy (NIRS) is a non-invasive, optical imaging technique that can be used to monitor relative changes in haemodynamic properties. These variables include: a general tissue saturation index (TSI%; (O₂Hb/O₂Hb + HHb) x 100), oxygenated haemoglobin (O₂Hb), deoxygenated haemoglobin (HHb), total haemoglobin (tHb; O₂Hb + HHb), and haemoglobin difference (HbDiff; O₂Hb - HHb). tHb has been suggested to be proportional to cerebral blood volume, while HbDiff reflects an increase in arteriolar vasodilation and subsequent increases in local cerebral blood flow and cerebral blood volume (Ferrari & Quaresima, 2012), due to the activity of the cerebrovascular reactivity mechanism. Research supports the use of NIRS as an accurate measure of cerebrovascular health (Fabiani et al., 2014; Highton et al., 2015; Tan et al., 2017), and as a valid tool to assess recovery in concussion patients (Bishop & Neary, 2018; Forcione et al., 2018; Hocke et al., 2018; Urban et al., 2014).

While it is supported that concussions do have an acute effect on CVR function (Bishop & Neary, 2018; Churchill et al., 2018; Len et al., 2013), there is little research on whether CVR is impacted long-term due to multiple concussions. The purpose of this study was twofold. First, to explore the effects that a history of multiple concussions (mTBI) can have on CVR in retired contact sport athletes when compared to retired
control athletes with no history of previous concussions (CTRL) by measuring prefrontal
cortex oxygenation with NIRS. Second, to determine if right and left prefrontal cortex
cerebral haemodynamics within both the mTBI and CTRL group show any differences.
We hypothesize that there will be significantly lower cerebral oxygenation variables
(O₂Hb, HHb, tHb, HbDiff) in the mTBI group when compared to the CTRL group.

Methodology

Testing for this study was conducted at the University of Regina and the
University of Victoria in Canada. Ethical approval from both institutional human
research ethics boards was obtained prior to collection of data (REB#2017-032;
REB#17-128). All procedures were conducted in accordance with the Declaration of
Helsinki for the ethical testing of human subjects.

Participants

Male volunteer participants (n=84; 40-75 yrs) were recruited in Victoria, BC,
Canada, and Regina, SK, Canada from December 2018 to March 2020. Out of these 84
participants, 55 of them were retired contact sport athletes who had sustained 3 or more
concussions in their playing careers (mTBI), and the remaining 29 were healthy controls
with no history of concussions (CTRL). Both groups were briefed on the testing protocol
and purpose before signing an informed consent form. To meet the inclusion criteria,
participants in both groups were required to have had played sports in their youth and
adulthood. Additionally, participants did not have to still be participating in a contact
sport but did have to maintain an active lifestyle up to the time of testing. The inclusion
criteria was selected to control for any differences in fitness and athlete personality
characteristics that might have occurred between the groups. Demographic information
and physical characteristics were collected at the time of testing (see Table 1). There was a significant difference in the age of the groups (p=0.01), with the CTRL group (mean=64±8 yr) being older than the mTBI group (mean=59±8 yr). Height (p=0.26) and body mass (p=0.051) were not significantly different between the two groups, while previous number of concussions were significantly different (P<0.001). In addition to height and body mass, a brief medical history, including concussion history, was collected as well as details on sleep, meals, medication, caffeine and alcohol consumption, and exercise for the 24 hours prior to testing. Subjects also completed the Sport Concussion Assessment Tool (SCAT) symptom scale (McCrory et al., 2017) to assess if they were currently experiencing ongoing concussion symptoms. Exclusion criteria included any caffeine intake within 6 hours, exercise within 4 hours, and alcohol within 12 hours, and any active concussions.

**Procedures**

Participants were given a simple explanation on the equipment functionality and then were properly fitted with the equipment. For the majority of participants, two PortaLite devices (Artinis Medical Systems, Einsteinweg, Netherlands) were used to monitor both the right and left prefrontal cortex hemispheres of the brain. For approximately 25% of the participants, the left side was monitored with an Oxymon NIRS device which has identical functionality as the PortaLite devices (Artinis Medical Systems, Einsteinweg, Netherlands). The PortaLite and Oxymon use near infrared light to assess cerebral oxygenation parameters. The probe on the devices use one receiver and 3 pairs of light emitting diodes (LED). The first pair of LEDs (760 and 843 nm) is located 30mm from the receiver with the second pair (761 and 845 nm) located 35 mm
from the receiver, and the third pair (762 and 848 nm) located 40 mm from the receiver. The probes were placed 1 cm above the participants’ eyebrows over the right and left prefrontal cortex on the lateral side of the supraorbital ridge to avoid the frontal sinus cavity (Bishop & Neary, 2018). Both probes were covered by a headband to secure it to the participant and to avoid external infrared light interfering with the signal. The devices were connected to the separate Oxysoft 3.0.97.1 software using Bluetooth connection for data collection.

The study utilized a modified Neary Protocol (Neary et al., 2019) to assess changes in CVR. Participants were seated and asked to remain still for a 5-minute resting phase to establish resting, baseline physiology. Following the resting phase, the subjects were given a 5 second countdown to prepare for the breath-hold manoeuvre. The 20-second breath-hold was followed by 40 seconds of recovery normal breathing, and this was repeated 5-times. Subjects were instructed to take a normal volume breath immediately prior to the start of each 20-second breath hold and avoid deep inspiration (Len et al., 2011, 2013), and then to forcefully expel the held air after the 20-second breath hold.

**Data Analysis**

All NIRS data for each participant were filtered using a low-pass filter to remove excess noise (Bishop and Neary, 2018) prior to being exported at 10 Hz into a custom-made Microsoft Excel spreadsheet template. Variables were analysed using the computed change between the average of the maximal and minimal values (ΔMAX) for each NIRS variable during the hypercapnic challenge. Z-scores and standard deviations (SD) were also calculated and analysed. Statistical analysis was performed using IBM
SPSS statistical software (IBM SPSS v.25, Chicago, IL) with tests for homogeneity of variance (Levene’s Test). Independent student t-tests were used to compare each NIRS variable between the two groups. Paired sample t-tests were used to compare within-group right and left prefrontal cortex differences. Statistical significance was set to \( p \leq 0.05 \).

Table 1. Participant Demographics and Physical Characteristics. Values are mean (standard deviation).

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Age (SD) (yrs)</th>
<th>Mean Height (SD) (cm)</th>
<th>Mean Body Mass (SD) (kg)</th>
<th>Mean number of previous concussions (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mTBI (n=55)</td>
<td>59 (8)*</td>
<td>177.4 (5.5)</td>
<td>91.0 (13.9)</td>
<td>4 (4)*</td>
</tr>
<tr>
<td>CTRL (n=29)</td>
<td>64 (8)*</td>
<td>175.8 (6.7)</td>
<td>84.6 (12.5)</td>
<td>0 (0)*</td>
</tr>
</tbody>
</table>

* \( p \leq 0.05 \) indicating significant differences

Results

Of the 55 previously concussed athletes, right-side data of 1 participant and left-side data of 6 participants was lost due to signal and equipment issues. Of the 29 control participants, the left-side data of 1 participant was lost. This resulted in the analysis of the right prefrontal cortex using 54 mTBI and 29 CTRL subjects, and the left analysis using 49 mTBI and 28 CTRL subjects. Right prefrontal cortex differences between the mTBI and CTRL groups are summarized in Table 2 and Figure 1. Left prefrontal cortex differences between the mTBI and CTRL groups are summarized in Table 3 and Figure 2. Within-group right vs. left prefrontal cortex differences are summarized in Table 4 and Figure 3 for the mTBI group, and Table 5 and Figure 4 for the CTRL group.
The only group difference found in the right prefrontal cortex was the significantly higher HbDiff ∆MAX in the mTBI group when compared to the CTRL group (p=0.045; Table 2). In the left prefrontal cortex (Table 3), the mTBI has significantly higher values in O$_2$Hb ∆MAX (p= 0.040), HHb Z-scores (p= 0.008), and HbDiff ∆MAX when compared to the CTRL group (p= 0.014).

Table 2. Right prefrontal cortex NIRS variables for individuals with multiple previous concussions (mTBI) and healthy control (CTRL) group. Values are mean (standard deviation).

<table>
<thead>
<tr>
<th>NIRS(µM)</th>
<th>mTBI n=54</th>
<th>CTRL n=29</th>
<th>Levene’s Test</th>
<th>(p≤0.05)</th>
<th>%DIFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>O$_2$Hb ∆MAX</td>
<td>1.45 (0.65)</td>
<td>1.21 (0.53)</td>
<td>0.21</td>
<td>0.089</td>
<td>19.83</td>
</tr>
<tr>
<td>O$_2$Hb Z$^#$</td>
<td>2.22 (1.74)</td>
<td>2.33 (1.65)</td>
<td>0.93</td>
<td>0.777</td>
<td>-4.72</td>
</tr>
<tr>
<td>O$_2$Hb SD</td>
<td>0.44 (0.24)</td>
<td>0.43 (0.44)</td>
<td>0.23</td>
<td>0.903</td>
<td>2.10</td>
</tr>
<tr>
<td>HHb ∆MAX</td>
<td>0.33 (0.16)</td>
<td>0.30 (0.15)</td>
<td>0.69</td>
<td>0.350</td>
<td>11.33</td>
</tr>
<tr>
<td>HHb Z$^#$</td>
<td>3.22 (1.58)</td>
<td>3.15 (1.09)</td>
<td>0.05</td>
<td>0.806</td>
<td>2.22</td>
</tr>
<tr>
<td>HHb SD</td>
<td>0.11 (0.08)</td>
<td>0.11 (0.09)</td>
<td>0.80</td>
<td>0.919</td>
<td>-1.87</td>
</tr>
<tr>
<td>tHb ∆MAX</td>
<td>1.50 (0.73)</td>
<td>1.28 (0.61)</td>
<td>0.30</td>
<td>0.178</td>
<td>17.19</td>
</tr>
<tr>
<td>tHb Z$^#$</td>
<td>2.61 (1.85)</td>
<td>2.65 (1.59)</td>
<td>0.56</td>
<td>0.925</td>
<td>-1.51</td>
</tr>
<tr>
<td>tHb SD</td>
<td>0.50 (0.30)</td>
<td>0.47 (0.52)</td>
<td>0.40</td>
<td>0.695</td>
<td>7.71</td>
</tr>
<tr>
<td>HbDiff ∆MAX</td>
<td>1.48 (0.64)</td>
<td>1.20 (0.50)</td>
<td>0.16</td>
<td>0.045*</td>
<td>23.33</td>
</tr>
<tr>
<td>HbDiff Z$^#$</td>
<td>1.80 (1.81)</td>
<td>1.75 (1.73)</td>
<td>0.86</td>
<td>0.902</td>
<td>2.86</td>
</tr>
<tr>
<td>HbDiff SD</td>
<td>0.40 (0.23)</td>
<td>0.42 (0.35)</td>
<td>0.34</td>
<td>0.709</td>
<td>-5.67</td>
</tr>
</tbody>
</table>

* p≤0.05 (If Levene’s test was significant, p values were used to reflect unequal variance). Percentage (%) DIFF is the difference between the groups (mTBI compared to CTRL). A positive % difference score indicates that the mTBI group was higher than the CTRL group. $^#$ Z-scores are unitless values.
Table 3. Left prefrontal cortex NIRS variables for the previously concussed (mTBI) and healthy control (CTRL) group. Values are mean (standard deviation).

<table>
<thead>
<tr>
<th>NIRS(µM)</th>
<th>mTBI n=49</th>
<th>CTRL n=28</th>
<th>Levene’s Test (p≤0.05)</th>
<th>% DIFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₂Hb ∆MAX</td>
<td>1.47 (0.67)</td>
<td>1.17 (0.50)</td>
<td>0.10</td>
<td>0.040*</td>
</tr>
<tr>
<td>O₂Hb Z#</td>
<td>1.92 (1.88)</td>
<td>1.79 (1.77)</td>
<td>0.50</td>
<td>0.766</td>
</tr>
<tr>
<td>O₂Hb SD</td>
<td>0.45 (0.22)</td>
<td>0.47 (0.38)</td>
<td>0.08</td>
<td>0.819</td>
</tr>
<tr>
<td>HHb ∆MAX</td>
<td>0.40 (0.29)</td>
<td>0.39 (0.16)</td>
<td>0.08</td>
<td>0.800</td>
</tr>
<tr>
<td>HHb Z#</td>
<td>3.22 (1.59)</td>
<td>2.22 (1.46)</td>
<td>0.73</td>
<td>0.008*</td>
</tr>
<tr>
<td>HHb SD</td>
<td>0.16 (0.18)</td>
<td>0.17 (0.13)</td>
<td>0.86</td>
<td>0.759</td>
</tr>
<tr>
<td>tHb ∆MAX</td>
<td>1.60 (0.83)</td>
<td>1.34 (0.65)</td>
<td>0.34</td>
<td>0.152</td>
</tr>
<tr>
<td>tHb Z#</td>
<td>2.56 (2.41)</td>
<td>1.98 (1.63)</td>
<td>0.57</td>
<td>0.263</td>
</tr>
<tr>
<td>tHb SD</td>
<td>0.54 (0.33)</td>
<td>0.56 (0.50)</td>
<td>0.06</td>
<td>0.857</td>
</tr>
<tr>
<td>HbDiff ∆MAX</td>
<td>1.43 (0.62)</td>
<td>1.10 (0.42)</td>
<td>0.08</td>
<td>0.014*</td>
</tr>
<tr>
<td>HbDiff Z#</td>
<td>1.27 (1.66)</td>
<td>1.45 (1.64)</td>
<td>0.52</td>
<td>0.642</td>
</tr>
<tr>
<td>HbDiff SD</td>
<td>0.39 (0.18)</td>
<td>0.39 (0.29)</td>
<td>0.05</td>
<td>0.949</td>
</tr>
</tbody>
</table>

* p≤0.05 (If Levene’s test was significant, p values were used to reflect unequal variance). Percentage (%) DIFF is the difference between the groups (mTBI compared to CTRL). A positive % difference score indicates that the mTBI group was higher than the CTRL group. #= Z-scores are unitless values.

As demonstrated in Tables 4 and 5, within-group differences between right and left prefrontal cortex showed both the mTBI and CTRL group had significantly higher left HHb ∆MAX and HHb SD when compared to the right side. The mTBI group had significantly lower HbDiff ∆MAX and HbDiff Z-scores on the left side compared with the right. The CTRL group had significantly lower left HHb Z-scores as well as left tHb Z-scores compared with the contralateral side.
<table>
<thead>
<tr>
<th>NIRS(µM)</th>
<th>Right n=48</th>
<th>Left n=48</th>
<th>(p≤0.05)</th>
<th>% DIFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>O$_2$Hb ∆MAX</td>
<td>1.47 (0.67)</td>
<td>1.48 (0.68)</td>
<td>0.867</td>
<td>-0.68</td>
</tr>
<tr>
<td>O$_2$Hb Z$#$</td>
<td>2.32 (1.72)</td>
<td>1.95 (1.89)</td>
<td>0.073</td>
<td>18.97</td>
</tr>
<tr>
<td>O$_2$Hb SD</td>
<td>0.44 (0.25)</td>
<td>0.45 (0.23)</td>
<td>0.673</td>
<td>-2.22</td>
</tr>
<tr>
<td>HHb ∆MAX</td>
<td>0.33 (0.16)</td>
<td>0.41 (0.29)</td>
<td>0.013$^*$</td>
<td>-17.73</td>
</tr>
<tr>
<td>HHb Z$#$</td>
<td>3.13 (1.62)</td>
<td>3.24 (1.60)</td>
<td>0.626</td>
<td>-3.40</td>
</tr>
<tr>
<td>HHb SD</td>
<td>0.11 (0.08)</td>
<td>0.16 (0.18)</td>
<td>0.007$^*$</td>
<td>-34.38</td>
</tr>
<tr>
<td>tHb ∆MAX</td>
<td>1.51 (0.74)</td>
<td>1.62 (0.84)</td>
<td>0.122</td>
<td>-6.79</td>
</tr>
<tr>
<td>tHb Z$#$</td>
<td>2.68 (1.87)</td>
<td>2.60 (2.42)</td>
<td>0.740</td>
<td>3.08</td>
</tr>
<tr>
<td>tHb SD</td>
<td>0.50 (0.31)</td>
<td>0.54 (0.33)</td>
<td>0.179</td>
<td>-7.93</td>
</tr>
<tr>
<td>HbDiff ∆MAX</td>
<td>1.51 (0.65)</td>
<td>1.44 (0.63)</td>
<td>0.048$^*$</td>
<td>4.86</td>
</tr>
<tr>
<td>HbDiff Z$#$</td>
<td>1.92 (1.77)</td>
<td>1.30 (1.66)</td>
<td>0.002$^*$</td>
<td>47.69</td>
</tr>
<tr>
<td>HbDiff SD</td>
<td>0.41 (0.23)</td>
<td>0.39 (0.18)</td>
<td>0.531</td>
<td>3.82</td>
</tr>
</tbody>
</table>

* = p≤0.05. Percentage (%) DIFF is the difference between the two sides of the prefrontal cortex. A positive % difference score indicates that the right side was higher than the left side. $#$ = Z-scores are unitless values.
Table 5. CTRL right vs. left prefrontal cortex NIRS variables. Values are mean (standard deviation).

<table>
<thead>
<tr>
<th>NIRS (µM)</th>
<th>Right n=28</th>
<th>Left n=28</th>
<th>(p≤0.05)</th>
<th>% DIFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₂Hb ΔMAX</td>
<td>1.22 (0.54)</td>
<td>1.17 (0.50)</td>
<td>0.345</td>
<td>4.27</td>
</tr>
<tr>
<td>O₂Hb Z#</td>
<td>2.32 (1.68)</td>
<td>1.79 (1.77)</td>
<td>0.112</td>
<td>29.61</td>
</tr>
<tr>
<td>O₂Hb SD</td>
<td>0.43 (0.44)</td>
<td>0.47 (0.38)</td>
<td>0.353</td>
<td>-7.92</td>
</tr>
<tr>
<td>HHb ΔMAX</td>
<td>0.30 (0.16)</td>
<td>0.39 (0.16)</td>
<td>0.002*</td>
<td>-22.74</td>
</tr>
<tr>
<td>HHb Z#</td>
<td>3.18 (1.09)</td>
<td>2.22 (1.46)</td>
<td>0.003*</td>
<td>43.24</td>
</tr>
<tr>
<td>HHb SD</td>
<td>0.11 (0.09)</td>
<td>0.17 (0.13)</td>
<td>0.008*</td>
<td>-38.24</td>
</tr>
<tr>
<td>tHb ΔMAX</td>
<td>1.29 (0.62)</td>
<td>1.34 (0.65)</td>
<td>0.509</td>
<td>-3.73</td>
</tr>
<tr>
<td>tHb Z#</td>
<td>2.66 (1.62)</td>
<td>1.98 (1.63)</td>
<td>0.042*</td>
<td>34.34</td>
</tr>
<tr>
<td>tHb SD</td>
<td>0.47 (0.53)</td>
<td>0.56 (0.50)</td>
<td>0.112</td>
<td>-16.67</td>
</tr>
<tr>
<td>HbDiff ΔMAX</td>
<td>1.21 (0.50)</td>
<td>1.10 (0.42)</td>
<td>0.089</td>
<td>10.00</td>
</tr>
<tr>
<td>HbDiff Z#</td>
<td>1.72 (1.75)</td>
<td>1.45 (1.64)</td>
<td>0.412</td>
<td>18.62</td>
</tr>
<tr>
<td>HbDiff SD</td>
<td>0.43 (0.36)</td>
<td>0.39 (0.29)</td>
<td>0.281</td>
<td>8.95</td>
</tr>
</tbody>
</table>

*=p≤0.05. Percentage (%) DIFF is the difference between the two sides of the prefrontal cortex. A positive % difference score indicates that the right side was higher than the left side. #= Z-scores are unitless values.
Figure 1. Between-group right prefrontal cortex NIRS variables. Values are mean ± standard deviation (µM). *=p≤0.05. Z-scores are unitless values.

Figure 2. Between-group left prefrontal cortex NIRS variables. Values are mean ± standard deviation (µM). *=p≤0.05. Z-scores are unitless values.
Figure 3. mTBI right vs. left prefrontal cortex NIRS variables. Values are mean ± standard deviation (µM). *=p≤0.05. Z-scores are unitless values.

Figure 4. CTRL right vs. left prefrontal cortex NIRS variables. Values are mean ± standard deviation (µM). *=p≤0.05. Z-scores are unitless values.
Discussion

This study explored the long-term effects that multiple concussions can have on CVR during a 20-second hypercapnic challenge. The breath-hold protocol has been used successfully in previous research (Bailey et al., 2013; Niftrik et al., 2016), with a 20-second hypercapnic response showing the large increases in arterial CO\textsubscript{2} to properly elicit the CVR response (Len & Neary, 2011; Len et al., 2013; Peacock et al., 2016). To the authors’ knowledge, this is the first study to explore the long-term effects of multiple concussions on CVR on retired contact sport athletes with a history of multiple concussions using NIRS. The main findings of this study showed that there are significant changes in haemodynamic measures in the mTBI group compared to the CTRL group, and within-subject right vs. left prefrontal cortex, suggesting that there are long-term effects on CVR in former contact sport athletes with a history of multiple concussions.

Cerebral autoregulation activates CVR to increase cerebral blood flow as an effective way of removing excess CO\textsubscript{2} from the brain and restore homeostasis. Previous research has shown that blood flow in the prefrontal cortex is impaired in different disease states, such as stroke, Alzheimer’s, traumatic brain injuries, and immediately following a concussion (Burzynska et al., 2012; Krainik et al., 2005; Len et al., 2011; Yezhuvath et al., 2012). The participants in the current research study who had a history of multiple concussions (mTBI) showed significantly higher right and left prefrontal cortex HbDiff ΔMAX, left O\textsubscript{2}Hb ΔMAX, left HHb Z-score and HbDiff ΔMAX during the CO\textsubscript{2} challenge protocol when compared to the CTRL group. HbDiff is thought to reflect the oxygen extraction rate and oxidative metabolism, as it compares the
difference between $O_2Hb$ and $HHb$ (Ferrari & Quaresima, 2012). Since HbDiff $\Delta MAX$ is higher in the mTBI group than the CTRL group, this suggests that there was a greater metabolic use of oxygen by the cells in both prefrontal regions in an attempt to regulate the excess $CO_2$ that accumulated due to the breath-hold protocol. This suggests that the cellular mechanisms in response to elevated $CO_2$ are not functioning properly, or as efficiently, in the mTBI group as the same processes seen in the CTRL group. CVR reflects the vasomotor control over cerebral vessels and since the response is higher in the mTBI, it may be that the blood vessels do not respond properly to the powerful vasoactive stimuli of $CO_2$. Since HbDiff relies on $O_2Hb$ and $HHb$, it is then logical that $O_2Hb$ $\Delta MAX$ would be elevated concomitant with HbDiff $\Delta MAX$ when there is no change in $HHb$ $\Delta MAX$, as seen in the results. However, the literature is unclear on whether an increase or decrease in these variables should be observed in retired contact sport athletes that have sustained multiple concussions. There is some thought that CVR is dampened following an acute concussion when compared to healthy controls (Bailey et al., 2013; Krainik et al., 2005; Len et al., 2013; Meier et al., 2015; Yezhuvath et al., 2012), but other research suggests that CVR is enhanced in the days immediately following a concussion, with NIRS studies showing higher $O_2Hb$ values in the concussed subjects (Bishop & Neary, 2018). If CVR is indeed reduced following an acute concussion it is plausible that the mTBI group in the current study had recovered from the immediate impairments suffered during their concussions experienced years prior to the study. Although speculative at this time, the greater HbDiff response in the mTBI group compared to the CTRL group could be interpreted as reflecting CVR and autoregulatory systems having adapted over time to overcompensate when the brain is
exposed to the stress of elevated CO\textsubscript{2} levels. The significantly higher normalized HHb Z-scores in the mTBI group reinforces the suggestion that there is an alteration in the oxygen extraction rate in the post-concussion state.

It is important to keep in mind that past studies on concussions and CVR have focused on the acute stages of concussions (i.e., days 1 to 4 post-concussion). Further, methodological differences may explain why some studies have reported lower CVR responses while the current study showed a higher response to the CO\textsubscript{2} challenge. Past research has often used functional magnetic resonance imaging to determine blood oxygen levels (fMRI-BOLD). In healthy populations, NIRS and fMRI-BOLD usually correlate well (Cui et al., 2011; Fabiani et al., 2014; Huppert et al., 2006). One major difference between fMRI-BOLD and the NIRS device used in this study is that fMRI-BOLD has the capacity to measure a greater subcortical region (Meier et al., 2015) whereas the current study was limited to examining the left and right prefrontal cortices. Additionally, fMRI-BOLD measures the oxygen volume in a determined space, while NIRS uses the absorbance of infrared light to calculate the relative changes in concentrations at the microcirculatory level, at the arterioles, capillaries and venules (Ferrari & Quaresima, 2012). Thus, fMRI-BOLD reflects a generalized area of the brain, while NIRS reflects a localized region.

Both mTBI and CTRL groups showed significant bilateral prefrontal cortex differences in HHb \textDelta MAX and HHb SD. The similar responses to the intervention by both mTBI and CTRL can be interpreted as evidence that there is a normal variability between right and left prefrontal cortices. The variables for which there was no significant bilateral differences in both the mTBI and CTRL group are worth further
exploration to determine if they represent a concussion induced long-term difference between right and left prefrontal cortices. The mTBI group showed significantly higher right prefrontal cortex HbDiff ΔMAX and HbDiff Z-score. The lack of a comparable bilateral difference in HbDiff ΔMAX in the CTRL group suggests that in a healthy brain, both sides of the brain experience the same CO₂ build up during the hypercapnic intervention and had similar rates of oxygen extraction from the oxygenated blood. In the mTBI group, the higher HbDiff ΔMAX values in the right side during the intervention suggest that there may have been an unequal CO₂ stress and resulting in a greater need for oxygen on the right side compared with the left. Due to equipment limitations, 25% of subject’s left prefrontal cortex measurements used the Oxymon NIRS device instead of the Portalite NIRS devices (both devices are made by the same manufacturer). Since both systems function in the same manner, the data gathered with one device would be consistent with the other. Long-term alterations in CVR, as observed in this study, are not unique to concussions. Unequal distribution of blood flow during CVR activation to regulate excess CO₂ has been observed in other long-term diseases and traumatic brain injuries, with stroke victims showing a decrease in oxygenation variables in the effected hemisphere during hyperventilation (Krainik et al., 2005), and type 2 diabetes patients showing decreases in blood oxygenation levels during CVR activation during consecutive breath-holds when compared to healthy controls (Last et al., 2007). To help address the reason for these side differences, future research should record the mechanism of action of injury and if possible, which region of the head experienced direct contact.
The link to the cardiovascular system is essential to consider when discussing changes in CVR. Axonal injuries, which have the potential to occur from a concussion (Giza & Hovda, 2014) may cause a disruption to the link between the neurological and cardiovascular systems. The autonomic nervous system controls many aspects of the cerebral autoregulation and cardiovascular function, therefore an injury to the brain may certainly have a negative impact on the cardiovascular system. Impairments to the cardiovascular system during the acute concussion stage have been reported (Gall et al., 2004; Goldstein et al., 1998), 20-months post-concussion (Hilz et al., 2011), and up to two years in traumatic brain injury patients (Baguley et al., 2006; Baguley et al., 1999). If the cardiovascular system is unable to regulate and adapt blood flow in response to CO₂, then this impairment would affect the CVR response. The dysregulation between the two systems could be a reason why there was a greater CVR response in the mTBI group in this study compared to CTRLs. It is possible that the mTBI experienced an overcompensation of oxygenated blood delivery to remove the hypercapnic induced accumulated CO₂. While these are preliminary data, more research is needed to determine if suffering multiple concussions can have a long lasting effect on the cardiovascular system and whether this would play a role in negatively impacting the CVR response later in life.

It is also important to note that CVR has been suggested to be affected by the normal aging process (Galvin et al., 2010; McKetton et al., 2018; Thomas et al., 2014). Research comparing old and young subjects showed that older individuals had a lower CVR response in frontal lobe grey matter and white matter regions (McKetton et al., 2018; Thomas et al., 2014), and lower levels of cerebral blood flow velocity and cerebral
oxygenation (Galvin et al., 2010). The authors of these studies suggested that decreases in CVR can be attributed to the changing vasculature in the brain, and the tendency for CO₂ to accumulate faster in older subjects than younger ones, suggesting an altered vasomotor tone with aging. Although the age between the groups were significantly different, the CTRL group was older so it could be assumed that since the mTBI group still showed significant differences, that age did not affect the results.

There were several limitations involved with this study. One of the risks involved with using a breath-holding maneuver was that the subjects would be unable to last the entire 20-second-time period without taking a breath. We were able to measure end-tidal respiratory gases through capnography during the procedure to determine if the participants were able to maintain the 20-second hold. Through the monitoring of end-tidal gases, we are confident that the participants were able to hold each breath as close to the required 20-seconds as possible.

Additionally, inaccuracies in the disclosure of caffeine consumption and participation of exercise prior to testing were also potential limitations. Although all participants were asked to refrain from the consumption of caffeine 6 hours prior to conducting the test, and refrain from participating in strenuous physical activity 12 hours prior to testing, some participants may not have followed these requirements. These confounding variables could have potentially altered the cerebrovascular measures that were recorded throughout the test.

In summary, this study aimed to determine if there were long-term impairments to the CVR mechanism in retired contact sport athletes who had experienced multiple concussions during their athletic career. The mTBI group was found to have significantly
greater HbDiff ∆MAX in both the right and left prefrontal cortex, and in left O₂Hb ∆MAX, and HHb Z-scores compared to the CTRL group, suggesting that the mTBI group had dysfunctions in the CVR, with them being unable to properly adapt blood haemodynamics in response to the accumulation of CO₂ during the hypercapnic challenge. Additionally, the mTBI group demonstrated bilateral prefrontal cortex differences in HbDiff ∆MAX which was not observed in the CTRL group. This suggests that there is unequal distribution of CO₂ stress between hemispheres in the mTBI group.

Providing insight into what occurs in the brains of previously concussed retired contact sport athletes can be a better guide to develop novel post-concussion treatment options, and more effectively target impaired systems for intervention with the treatment options that are currently available. These options have the potential to not only be for the older athlete but could also guide treatment and preventative options for younger athletes to avoid any potential long-term impairments to the CVR mechanism.

Future research is needed to determine the severity of haemodynamic impairments during CVR activation that occur on a long-term basis in retired athletes. This could be best approached by conducting longitudinal studies of athletes throughout their playing careers, into their recreational careers and retirement from sports. Additional studies are needed to explore the long-term effects of concussions on the performance of various physiological systems by using an integrative physiology approach (e.g., cerebral blood flow response, blood pressure, electrocardiography, and expired gas analysis) to fully assess the extent of any potential impairments. Finally, a focus should also be made to determine the effects that multiple concussions can have on the physiology of female athletes to determine any long-term effects concussions may
have in this group. Providing greater insight into how these systems are affected over
time presents an opportunity to the development of potential solutions for retired athletes
who are currently experiencing impairments and can better direct intervention strategies
eyearly in the concussion recovery process to minimize the effects that concussions can
have long-term.

Acknowledgements

The authors would like to sincerely thank all the participants for their time and
dedication in support of this data collection. Funding from the Canadian Academy of
Sport and Exercise Medicine (CASEM) is greatly acknowledged. We would also like to
thank Cameron Bowers, Jake Bryan, Marisa Harrington, and Kathleen Leahy for their
assistance in this project.
References


sport concussion with near-infrared spectroscopy. *Clinical Physiology and

Parasympathetic baroreflexes and heart rate variability during acute stage of sport
https://doi.org/10.1080/02699052.2016.1226385

Burzynska, A. Z., Nagel, I. E., Preuschoff, C., Gluth, S., Bäckman, L., Li, S.-C.,
Lindenberger, U., & Heekeren, H. R. (2012). Cortical thickness is linked to
executive functioning in adulthood and aging. *Human Brain Mapping, 33*(7),
1607–1620. https://doi.org/10.1002/hbm.21311

Multiple Concussions on Retired National Hockey League Players. *Journal of
Sport and Exercise Psychology*. https://doi.org/10.1123/jsep.35.2.168

Evaluating Cerebrovascular Reactivity during the Early Symptomatic Phase of
https://doi.org/10.1089/neu.2018.6024

comparison of NIRS and fMRI across multiple cognitive tasks. *NeuroImage,

Cummings, K. J., Swart, M., & Ainslie, P. N. (2007). Morning attenuation in
cerebrovascular CO2 reactivity in healthy humans is associated with a lowered

https://doi.org/10.1152/japplphysiol.01437.2006


https://doi.org/10.1111/psyp.12288


https://doi.org/10.1136/bjsm.2003.009530

*Neurosurgery, 75*, S24–S33. https://doi.org/10.1227/NEU.0000000000000505

of the autonomic and cardiovascular systems in acute brain injury. *American 
Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 

Highton, D., Ghosh, A., Tachtsidis, I., Panovska-Griffiths, J., Elwell, C. E., & Smith, M. 
(2015). Monitoring Cerebral Autoregulation After Brain Injury: Multimodal 
Assessment of Cerebral Slow-Wave Oscillations Using Near-Infrared 
Spectroscopy. *Anesthesia and Analgesia, 121*(1), 198–205. 
https://doi.org/10.1213/ANE.0000000000000790

Autonomic Dysfunction after Mild Traumatic Brain Injury. *Journal of 

Reduced Functional Connectivity in Adults with Persistent Post-Concussion 
Symptoms: A Functional Near-Infrared Spectroscopy Study. *Journal of 

A temporal comparison of BOLD, ASL, and NIRS hemodynamic responses to 
https://doi.org/10.1016/j.neuroimage.2005.08.065


analysis models. *Brain and Behavior, 6*(2), e00426.
https://doi.org/10.1002/brb3.426


Chapter 5: Long-term Effects of Multiple Concussions on Dynamic Cerebral Autoregulation in Retired Contact Sport Athletes

Abstract

It has been suggested that concussions can have long-term impact on both autonomic function and cerebral autoregulation. This study aimed to investigate the long-term effects of multiple concussions on dynamic cerebral autoregulation by using NIRS to measure cerebral haemodynamics during a 5-minute Squat-stand test (10 seconds squat, 10-second stand, 0.05Hz; repeated 15 times) in 55 male retired contact sport athletes with previous multiple concussions (mTBI), and 29 healthy retired athletes with no prior concussions (CTRL). Oxygenation parameters (oxy-haemoglobin, O$_2$Hb; deoxy-haemoglobin, HHb; total-haemoglobin, tHb; haemoglobin-difference, HbDiff) were monitored from the right and left prefrontal cortex and computed as the average of the maximal and minimal values (ΔMAX), Z-scores, and standard deviations recorded during the protocol. Independent t-tests were used to compare the mTBI to CTRL groups, and paired sample t-tests were used to compare right and left prefrontal cortices within both groups. Right prefrontal cortex findings showed no significant differences between groups. Left prefrontal cortex measures showed significantly higher O$_2$Hb ΔMAX (p = 0.046) and Hbdiff ΔMAX (p = 0.018) in the mTBI group compared with CTRL. Within-group bilateral prefrontal cortex differences showed the mTBI group had significantly higher left HHb ΔMAX (p = 0.003) and lower left HbDiff Z-scores (p = 0.010) compared with the right cortex. CTRL group showed lower left HbDiff SD (p = 0.039), tHb Z-scores (p = 0.030), and HbDiff ΔMAX (p = 0.037). Significant increases in left HbDiff and O$_2$Hb in the mTBI group suggests autonomic dysregulation in the brain’s
ability to metabolically adapt to changing cerebral perfusion pressure. This research suggests that there are potential long-term effects on dynamic cerebral autoregulation due to past multiple concussions in former contact sport athletes.

**Introduction**

The long-term consequences of concussions are beginning to be studied with research linking concussions to several impairments later in life. Multiple concussions have been linked to an increased risk of neurodegenerative diseases (Daneshvar et al., 2011; McKee et al., 2009; Omalu et al., 2010), cerebral structural changes (Bendlin et al., 2008; Goswami et al., 2016; Koerte et al., 2015; McKee et al., 2009; Tremblay et al., 2013), as well as reports of impairments in cognition (Guskiewicz et al., 2005; Iverson et al., 2012; Montenigro et al., 2016) and corticomotor function (Lewis et al., 2017), as well as changes in behaviour later in life (Guskiewicz et al., 2007; Montenigro et al., 2016). As the current incidence rates of concussions in younger athletes is large and continues to increase worldwide (Finch et al., 2013; Ilie et al., 2013; Kazl & Torres, 2019; Veliz et al., 2017), it is imperative to fully assess and understand the long-term impact that sustaining multiple concussions can have on an individual as they age.

The immediate effects of concussions are well documented, with more physiological research being conducted to determine the mechanisms that are disturbed in the brain acutely. There have been reports of impairments in the static (sCA) and dynamic cerebral autoregulation (dCA), cerebrovascular reactivity, and neurovascular coupling systems immediately following a concussion (Bishop et al., 2017; Bishop & Neary, 2018; Kurowski et al., 2017; Len et al., 2011; Sharma et al., 2020; Wright et al., 2018). Usually these impairments are short lived and resolve within days to weeks.
However, many older retired athletes with a history of multiple concussions continue to report changes in their cognition later in life that are inconsistent with that experienced by age-matched individuals with no history of prior concussions (Caron et al., 2013; Iverson et al., 2012; Montenigro et al., 2016). Very little research has been conducted specifically on the long-term physiological impact that concussion might have in individuals with a history of multiple concussions. These physiological impairments could be influencing the dysfunctions that many retired previously concussed contact-sport athletes report (Giza & Hovda, 2014).

Cerebral autoregulation is the ability of the brain to control cerebral blood flow and pressure through changes in cerebral perfusion pressure and cerebrovascular resistance (Golding et al., 1999). Controlling cerebral blood flow is critical to maintaining homeostasis and avoiding damage to the sensitive cells in the brain. Changes in cerebral blood flow can activate different mechanisms of the autoregulatory system including dCA, cerebrovascular reactivity, and neurovascular coupling, each of which are activated under different circumstances (Ainslie & Duffin, 2009; Bishop et al., 2017; Bishop et al., 2018; Len & Neary, 2010; Willie et al., 2011). Research is beginning to report long-term impairments in some of these mechanisms in those with a history of concussion, and specifically there have been findings of haemodynamic dysfunctions in dCA (Bailey et al., 2013).

dCA is the capacity of cerebrovasculature to accommodate rapid changes in arterial blood pressure while maintaining adequate cerebral blood flow and circulation (La Rovere et al., 2008). Impairments in this system cause increases in the sensitivity of the brain to blood pressure surges and can lead to alterations in baroreflex sensitivity.
Invoking the baroreflex mechanism allows for the assessment of dynamic autoregulatory control. Baroreceptors and chemoreceptors at the aortic and carotid arteries allow the peripheral nervous system to monitor the blood pressure and metabolic status of the body. The signals converge in the medulla oblongata of the central nervous system, and allow for the constant monitoring and adjusting of flow-pressure during rest and exercise (Ainslie & Duffin, 2009). When flow-pressure re-adjustment is needed, heart rate, blood pressure, peripheral resistance, and cerebral perfusion will be altered accordingly to maintain steady pressure levels (Bishop et al., 2017; Smirl, Hoffman, Tzeng, Hansen, & Ainslie, 2015; Wright et al., 2018). Using a squat-stand protocol (10-second squat; 10-second stand; 0.05Hz) has been shown to stimulate the baroreflex and by using this intervention method it is possible to monitor cerebral circulation for any dysregulation of dCA (Bishop et al., 2017; Smirl et al., 2015).

Near infrared spectroscopy (NIRS) is a non-invasive, optical imaging technique that can be used to monitor relative changes in cerebral haemodynamics (Ferrari & Quaresima, 2012). NIRS variables included: a general tissue saturation index (TSI%; \( \frac{O_2Hb}{O_2Hb + HHb} \times 100 \)), oxygenated haemoglobin (\( O_2Hb \)), deoxygenated haemoglobin (HHb), total haemoglobin (tHb; \( O_2Hb + HHb \)), and haemoglobin difference (HbDiff; \( O_2Hb - HHb \)). tHb has been suggested to be proportional to cerebral blood volume, while HbDiff reflects an increase in arteriolar vasodilation and subsequent increases in local cerebral blood flow and cerebral blood volume, due to the activity of the neurovascular coupling mechanism. Research supports the use of NIRS as an accurate measure of cerebrovascular health (Fabiani, Low, et al., 2014; Tan et al., 2017),
and as a valid tool to assess recovery in concussion patients (Bishop & Neary, 2018; Forcione et al., 2018; Hocke et al., 2018; Urban et al., 2014)

Currently, the long-term effects of sustaining multiple concussions on dCA are unknown. The purpose of this study was twofold. First, to explore the effects that multiple concussions have on dCA in retired contact sport athletes with a history of concussion (mTBI) when compared to retired athletes with no history of previous concussions (CTRL) by measuring prefrontal cortex oxygenation with NIRS. Second, to determine if there are any differences between right and left prefrontal cortex cerebral haemodynamics within both the mTBI group and an age-matched CTRL group. We hypothesize that all cerebral oxygenation variables (O$_2$Hb, HHb, tHb, HbDiff) measured using NIRS would be higher in the mTBI group compared to the CTRL group.

**Methodology**

Testing for this study was conducted at the University of Regina and the University of Victoria in Canada. Ethical approval from both university’s research ethics boards was obtained prior to data collection (REB#2017-032; REB#17-128). All procedures were conducted in accordance with the Declaration of Helsinki for the ethical testing of human subjects.

**Participants**

Male volunteer participants (n=84; 40-75 yrs) were recruited in Victoria, BC, Canada, and Regina, SK, Canada, from December 2018 to March 2020. Out of these 84 participants, 55 of them were retired contact sport athletes who had sustained 3 or more concussions in their playing careers (mTBI), and the remaining 29 were healthy controls with no history of concussions (CTRL) were briefed on the testing protocol and purpose
before signing an informed consent form. Inclusion criteria required participants in both groups to have had played sports in their youth. All participants did not have to still be participating in a sport currently but did have to maintain an active lifestyle up to the time of testing. The inclusion criteria was selected to control for any differences in fitness and athlete personality characteristics that might have occurred between the groups. Demographic information and physical characteristics were collected at the time of testing (see Table 1). There was a significant difference in the age of the groups (p=0.01), with the CTRL group (mean=64±8 yrs) being older than the mTBI group (mean=59±8 yrs). Height (p=0.26) and body mass (p=0.051) were not significantly different between the two groups, while previous number of concussions were significantly different (P<0.001). In addition to height and body mass, a brief medical history, including concussion history, was collected as well as details on sleep, meals, medication, caffeine and alcohol consumption, and exercise for the 24 hours prior to testing. Subjects also completed the Sport Concussion Assessment Tool (SCAT) symptom scale (McCrorry et al., 2017) to assess if they were currently experiencing ongoing concussion symptoms. Exclusion criteria included any caffeine intake within 6 hours, exercise within 4 hours, and alcohol within 12 hours, and any active concussions.

*Procedures*

Participants were given a simple explanation on the equipment functionality and then were properly fitted with the equipment. For most participants, two PortaLite devices (Artinis Medical Systems, Einsteinweg, Netherlands) were used to monitor both the right and left prefrontal cortex hemispheres of the brain. For approximately 25% of the participants, the left side was monitored with an OxyMon NIRS device which has
identical functionality as the PortaLite devices (Artinis Medical Systems, Einsteinweg, Netherlands). The PortaLite and Oxymon use near infrared light to assess cerebral oxygenation parameters. The probe on the devices use one receiver and 3 pairs of light emitting diodes (LED). The first pair of LEDs (760 and 843 nm) is located 30mm from the receiver with the second pair (761 and 845 nm) located 35 mm from the receiver, and the third pair (762 and 848 nm) located 40 mm from the receiver. The probes were placed 1 cm above the participant’s eyebrows over the right and left prefrontal cortex on the lateral side of the supraorbital ridge to avoid the frontal sinus (Bishop & Neary, 2018). Both probes were covered by a headband to secure its position on the participant and to avoid external infrared light interfering with the signal. The devices were connected to the separate Oxysoft 3.0.97.1 software using Bluetooth connection for data collection.

The study used a modified Neary Protocol (Neary et al., 2019) to assess differences in dCA control between mTBI and CTRL groups. Participants were seated and asked to remain still for a 5-minute resting phase to establish resting physiology. Prior to the squat-stand maneuver, the participants stood up for 1-minute to allow the body to adjust to standing position so that a new baseline flow-pressure equilibrium ‘set point’ could be established (Bishop et al., 2017). Following the 1-minute of quiet standing, participants completed a squat-stand maneuver which consisted of a cyclical 10-second squat followed by 10-seconds of standing (squat rate of 0.05Hz), repeated for 5 minutes for a total of 15 squats. Each participant was instructed to keep their head in a level plane by “looking straight forward” during each squat. A goniometer was used to
ensure that the participant squatted to 90° at the knees. If a squat was performed incorrectly the participant was corrected and reminded of the protocol details.

**Data Analysis**

All NIRS data for each participant were filtered using a low-pass filter to remove excess noise (Bishop & Neary, 2018) prior to being exported at 10 Hz into a custom-made Microsoft Excel spreadsheet template. Variables were analysed using the computed change between the average of the maximal and minimal values (ΔMAX) for each NIRS variable during the squat-stand maneuver. Z-scores and standard deviations (SD) were also calculated and analysed. Statistical analysis was performed using IBM SPSS statistical software (IBM SPSS v.25, Chicago, IL) with tests for homogeneity of variance (Levene’s Test). Independent student t-tests were used to compare each NIRS variable between the two groups. Paired sample t-tests were used to compare within group right and left prefrontal cortex differences. Statistical significance was set at p≤0.05.

Table 1. Participant Demographic and Physical characteristics. Values are mean (standard deviation).

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Age (SD) (yrs)</th>
<th>Mean Height (SD) (cm)</th>
<th>Mean Body Mass (SD) (kg)</th>
<th>Mean number of previous concussions (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mTBI (n=55)</td>
<td>59 (8)*</td>
<td>177.4 (5.5)</td>
<td>91.0 (13.9)</td>
<td>4 (4)*</td>
</tr>
<tr>
<td>CTRL (n=29)</td>
<td>64 (8)*</td>
<td>175.8 (6.7)</td>
<td>84.6 (12.5)</td>
<td>0 (0)*</td>
</tr>
</tbody>
</table>

* p≤0.05 indicating significant differences
Results

Of the 55 previously concussed athletes, left-side data of 5 participants was lost due to signal and equipment issues. Of the 29 control participants, left-side data of 1 participant was lost. This resulted in the analysis of the right prefrontal cortex using 55 mTBI and 29 CTRL subjects, and the left analysis using 50 mTBI and 28 CTRL subjects. Right prefrontal cortex differences between the mTBI and CTRL groups are summarized in Table 2 and Figure 1. Left prefrontal cortex differences between the mTBI and CTRL groups are summarized in Table 3 and Figure 2. Within-group right vs. left prefrontal cortex differences are summarized in Table 4 and Figure 3 for the mTBI group, and Table 5 and Figure 4 for the CTRL group.

Right prefrontal cortex findings showed no significant differences in any haemodynamic ∆MAX, Z-scores, or standard deviations between the mTBI and CTRL groups (Table 2). Left prefrontal cortex O₂Hb ∆MAX, HHb Z, HbDiff ∆MAX, and HbDiff SD measures were significantly higher in the mTBI group when compared to the CTRL group (Table 3).
Table 2. Right prefrontal cortex NIRS variables for those with a history of previous concussion (mTBI) and healthy control (CTRL) group. Values are mean (standard deviation).

<table>
<thead>
<tr>
<th>NIRS(µM)</th>
<th>mTBI n=55</th>
<th>CTRL n=29</th>
<th>Levene’s Test</th>
<th>(p≤0.05)</th>
<th>% DIFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₂Hb ∆MAX</td>
<td>5.56 (2.32)</td>
<td>5.14 (2.15)</td>
<td>0.87</td>
<td>0.682</td>
<td>8.17</td>
</tr>
<tr>
<td>O₂Hb Z#</td>
<td>11.56 (7.80)</td>
<td>11.56 (6.24)</td>
<td>0.47</td>
<td>0.659</td>
<td>0.00</td>
</tr>
<tr>
<td>O₂Hb SD</td>
<td>2.37 (0.85)</td>
<td>2.18 (0.77)</td>
<td>0.82</td>
<td>0.490</td>
<td>8.72</td>
</tr>
<tr>
<td>HHb ∆MAX</td>
<td>1.01 (0.58)</td>
<td>0.95 (0.50)</td>
<td>0.58</td>
<td>0.418</td>
<td>6.54</td>
</tr>
<tr>
<td>HHb Z#</td>
<td>8.13 (5.19)</td>
<td>7.51 (4.60)</td>
<td>0.28</td>
<td>0.999</td>
<td>8.26</td>
</tr>
<tr>
<td>HHb SD</td>
<td>0.46 (0.26)</td>
<td>0.46 (0.17)</td>
<td>0.32</td>
<td>0.321</td>
<td>0.65</td>
</tr>
<tr>
<td>tHb ∆MAX</td>
<td>5.76 (2.70)</td>
<td>5.17 (2.38)</td>
<td>0.54</td>
<td>0.614</td>
<td>11.41</td>
</tr>
<tr>
<td>tHb Z#</td>
<td>12.01 (8.46)</td>
<td>11.48 (7.63)</td>
<td>0.65</td>
<td>0.592</td>
<td>4.62</td>
</tr>
<tr>
<td>tHb SD</td>
<td>2.52 (1.05)</td>
<td>2.27 (0.85)</td>
<td>0.33</td>
<td>0.941</td>
<td>11.01</td>
</tr>
<tr>
<td>HbDiff ∆MAX</td>
<td>5.49 (2.16)</td>
<td>5.22 (2.19)</td>
<td>0.57</td>
<td>0.320</td>
<td>5.17</td>
</tr>
<tr>
<td>HbDiff Z#</td>
<td>10.40 (6.87)</td>
<td>10.68 (5.24)</td>
<td>0.43</td>
<td>0.781</td>
<td>-2.62</td>
</tr>
<tr>
<td>HbDiff SD</td>
<td>2.29 (0.77)</td>
<td>2.17 (0.80)</td>
<td>0.56</td>
<td>0.271</td>
<td>5.53</td>
</tr>
</tbody>
</table>

* p≤0.05 (If Levene’s test was significant, p values were used to reflect unequal variance). Percentage (%) DIFF is the difference between the groups (mTBI compared to CTRL). A positive % difference score indicates that the mTBI group was higher than the CTRL group. # = Z-scores are unitless values.
Table 3. Left prefrontal cortex NIRS variables for those with a history of previous concussion (mTBI) and healthy control (CTRL) group. Values are mean (standard deviation).

<table>
<thead>
<tr>
<th>NIRS(µM)</th>
<th>mTBI n=50</th>
<th>CTRL n=28</th>
<th>Levene’s Test</th>
<th>(p≤0.05)</th>
<th>% DIFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₂Hb ΔMAX</td>
<td>5.76 (2.32)</td>
<td>4.75 (1.71)</td>
<td>0.20</td>
<td>0.046*</td>
<td>21.26</td>
</tr>
<tr>
<td>O₂Hb Z#</td>
<td>11.05 (7.85)</td>
<td>8.97 (6.64)</td>
<td>0.89</td>
<td>0.239</td>
<td>23.19</td>
</tr>
<tr>
<td>O₂Hb SD</td>
<td>2.42 (0.82)</td>
<td>2.12 (0.74)</td>
<td>0.49</td>
<td>0.115</td>
<td>14.15</td>
</tr>
<tr>
<td>HHb ΔMAX</td>
<td>1.16 (0.58)</td>
<td>1.16 (0.78)</td>
<td>0.05</td>
<td>0.999</td>
<td>0.00</td>
</tr>
<tr>
<td>HHb Z#</td>
<td>7.67 (4.94)</td>
<td>5.44 (4.19)</td>
<td>0.46</td>
<td>0.048*</td>
<td>40.99</td>
</tr>
<tr>
<td>HHb SD</td>
<td>0.56 (0.25)</td>
<td>0.67 (0.47)</td>
<td>0.03</td>
<td>0.252</td>
<td>-16.57</td>
</tr>
<tr>
<td>tHb ΔMAX</td>
<td>6.13 (2.75)</td>
<td>5.28 (2.06)</td>
<td>0.09</td>
<td>0.162</td>
<td>16.10</td>
</tr>
<tr>
<td>tHb Z#</td>
<td>11.77 (8.93)</td>
<td>8.61 (6.93)</td>
<td>0.49</td>
<td>0.110</td>
<td>36.70</td>
</tr>
<tr>
<td>tHb SD</td>
<td>2.65 (1.02)</td>
<td>2.48 (1.08)</td>
<td>0.54</td>
<td>0.484</td>
<td>6.85</td>
</tr>
<tr>
<td>HbDiff ΔMAX</td>
<td>5.55 (2.11)</td>
<td>4.40 (1.82)</td>
<td>0.70</td>
<td>0.018*</td>
<td>26.14</td>
</tr>
<tr>
<td>HbDiff Z#</td>
<td>9.52 (6.68)</td>
<td>8.61 (5.65)</td>
<td>0.69</td>
<td>0.544</td>
<td>10.57</td>
</tr>
<tr>
<td>HbDiff SD</td>
<td>2.28 (0.71)</td>
<td>1.90 (0.72)</td>
<td>0.99</td>
<td>0.025*</td>
<td>20.00</td>
</tr>
</tbody>
</table>

* p≤0.05 (If Levene’s test was significant, p values were used to reflect unequal variance). Percentage (%) DIFF is the difference between the groups (mTBI compared to CTRL). A positive % difference score indicates that the mTBI group was higher than the CTRL group. # = Z-scores are unitless values.

Within-group differences in bilaterally measured prefrontal cortex valued showed both the mTBI and CTRL group had significantly higher left HHb SD compared with the right prefrontal cortex (Tables 4 and 5). The mTBI group also had significantly higher HHb ΔMAX on the left side compared to the right, and significantly lower HbDiff Z-scores on the left compared to the right (Table 4). The CTRL group also demonstrated significantly lower left O₂Hb Z-scores and HbDiff ΔMAX compared to the right side.
Table 4. mTBI right vs. left prefrontal cortex NIRS variables. Values are mean (standard deviation).

<table>
<thead>
<tr>
<th>NIRS(µM)</th>
<th>Right n=49</th>
<th>Left n=49</th>
<th>(p≤0.05)</th>
<th>% DIFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>O$_2$Hb ∆MAX</td>
<td>5.72 (2.35)</td>
<td>5.73 (2.34)</td>
<td>0.953</td>
<td>-0.17</td>
</tr>
<tr>
<td>O$_2$Hb Z#</td>
<td>12.13 (8.02)</td>
<td>11.17 (7.89)</td>
<td>0.095</td>
<td>8.59</td>
</tr>
<tr>
<td>O$_2$Hb SD</td>
<td>2.41 (0.84)</td>
<td>2.41 (0.83)</td>
<td>0.983</td>
<td>0.00</td>
</tr>
<tr>
<td>HHb ∆MAX</td>
<td>0.96 (0.51)</td>
<td>1.17 (0.58)</td>
<td>0.003*</td>
<td>-17.78</td>
</tr>
<tr>
<td>HHb Z#</td>
<td>7.81 (4.96)</td>
<td>7.74 (4.97)</td>
<td>0.913</td>
<td>0.90</td>
</tr>
<tr>
<td>HHb SD</td>
<td>0.43 (0.18)</td>
<td>0.54 (0.23)</td>
<td>p&lt;0.001*</td>
<td>-21.69</td>
</tr>
<tr>
<td>tHb ∆MAX</td>
<td>5.87 (2.68)</td>
<td>6.11 (2.78)</td>
<td>0.248</td>
<td>-3.93</td>
</tr>
<tr>
<td>tHb Z#</td>
<td>12.46 (8.69)</td>
<td>11.90 (8.97)</td>
<td>0.391</td>
<td>4.71</td>
</tr>
<tr>
<td>tHb SD</td>
<td>2.51 (0.99)</td>
<td>2.63 (1.01)</td>
<td>0.189</td>
<td>-4.56</td>
</tr>
<tr>
<td>HbDiff ∆MAX</td>
<td>5.69 (2.17)</td>
<td>5.50 (2.11)</td>
<td>0.106</td>
<td>3.45</td>
</tr>
<tr>
<td>HbDiff Z#</td>
<td>10.96 (6.93)</td>
<td>9.60 (6.72)</td>
<td>0.010*</td>
<td>14.17</td>
</tr>
<tr>
<td>HbDiff SD</td>
<td>2.36 (0.76)</td>
<td>2.28 (0.72)</td>
<td>0.089</td>
<td>3.51</td>
</tr>
</tbody>
</table>

*=p≤0.05. Percentage (%) DIFF is the difference between the two sides of the prefrontal cortex. A positive % difference score indicates that the right side was higher than the left side. #=Z-scores are unitless values.
Table 5. CTRL right vs. left prefrontal cortex NIRS variables. Values are mean (standard deviation).

<table>
<thead>
<tr>
<th>NIRS(µM)</th>
<th>Right n=28</th>
<th>Left n=28</th>
<th>(p≤0.05)</th>
<th>% DIFF</th>
</tr>
</thead>
<tbody>
<tr>
<td>O$_2$Hb ΔMAX</td>
<td>4.96 (1.95)</td>
<td>4.75 (1.71)</td>
<td>0.559</td>
<td>4.42</td>
</tr>
<tr>
<td>O$_2$Hb Z#</td>
<td>11.15 (5.94)</td>
<td>8.97 (6.64)</td>
<td>0.039*</td>
<td>24.30</td>
</tr>
<tr>
<td>O$_2$Hb SD</td>
<td>2.12 (0.70)</td>
<td>2.12 (0.74)</td>
<td>0.999</td>
<td>0.00</td>
</tr>
<tr>
<td>HHb ΔMAX</td>
<td>0.90 (0.42)</td>
<td>1.16 (0.78)</td>
<td>0.117</td>
<td>-22.84</td>
</tr>
<tr>
<td>HHb Z#</td>
<td>6.88 (3.14)</td>
<td>5.44 (4.19)</td>
<td>0.054</td>
<td>26.47</td>
</tr>
<tr>
<td>HHb SD</td>
<td>0.45 (0.16)</td>
<td>0.67 (0.47)</td>
<td>0.019*</td>
<td>-33.28</td>
</tr>
<tr>
<td>tHb ΔMAX</td>
<td>4.92 (2.02)</td>
<td>5.28 (2.06)</td>
<td>0.454</td>
<td>-6.82</td>
</tr>
<tr>
<td>tHb Z#</td>
<td>10.97 (7.25)</td>
<td>8.61 (6.93)</td>
<td>0.030*</td>
<td>27.41</td>
</tr>
<tr>
<td>tHb SD</td>
<td>2.19 (0.73)</td>
<td>2.48 (1.08)</td>
<td>0.235</td>
<td>-11.69</td>
</tr>
<tr>
<td>HbDiff ΔMAX</td>
<td>5.09 (2.11)</td>
<td>4.40 (1.82)</td>
<td>0.037*</td>
<td>15.68</td>
</tr>
<tr>
<td>HbDiff Z#</td>
<td>10.35 (5.02)</td>
<td>8.61 (5.65)</td>
<td>0.067</td>
<td>20.21</td>
</tr>
<tr>
<td>HbDiff SD</td>
<td>2.13 (0.78)</td>
<td>1.90 (0.72)</td>
<td>0.088</td>
<td>12.11</td>
</tr>
</tbody>
</table>

*=p≤0.05. Percentage (%) DIFF is the difference between the two sides of the prefrontal cortex. A positive % difference score indicates that the right side was higher than the left side. #=Z-scores are unitless values.
Figure 1. Right prefrontal cortex NIRS variables. Values are mean ± standard deviation (µM). Z-scores are unitless values.

Figure 2. Left prefrontal cortex NIRS variables. Values are mean ± standard deviation (µM). *=p≤0.05. Z-scores are unitless values.
Figure 3. mTBI right vs. left prefrontal cortex NIRS variables. Values are mean ± standard deviation (µM). *=p≤0.05. Z-scores are unitless values.

Figure 4. CTRL right vs. left prefrontal cortex NIRS variables. Values are mean ± standard deviation (µM). *=p≤0.05. Z-scores are unitless values.
Discussion

This study explored the long-term effects that multiple concussions can have on dCA using a squat-stand maneuver. This protocol has been used in previous research (Bishop et al., 2017; Smirl et al., 2015), and was shown to effectively activate the baroreflex mechanism. To the authors’ knowledge, this is the first study to explore the long-term effects of multiple concussions on dCA in retired contact sport athletes with a history of multiple concussions using NIRS. The main findings of this study showed that there are significant differences in haemodynamic measures in the mTBI group compared to the CTRL group and within participants’ right and left prefrontal cortex oxygen utilization, suggesting that a history of multiple concussions has a long-term effect on dCA in former contact sport athletes.

In instances where there are rapid blood pressure changes, such as those initiated by movement or exercise, the dynamic cerebral autoregulatory system and the baroreflex mechanism are activated to maintain blood pressure homeostasis to avoid impairments to the pressure sensitive cerebrovasculature (Bishop et al., 2017; Kurowski et al., 2017; Leddy et al., 2016; Len & Neary, 2010; Wright et al., 2018). Using NIRS to measure the haemodynamic alterations that occur under these conditions, it is possible to collect indirect measures reflecting how, or if, the brain vasculature adapts appropriately. There were no significant differences in the right prefrontal cortex of any NIRS variable suggesting that the mTBI and CTRL groups responded similarly to the squat-stand maneuver. However, differences were observed in the left prefrontal cortex, showing significantly higher O$_2$Hb $\Delta$MAX and HbDiff $\Delta$MAX in the mTBI group compared with the CTRLs. Although 25% of subject’s left prefrontal cortex measurements used the
Oxymon NIRS device instead of the Portalite NIRS devices, both systems function in the same manner (both devices are made by the same manufacturer) and the data gathered with one device would therefore be consistent with the other. It has been suggested that the HbDiff represents a cellular measure of metabolic rate and oxygen extraction from arterial blood as HbDiff reflects the difference between \( O_2 \)Hb and HHb (Ferrari & Quaresima, 2012). The higher HbDiff in the mTBI group suggests that as their brain adjusted to the metabolic stresses of the squat-stand maneuver there was a greater demand for oxygen than in the non-concussion CTRL group. This may indicate that in the mTBI group, there was an overcompensation resulting in an increased neural oxygen metabolic activity in an attempt to maintain homeostasis in the brain. Since HbDiff is reliant on \( O_2 \)Hb and HHb, if HHb was not different between the groups, the impact on HbDiff must have been exclusively due to an increase in \( O_2 \)Hb. The significantly elevated mTBI \( O_2 \)Hb values further support the notion that there is autoregulatory dysfunction in the mTBI group as there is a higher demand for oxygenated blood during the squat-stand maneuver. Higher levels of both \( O_2 \)Hb and HbDiff in the mTBI group suggest that the autoregulatory system was inappropriately reacting to the dynamic shifts in cerebral perfusion pressures that are occurring during the squat-stand maneuver. Further evidence for this is the significantly higher left HbDiff SD or variance in the mTBI group. In healthy physiological systems, there is a certain level of variance or randomness in their measures which indicate the system is functionally properly (Bishop et al., 2017; Lang et al., 2003; Pincus, 1991; Urban et al., 2014; Zweifel et al., 2008). However, the higher variance in the mTBI group continues to suggest that their
autoregulatory system is unable to properly react to the dynamic shifts that are occurring in the brain.

The majority of research confirms that any impairments to cerebral autoregulation immediately following a concussion (i.e., days 1 to 4 post-concussion) do eventually resolve over time and show no discernable impairments to autoregulation once they have recovered (Moir et al., 2018; Wright et al., 2018). However, to date no studies have evaluated cerebral autoregulation in retired (previously concussed multiple times) contact sport athletes as they age. Even if an individual has recovered from the concussion, the differences compared to non-concussed retired athletes reported in this study might be subtle enough that the younger individuals might not notice any effects once they have recovered from their acute concussion. These differences might only become fully realised when the healthy systems in the body begin to age and are unable to efficiently manage the impairments in the autoregulatory system. Therefore, more research is needed to determine how these impairments effect the daily function of individuals when they are younger and as they age to determine the impact that can occur. It is important to mention the impact that normal aging has on dCA, especially considering the population that was used in this study. Numerous studies support the age related decreases in cerebral blood flow (Leenders et al., 1990; Wen & Wong, 2019) and baroreflex sensitivity (Huang et al., 2007), with additional research suggesting that aging individuals have delayed cerebrovascular adjustments to the changing cerebral perfusion pressure at the onset of dynamic exercise (Fisher et al., 2017). Although many of the cerebral haemodynamic aspects do tend to change as an individual ages, most research suggests that dCA itself is not greatly affected by the aging process (Carey et al., 2000;
Carey et al., 2003; Yam et al., 2005). If this is the case, any abnormalities in dCA could be interpreted as being the result of something other than age.

Since both mTBI and CTRL groups demonstrated significant bilateral differences in HHb SD, it is assumed that there is a normal difference in the variance between right and left prefrontal cortices regarding HHb SD. Many cerebral processes are lateralized to a specific region in the brain (Cerqueira et al., 2008), therefore differences between right and left prefrontal cortices would be consistent with previous research (Gur & Reivich, 1980; Halsey et al., 1979; Tempest et al., 2014). It is worth exploring the variables that responded differently in the mTBI and CTRL group to determine if there are long-term differences between right and left prefrontal cortices due to multiple concussions. The mTBI group showed significantly higher values in HHb ∆MAX but lower HbDiff Z-score in the left prefrontal cortex. Since these bilateral differences were not observed in the CTRL group, the elevated deoxygenated blood in the left prefrontal cortex of the mTBI relative to their right side represents a pathology not seen in a healthy brain, and may be due to the exercise induced increase in perfusion pressure during the squat-stand maneuver. Additionally, the CTRL group did show significant differences in HbDiff ∆MAX which was not seen in the mTBI group. This may indicate that within a healthy individual, the right prefrontal cortex plays a larger role in oxygen metabolism during baroreflex activation. This has been suggested in previous research showing that the right prefrontal cortex plays a greater role during some of the stages of baroreceptor stimulus processing in adults, (Weisz et al., 2001) and children (Kamiyama et al., 2014). Since no differences were observed in the mTBI group in HbDiff ∆MAX, this points towards the brain being unable to regulate the oxygen metabolic demands as efficiently
as a healthy brain. To help address the reason for the differences between prefrontal cortices, future research should record the mechanism of action of injury and the location where the concussion damage occurred, as well as functional imaging methods to determine if there are differences in functional connectivity between regions.

There are several limitations to acknowledge in this study. Due to the age of the participants the NIRS differential path-length factor (DPF) calculation were not able to be specific for each participant. DPF calculations consist of values up until the age of 50 (Duncan et al., 1995; Kohl et al., 1998). However, since the majority of participants in this study were above the age of 50 the maximal possible DPF value was used for all participants in both groups to maintain consistency. Using the same DPF factor for each participant could have led to an underestimation of the NIRS variables and thus the actual results could be of a greater magnitude than what was recorded. The ability to properly perform the squat-stand maneuver was also a potential limitation for a number of participants. Participants were instructed how to properly squat to 90° at the knees while keeping their heels on the floor and keeping their head level throughout to optimize the NIRS signal. However, some participants had difficulties either looking ahead or reaching the required squat depth to fully elicit the baroreflex response.

The participants were asked to self-report information on prior concussions before participating. This could have led to inaccuracies on the reported number of concussions that were experienced over the course of their life and influenced the study eligibility of participants. Additionally, disclosure of caffeine consumption and participation of previous exercise prior to testing could also have influenced the results. Although all participants were asked to refrain from the consumption of caffeine 6 hours
and participating in strenuous physical activity 12 hours prior to testing, some participants may not have disclosed the amount or timing of caffeine consumption or exercise participation. These confounding variables could have potentially altered the cerebrovascular measures that were recorded throughout the test.

In summary, this study aimed to determine if there were long-term impairments to the dCA in retired contact sport athletes who have experienced multiple concussions throughout their lives. Significant differences were observed in the left prefrontal cortex showing increases in $O_2$Hb $\Delta$MAX, HbDiff $\Delta$MAX, and HbDiff SD in the mTBI group compared to the CTRL group during the squat-stand maneuver, suggesting that the dCA experiences dysfunction in its ability to adapt the metabolic demands to the changes in cerebral perfusion pressure. Additionally, the mTBI group showed uniquely higher left HHb $\Delta$MAX, while the CTRL group showed uniquely higher right prefrontal cortex HbDiff $\Delta$MAX. Giving insight into the changes that are occurring in these retired contact sport athletes can better guide the development of novel treatment options for not only the older athletes but can also guide treatment and preventative options for younger athletes to target impaired systems with treatment to avoid any potential long-term impairments to dCA.

Future research should focus on determining the severity of cerebral haemodynamic impairments that occur on a long-term basis in retired athletes. This could be accomplished by conducting longitudinal studies to follow athletes from their competitive playing careers, into their recreational careers and beyond. Determining any long-term effects of multiple concussions on the physiology of female athletes should also be explored. Finally, additional studies using an integrative physiology approach
(e.g., cerebral blood flow response, blood pressure, electrocardiography, and expired gas analysis) are needed to determine the impact of multiple concussions on long-term performance of these physiological systems. Gaining greater insight into how these systems are affected over time could lead to the development of potential solutions for retired athletes who are currently experiencing impairments, and can better direct intervention strategies early in the concussion recovery process to minimize the effects that concussions can have long-term.

Acknowledgements

The authors would like to sincerely thank all the participants for their time and dedication in support of this data collection. Funding from the Canadian Academy of Sport and Exercise Medicine (CASEM) is greatly acknowledged. We would also like to thank Cameron Bowers, Jake Bryan, Marisa Harrington, and Kathleen Leahy for their assistant in this project.
References


https://doi.org/10.1080/02699052.2016.1226385


https://doi.org/10.1161/01.str.31.12.2895


https://doi.org/10.1161/01.STR.0000081981.99908.F3


https://doi.org/10.1016/j.bbi.2008.01.005


Iverson, G. L., Echemendia, R. J., LaMarre, A. K., Brooks, B. L., & Gaetz, M. B. (2012). *Possible Lingering Effects of Multiple Past Concussions* [Research article]. Rehabilitation Research and Practice. https://doi.org/10.1155/2012/316575


https://doi.org/10.1136/bjsports-2017-097699


pressure and arterial compliance over the adult lifespan with optical imaging.

*PLoS ONE, 12*(2). https://doi.org/10.1371/journal.pone.0171305


https://doi.org/10.1371/journal.pone.0095924


https://doi.org/10.1093/cercor/bhs102


https://doi.org/10.1097/00001756-200110290-00018

Willie, C. K., Colino, F. L., Bailey, D. M., Tzeng, Y. C., Binsted, G., Jones, L. W.,
Haykowsky, M. J., Bellapart, J., Ogoh, S., Smith, K. J., Smirl, J. D., Day, T. A.,
https://doi.org/10.1016/j.jneumeth.2011.01.011


https://doi.org/10.3171/FOC.2008.25.10.E2
Chapter 6: Discussion

This thesis aimed to shed light on the long-term physiological changes that are potentially occurring in each of the three major cerebral autoregulatory mechanisms following concussion injury. Cerebral autoregulation is imperative for maintaining homeostasis in the brain during different instances of stress on the system (Golding et al., 1999), is multifactorial, and mediated by myogenic, autonomic and metabolic processes. Neurovascular coupling activates during increased neural activity, cerebrovascular reactivity activates during the accumulation of CO$_2$ in brain tissue, and dynamic cerebral autoregulation activates during rapid changes in cerebral perfusion pressure. All three systems act through adapting cerebral blood flow to the various demands of cerebral tissues during these various scenarios. Cerebral autoregulation itself is control by the autonomic nervous system and it would be logical to assume that if the autonomic nervous system is impaired, there will be downstream effects on cerebral autoregulation, and in turn, all three autoregulatory mechanisms. The collected data in this thesis suggests that there are significant differences in cerebral haemodynamics in the retired contact sport athletes with a history of concussions (mTBI) compared with the control athletes with no history of concussions (CTRL) in all three cerebral autoregulatory mechanisms. Seeing changes in all three mechanisms provides evidence that the autonomic nervous system itself experiences long-term impairments due to multiple concussions experienced throughout the lifespan of competitive contact sport athletes.

There is mounting research to provide evidence that the autonomic nervous system is impaired in the short-term following a concussion (i.e., 1 to 4 days post-concussion). Impairments in the autonomic nervous system have been reported to last
from days to several years (Hanna-Pladdy et al., 2001; Hilz et al., 2011, 2016, 2017; Jünger et al., 1997), with the majority of research suggesting impairments in the parasympathetic branch of the autonomic nervous system (Abaji et al., 2015; Gall et al., 2004; Hutchison et al., 2017; La Fountaine et al., 2011; Senthinathan et al., 2017). The majority of this research used heart rate variability (HRV) to assess autonomic nervous system function, however, direct measures of cerebral blood flow via transcranial Doppler have been used (Pertab et al., 2018), and are in agreement with the HRV studies that reported impairments of the autonomic nervous system following a concussion (Bailey et al., 2013; Jünger et al., 1997; Strebel et al., 1997; Vavilala et al., 2004). Although this thesis used NIRS to measure cerebral haemodynamics, NIRS and other cerebral blood flow measures have generally been well correlated (Steiner et al., 2008; Vernieri Fabrizio et al., 2004), and has been used to reflect changes in cerebral autoregulation (Highton et al., 2015). Since the evidence suggests that autonomic dysfunction occurs immediately following a concussion, it is possible that these dysfunctions are never properly remedied through the healing process and that there are subtle dysfunctions still present, especially in individuals experiencing multiple concussions, or continuous subconcussive blows, in those that continued to engage in contact sport throughout their life.

It is important to keep in mind that other disease states can also impair autonomic function and these impairments are not unique to concussions. Cardiovascular disease have been reported to include autonomic dysfunction in coronary blood vessels that contributes to the morbidity and mortality of cardiovascular disease (Carney et al., 2005; Harris & Matthews, 2004). There are also numerous mental and cerebral diseases which
impair autonomic function. Individuals suffering with depression also show to have autonomic changes that are characterized by reduction in HRV variables that measure the parasympathetic branch (Carney et al., 2005; Sgoifo et al., 2015; Udupa et al., 2007; van der Kooy et al., 2006). Parkinson disease patients have shown impairments in performance in autonomic function tests, showing reduction in cardiovascular reflex in these patients (Awerbuch & Sandyk, 1994; Kim et al., 2014; Oh et al., 2011). Finally, patients with dementia and Alzheimer’s disease are also suggested to experience autonomic dysfunction affecting parasympathetic and vasomotor pathways (Algotsson et al., 1995; Allan et al., 2007). Although there could be overlap in these diseases with the participants in this thesis due to their age, all participants were asked to self-report any medical conditions, including history of mental disorders. This way it was possible to keep account of any potential co-factors that could have arisen due to different diseases. However, self-reporting is not always fully reliable, and some participants might not have fully disclosed the necessary information.

Common themes emerged from the results of the three studies. The major theme was that the left prefrontal cortex was more effected in the mTBI group than the right prefrontal cortex. The only observed differences in the right prefrontal cortex was a significant increase in HbDiff ∆MAX in the mTBI group during the 20-second breath-hold protocol that assessed CVR. There were some similarities between significant measures between the three tests as well. HbDiff ∆MAX and O₂Hb ∆MAX were shown to be higher in both assessments of CVR and dCA in the mTBI group. These variables were not shown to be different in the assessment of NVC. If autonomic dysfunction is occurring and there is an increase in demand for nutrients to adapt to the different
stresses, it makes sense that the metabolic rate, as measured by HbDiff ΔMAX would be increased in the mTBI group. Since HbDiff ΔMAX calculations are reliant on O₂Hb and HHb, it would be expected that one of these would increase if HbDiff is also increasing. In the case of this thesis, O₂Hb was the value that increased. The breath-hold and squat-stand tests are generally more physiologically challenging than the object identification protocol and could be why the changes in HbDiff and O₂Hb are significantly different in CVR and dCA, and not NVC. It is important to mention that even though the O₂Hb variables in the left prefrontal cortex during the NVC assessment were not significant, there were large trending physiological differences between the mTBI and CTRL groups. For example, O₂Hb ΔMAX was 23% lower in mTBI group (p=0.095), O₂Hb Z-scores were 27% lower in the mTBI group (p=0.151), and O₂Hb SD was 41% lower in the mTBI group (p=0.068). With these trending and significant differences in O₂Hb across all three tests, future research could further explore this relationship to determine if O₂Hb has the potential to be a reliable measure of haemodynamic dysfunction in individuals with a history of past concussions.

As to why the left prefrontal cortex is more affected than the right side is difficult to elucidate from these results. One idea is that the side of the head that was hit during the concussion could play a role in the long-term impairments observed later in life. Unfortunately, this information could be difficult to collect on a retrospective basis as participants could remember the details differently over time, and could forget potentially minor details such as the location of where they were hit. The location of injury has been shown in other diseases to influence the autonomic nervous system in different hemispheres. For example, stroke patients impacted in specific hemispheres
showed imbalances in the autonomic nervous system, especially on the affect side (Al-Qudah et al., 2015; Barron et al., 1994; Sykora et al., 2008). Another potential explanation is the effects that concussion may have on long-term structure and function of the prefrontal cortex. In mice studies, exposure to chronic stress showed a decrease in the measured volume of the medial portion of the left prefrontal cortex (Cerqueira et al., 2005), with similar findings shown in human MRI studies as a result of healthy aging (MacLullich et al., 2006). A reduced volume could result in the left prefrontal cortex having less impact on the inhibition of the stress response in the right prefrontal cortex. Most processes in the healthy brain are lateralized (Cerqueira et al., 2008) as in the left and right hemispheres are specialized and responsible for different functions. In different disease states, this lateralization becomes disrupted and research has shown that the specialization of the hemispheres dissipates (Davidson, 1998; Johnstone et al., 2007). Multiple concussions may be playing a role in the shifting of the control centres in the brain away from the preferred prefrontal cortex side. This may be a potential explanation as to why the results of this thesis show that some of the cerebral haemodynamic measures increased, as the one side had to overcompensate for the lack of specialized processes and would require more nutrients to function. The over-activated side might not be optimized to handle the control of that specific task as well as the side showing no differences between groups. However, more research is needed to determine the exact inner workings of this process and provide greater insight into why there are differences between right and left prefrontal cortices.

One limitation with this thesis is the assumption that any changes observed in cerebral autoregulation is the result of a concussion that the former athletes had suffered
during their playing career. Long-term alterations in cerebral measures, in general, are often complex and many additional factors may play a role in influencing these long-term changes manifesting later in life. Therefore, previous concussions may not be the driving force behind why autoregulation impairments are being observed in this population. There have been links between depression and left prefrontal cortex autoregulation impairments (Florence et al., 1994; George et al., 1994; Koenigs & Grafman, 2009). Although dysregulation was noted in these thesis studies collectively, we did not assess levels of depression in our participants, but this may be an alternative explanation as to why the results of this thesis showed the left prefrontal cortex to be predominantly affected. Additionally, studies suggest that the impairments seen in concussions are not unique and are often shared with individuals who suffer from chronic pain (Gasquoine, 2000; Smith-Seemiller et al., 2003; Snell et al., 2018). This study did not assess the presence or severity of chronic pain in the participants and it is likely that through playing contact sports throughout their life, there would be some prevalence of chronic pain within this population and this may be a reason as to why changes in autoregulation are occurring. Future research should explore these additional factors further to clarify the effect that they may have in conjunction with previous concussions.

To assess all three mechanisms at one point of time can be a challenge as they require different testing methods to evaluate each system. The Neary Protocol (2019) is an excellent option to assess the three mechanisms during the same procedure, as it combines all three mechanisms into a single protocol that can be followed with participants. Although this makes the protocol require a large time commitment to
complete, based on the results of these studies, assessing all three mechanisms was important as they explore different aspects of cerebral autoregulation; i.e., from a chemical (CVR), metabolic (NVC), and pressure (dCA) perspective. However, the 20-second breath-hold maneuver assessing CVR showed the most changes out of all three mechanisms in both the right and left prefrontal cortices and could be an excellent candidate for a test to assess autonomic dysfunction if time was a limitation. Looking at CVR can provide a more detailed insight into the specific vasomotor control of blood vessels in the prefrontal cortex which is heavily controlled by the autonomic nervous system. Providing insight into this mechanism could allow researchers to gain an understanding of any impairments to the autonomic nervous system. The 20-second breath-hold test is also simple to perform, and other than a NIRS device to measure cerebral haemodynamics, requires no outside equipment to conduct. There is also published literature to support its use for acute concussion injury (Bishop & Neary, 2018; Len et al., 2011, 2013). Additionally, unlike the squat-stand maneuver, there is very minimal physical requirement from the participants as the protocol could all be conducted in a seated position. However, both the 5-minute object identification protocol and the 10-second squat-stand test both have potential advantages. The 5-minute object identification protocol can give potential insight into instances of high metabolic demands which, when impaired, are often the cause of focusing and concentration issues seen in the acute and long-term dysfunctions of concussions (Chen et al., 2007; Guskiewicz et al., 2007; Montenigro et al., 2016; Smirl et al., 2016). The 10-second squat-stand showed the most differences between the right and left prefrontal cortices in the CTRL group which could be beneficial for gaining insight into the normal
physiological response to pressure changes and baroreflex sensitivity. In the end though, it is still recommended to utilize all three tests to have a complete picture of the potential changes that are occurring in the autonomic nervous system.
Chapter 7: Conclusion and Future Direction

This thesis aimed to use NIRS to objectively measure the changes in cerebral haemodynamics in the three main autoregulatory mechanisms in retired contact sport athletes with a history of multiple concussions during their life in contact sport. Three studies, separately exploring the neurovascular coupling mechanism, cerebrovascular reactivity, and dynamic cerebral autoregulation showed that there are long-term impairments to cerebral autoregulation in individuals with a history of multiple concussions, and thus suggests the presence of autonomic nervous system dysfunction. Giving insight into the changes that are occurring in these retired contact sport athletes can better guide the development of treatment options for not only the older athletes but can also guide treatment and preventative options for younger athletes to avoid any potential long-term impairments to cerebral autoregulation that may occur.

Future research is warranted to determine the severity of cerebral haemodynamic impairments that are occurring on a long-term basis in retired athletes by conducting longitudinal studies following athletes from their playing careers, into their recreational careers and retirement from sports. A focus should also be made to determine the effects that multiple concussions can have on the physiology of female athletes to determine any long-term effects concussions may have. Additional studies exploring using an integrative physiology approach (e.g., cerebral blood flow response, blood pressure, electrocardiography, and expired gas analysis) are needed to fully assess the impact that concussions can have on long-term performance of physiological systems. Finally, research is needed to determine the underlying mechanisms that are seen in the results of this thesis. Specifically, to answer the question as to why the left prefrontal cortex seems
to be more impaired than the right side. By providing greater insight into how these systems are affected long-term, this could present an opportunity to improve the target systems for interventions early in the concussion recovery process and potential solutions for retired athletes who have current impairments.
References for Introduction, Review of Literature, and Discussion

https://doi.org/10.1161/01.STR.20.1.45


https://doi.org/10.1152/ajpregu.91008.2008

https://doi.org/10.1016/j.neulet.2009.01.030

https://doi.org/10.3109/02699052.2015.1031699
https://doi.org/10.1111/j.1600-0404.1995.tb05836.x

https://doi.org/10.1136/jnnp.2006.102343


https://doi.org/10.1007/s40279-016-0532-y

https://doi.org/10.3200/JACH.57.5.521-526

https://doi.org/10.1001/jamaneurol.2017.2396


https://doi.org/10.1523/JNEUROSCI.0041-13.2013


https://doi.org/10.1176/appi.ajp.2009.09050617


https://doi.org/10.15252/embj.201695810


*Radiology, 216*(3), 672–682. https://doi.org/10.1148/radiology.216.3.r00au37672


https://doi.org/10.3389/fnhum.2019.00294


https://doi.org/10.3109/02699052.2014.965207


https://doi.org/10.1016/j.neuroimage.2013.04.113


Iverson, G. L., Echemendia, R. J., LaMarre, A. K., Brooks, B. L., & Gaetz, M. B. (2012). *Possible Lingering Effects of Multiple Past Concussions* [Research article]. Rehabilitation Research and Practice. https://doi.org/10.1155/2012/316575


https://doi.org/10.1016/S0197-4580(01)00217-2


https://doi.org/10.1523/JNEUROSCI.2063-07.2007


https://doi.org/10.3171/jns.1997.86.3.0425


https://doi.org/10.1016/j.spen.2019.03.003


https://doi.org/10.1148/radiol.13130545

https://doi.org/10.1227/01.NEU.0000149008.73513.44

https://doi.org/10.1089/neu.2015.4323

https://doi.org/10.1207/s15324826an1303_4

https://doi.org/10.1210/jc.2005-2610


https://doi.org/10.1016/B978-0-444-52892-6.00004-0

https://doi.org/10.1080/026990596124016

https://doi.org/10.1136/jnnp-2016-314279

https://doi.org/10.1016/j.jsams.2014.07.008
https://doi.org/10.2190/CUMK-THT1-X98M-WB4C

https://doi.org/10.1001/jama.2017.8334


https://doi.org/10.1227/01.NEU.0000166663.98616.E4

https://doi.org/10.1371/journal.pone.0102181


Copies of Ethics Forms

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**Title of Project:**

**Global Rugby Health Research Programme:** *Integration of multimodal imaging techniques for assessment and diagnosis of concussion or mild traumatic brain injury (mTBI) REB 2017-032*

**Principle Investigator(s):**

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The university and those conducting this project subscribe to the ethical conduct of research and to the protection at all time of the interests, comfort, and safety of participants, and this project has been approved by the University of Regina Ethics Board (#2017-032). This form and the information it contains are given to you for your own protection and full understanding of the procedures in this research. Your signature on this form will signify that you have received a document that describes the procedures, objectives, possible risks, and benefits of this research project, that you have received an adequate opportunity to consider the information in the document, and that you voluntarily agree to participate in the project.

**Purpose:** This project is aimed at understanding the physiological responses during rest, during changes in respiration (breathing) rate, and during exercise following a concussion in retired rugby, ice hockey and football players. It is hoped that this research will provide an indication of the long-term effects of multiple concussions on brain and heart function (physiology).

[February 5, 2019]
Procedures: This testing will be performed in a laboratory setting that will ensure your privacy. This testing will last approximately one-hour and you will be asked to complete some medical information forms. Then the following protocol will be completed:

- Seated rest for 5 minutes,
- Bent Over Position Test for 2 minutes (lower your head between your knees, elbow on knees), or 6 breaths per minute for 5 minutes,
- Washout period for 2 minutes to recover to baseline,
- Neurovascular coupling test (“Where’s Waldo”), 5 minutes (20 sec eyes closed: 40 sec eyes open x 5 repeats)
- Washout period for 2 minutes to recover to baseline,
- Repeated breath-holds for 5 minutes (20 sec hold: 40 sec normal breathing x 5 repeats)
- Washout period for 2 minutes to recovery to baseline,
- Stand for 1 minute
- Squat-Stand maneuver (squat for 5 or 10 sec, stand for 5 or 10 sec, repeated for 5 minutes).

During testing, you will be connected to medical equipment. One device is a probe that will measure your brain oxygenation levels and blood vessels changes (called near infrared spectroscopy). ECG electrodes to measure heart rate, a finger blood pressure monitor, and a mouthpiece connected to a gas analysis system to record expired gases when you breathe. All equipment is non-invasive and is completely safe, and has been used in hundreds of research projects. You will be assessed on a single occasion in the Concussion Testing Centre laboratory which will take approximately 45-50 minutes. As well you will be asked to complete two separate on-line questionnaire to gather information about your general health and a neurocognitive test. The questionnaires can be found at: [http://www.leedsbeckett.ac.uk/ukrugbyhealth/](http://www.leedsbeckett.ac.uk/ukrugbyhealth/) and [www.cnsvs.com](http://www.cnsvs.com). Once you have signed up to the research online via the project webpage (www.leedsbeckett.ac.uk/ukrugbyhealth) you will receive an automatic email which provides you with a link to the neurocognitive test webpage (www.cnsvs.com) and the general health questionnaire.

Potential Risks and Discomforts: The protocol described above may cause you to feel fatigued or tired and this is a normal response. However, If you feel, at any time, that you need to stop any of the tests being conducted you may do so without penalty. The research and techniques that we will use in this testing have been used in our previous research conducted by Dr. Patrick Neary at the University of Regina.

Possible Benefits to Subject and/or Society: This research study may provide you with an opportunity to gain some knowledge and education about concussions in sport. Your participation in this study may also assist us, the researchers, to determine the long-term effects of multiple concussions on brain and heart physiology. However, our data cannot be used to confirm the diagnosis of a concussion.

Confidentiality: Any information that is obtained during this study will be kept confidential to the full extent permitted by law. Participants will be given a unique identification number and any information provided will be marked with this ID number. All confidential materials will be locked in a filing cabinet in Dr. Patrick Neary’s laboratory or office. Graduate students and research assistants working under the supervision of Dr. Neary will also have access to the coded data for

[February 5, 2019]
their research. If we publish our data, only average results will be reported and thus you will not be identified and will remain anonymous. Our anonymised research results will also be shared with Dr. Karen Hind and colleagues at Leeds Beckett University, in Leeds UK, Dr. Steve Martin at the University of Victoria, Dr. Patricia Hume, Matthew Wood, and Josh McGeown and colleagues from New Zealand, Drs. Fraser and Pears in Australia, and Drs. Winklewski, Gruszecki, Piskorski from Poland.

Participants and Withdrawal: If you feel, at any time, that you would like to withdraw from the research study, you may do so freely and without consequence. Your right to withdraw data from the study will apply for 3 months from the date of your involvement in the study. After this time it is possible that some form of research dissemination will have already occurred and it may not be possible to withdraw your data.

Feedback: You will have full access to your individual test results, upon request, once the data has been analyzed.

Questions: This project was approved by the Research Ethics Board, University of Regina. If research participants have any questions or concerns about their rights or treatment as participants, they may contact the Chair of the Research Ethics Board at (306) 585-4775 or by e-mail at research.ethics@uregina.ca. If, at any time, you have questions about this study, feel free to ask any of the investigators.

Participation is voluntary and you may withdraw at any time without penalty. Refusal to participate in this study will not influence or affect the management of your injury and your return to activity. Please complete the section on the following page. Thank you for your participation!
Consent for Subjects to Participate in this Research Project

Title of Project: Global RugbyHealth Research Programme: Integration of multimodal imaging techniques for assessment and diagnosis of concussion or mild traumatic brain injury (mTBI) (REB 2017-032).

- I understand that my participation in this study is voluntary and that I may withdraw my participation in this experiment at any time, without any consequences.

- I am aware that I will be expected to disclose any and all previous concussion injuries I have sustained to the best of my ability.

- I have been informed that all information collected from me will be treated confidentially, and will be locked in the filing cabinet of the principle investigator(s), Dr. J. Patrick Neary, and graduate students involved in this study from the University of Regina and other academic institutions involved in this research will also have direct access to your data for their research. Other colleagues mentioned in the information sheet will only have access to your coded data.

- I have been assured that I may contact Dr. Patrick Neary at patrick.neary@uregina.ca (306-585-4844) at any time if I have questions or would like more information directly related to this study. I may obtain a copy of my results, upon completion of the study, by contacting the above person.

- This project was approved by the Research Ethics Board, University of Regina. If I have any questions or concerns about my rights or treatment as a participant, I may contact the Chair of the Research Ethics Board at (306) 585-4775 or by e-mail at research.ethics@uregina.ca.

- I understand the contents of this form, and I agree to participate in this research study.

- I have received a copy of the information sheet and informed consent form for my records.

NAME (Please print legibly): _____________________________

ADDRESS: _______________________________________

________________________________ Phone: ________

SIGNATURE: ___________________ DATE: ____________

WITNESS: ________________________


Please Note: A future objective of Dr. Neary is to consult to professional sport, and therefore information learned in this research could be used to develop novel software for the diagnosis of concussion and possible commercialization for financial gain. However, your data will remain completely anonymous. You are entitled to ask any questions regarding this research for financial gain.

[February 5, 2019]
DR. PATRICK NEARY
Faculty of Kinesiology and Health Studies

TITLE: Global Rugby Health Programme

AMENDMENT APPROVAL OF: Revised consent form to reflect changes in access of coded data

NEXT RENEWAL DATE: March 8, 2019

AMENDMENT APPROVAL DATE: January 31, 2019

AMENDMENT CERTIFICATION
The University of Regina Research Ethics Board has reviewed the changes to the above-named research project as outlined in your memos dated January 21, 2018 and February 6, 2019, and they are approved.

ONGOING REVIEW REQUIREMENTS
In order to receive annual renewal, a status report must be submitted to the REB Chair for Board consideration within one month of the current expiry date each year the study remains open, and upon study completion. Please refer to the following website for the renewal and closure forms:

https://www.uregina.ca/research/for-faculty-staff/ethics-compliance/human/ethicsforms.html

Ara Steininger
Research Ethics Board

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# Certificate of Approval

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**PROJECT TITLE:**
Canada - UK Rugby Health Project: Integration of multimodal imaging techniques for assessment and diagnosis of concussion or mild traumatic brain injury (mTBI) (Functional near-infra red spectroscopy of the right and left prefrontal cortex in retired rugby players with concussion and retired non contact athletes)

**RESEARCH TEAM MEMBER**
Co-Leads: Dr. Catherine Gaul (UVic), Dr. Lynne Stuart-Hill (UVic), Dr. J. Patrick Neary (University of Regina), Ryan Dech (Graduate Student, University of Regina), Jyotpal Singh (Graduate Student, University of Regina)

**DECLARED PROJECT FUNDING:** CASEM (pending)

## CONDITIONS OF APPROVAL

This Certificate of Approval is valid for the above term provided there is no change in the protocol.

** Modifications**
To make any changes to the approved research procedures in your study, please submit a "Request for Modification" form. You must receive ethics approval before proceeding with your modified protocol.

**Renewals**
Your ethics approval must be current for the period during which you are recruiting participants or collecting data. To renew your protocol, please submit a "Request for Renewal" form before the expiry date on your certificate. You will be sent an emailed reminder prompting you to renew your protocol about six weeks before your expiry date.

**Project Closures**
When you have completed all data collection activities and will have no further contact with participants, please notify the Human Research Ethics Board by submitting a "Notice of Project Completion" form.

## Certification

This certifies that the UVic Human Research Ethics Board has examined this research protocol and concluded that, in all respects, the proposed research meets the appropriate standards of ethics as outlined by the University of Victoria Research Regulations Involving Human Participants.

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Dr. Rachael Scarth  
Associate Vice-President Research Operations

Certificate Issued On: 02-Jun-17