A PROSPECTIVE, MULTIDISCIPLINARY APPROACH TO UNDERSTANDING SPORT-RELATED HEAD TRAUMA: NOVEL INSIGHTS INTO THE EFFECTS ON MYELIN AND CEREBROVASCULAR FUNCTION

by

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Abstract

Sport-related concussion occurs at an alarmingly high rate, affecting millions each year. Concern is growing over the effects of repetitive subconcussive head trauma towards the development of long-term neurological deficits. There are calls within sports medicine to shift from symptom-based assessments towards reliable, objective tools to improve identification and management of dangerous levels of sport-related head trauma. The overall objective across the five studies of this thesis was to elucidate the roles of brain myelination and cerebrovascular dysfunction within this context. In study 1, myelin water imaging permitted the first direct evaluation of the effect of sport-related head trauma on myelin integrity. While transient post-concussion disruptions in myelin were observed for at least 2-weeks in multiple brain areas, no myelin changes were observed as a consequence of repetitive subconcussive trauma. Study 2 used transcranial Doppler ultrasound to assess the effects of concussion on indices of dynamic cerebral autoregulation (CA), demonstrating a delay in the CA response to blood pressure alterations persisting beyond symptom resolution that is suggestive of autonomic dysregulation of the cerebrovasculature; deficits did not appear to be cumulative across multiple injuries. Study 3 revealed detrimental effects of one season of contact sport participation on both the timing and magnitude of CA responses related to the degree of exposure to repetitive subconcussive head trauma. Study 4 assessed the effect of concussion on neurovascular coupling (NVC) dynamics within the artery supplying the occipital cortex following visual stimulation, and revealed delays in achieving peak response rate in concert with an elevated response magnitude acutely post-injury with resolution by 1-month; deficits did not appear to accumulate across multiple injuries. Study 5 revealed no changes in NVC dynamics as a function of exposure to one season of participation in contact or non-contact sport, suggesting subconcussive trauma may not impair

NVC. Collectively, these results suggest sport-related head trauma can impair myelin integrity and cerebrovascular function; the potential role for autonomic dysregulation towards these findings is discussed. While the effect of repetitive subconcussive trauma on susceptibility to injury remains unclear, the disruptions observed following acute concussion highlight the emerging distinction between clinical and physiological recovery.

Lay Abstract

Lessening the health risks of sport-related head trauma requires a better characterization of the disruptions it causes to the brain, including microstructural structural damage and control of brain blood flow. Study 1 of this thesis showed concussions cause disruptions to the myelin sheath that surrounds brain fibers lasting at least two weeks after the injury. Studies 2-5 in this thesis show concussions, as well as exposure to head hits experienced just by playing hockey and football, may disrupt two important mechanisms controlling blood flow to the brain. Some disruptions in physiology after concussion may last longer than symptoms and / or outwardly observable behaviour, suggesting clinical and physiological recovery may be distinct. The potential role towards these findings for alterations in autonomic nervous system behaviour is discussed.

Preface

Chapter 2 is based on work conducted at the UBC MRI Research Centre by Dr. A. Rauscher and has been published: Wright AD, Jarrett M, Vavasour I, Shahinfard E, Kolind S, van Donkelaar P, Taunton J, Li D, Rauscher A (2016) Myelin water fraction is transiently reduced after a single mild traumatic brain injury – a prospective cohort study in collegiate hockey players. PLoS ONE 11(2): e0150215. AR, JT and DL designed the study. AR and DL designed the imaging protocol. MJ, ADW, IV, ES and SK performed data analysis under the supervision of AR. All authors interpreted the data. ADW, MJ and AR wrote the manuscript. ADW, MJ, and AR completed all revisions following reviewers' comments and completed the final version accepted for publication. This study was approved by the University of British Columbia Clinical Research Ethics Board (H11-00423). Copyright approval was obtained from the Public Library of Science for reproduction of figures and text.

The methods used in Chapters 3-6 of this thesis were approved by the Clinical Research Ethics Board at the University of British Columbia, as part of a larger multidisciplinary study assessing the physiological effects of sport-related head trauma involving a variety of methods and hypotheses (H14-02996). Data were collected at the University of British Columbia (Kelowna, BC) by Alexander D. Wright, Kelsey Bryk, Krista Fjeld, Jillian Dierijck, Kevin Bouliane, and Jonathan D. Smirl. In-season impact sensor data were collected by Alexander D. Wright and Kelsey Bryk.

A version of Chapter 3 has been submitted and is currently under review: Wright AD, Smirl JD, Bryk K, Fraser SK, van Donkelaar P (2017). A transcranial Doppler ultrasound-based evaluation

of the acute and cumulative effects of sport-related concussion on indices of dynamic cerebral autoregulation. This chapter describes work conducted in Kelowna, BC at the UBC Sport Concussion Research Lab. ADW and PvD designed the study. ADW, JDS, and KB performed data collection, and ADW, JDS, and SF performed the analyses. ADW and JDS interpreted the data. ADW wrote the manuscript. All authors had full access to the data, and helped to critically revise the manuscript before reviewing and approving the final version.

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Glossary

Symbol	Definition (units)
Δ	Delta, change in the suffixed parameter
AD	Axial diffusivity
ANOVA	Analysis of variance
ASL	Arterial spin labelling
AUC_{25}	Area-under-the-curve to 25 seconds (cm)
BESS	Balance error scoring system
BMI	Body mass index (kg/m^2)
BOLD	Blood oxygen level dependent
BP	Blood pressure
CA	Cerebral autoregulation
95%CI	95% confidence interval
CBF	Cerebral blood flow
CBFv	Cerebral blood flow velocity (cm/s)
CC	Corpus callosum
CO_2	Carbon dioxide
cPLA	Cumulative peak linear acceleration (g)
cPRA	Cumulative peak rotational acceleration (rad/s^2)
CT	Computed tomography
DAI	Diffuse axonal injury
DLPFC	Dorsolateral prefrontal cortex
DTI	Diffusion tensor imaging
ECG	Electrocardiogram
FA	Fractional anisotropy
fMRI	Functional magnetic resonance imaging
HIC	Head injury criterion
HITS	Head Impact Telemetry System™
HR	Heart rate (beats/min)
ICA	Internal carotid artery

ICP	Intracranial pressure (mmHg)
LF:HF	Low frequency to high frequency power ratio
MAP	Mean arterial pressure (mmHg)
MBP	Myelin basic protein
MCA	Middle cerebral artery
MCAv	Middle cerebral artery blood velocity (cm/s)
MD	Mean diffusivity
MRI	Magnetic resonance imaging
mTBI	Mild traumatic brain injury
MWF	Myelin water fraction (%)
MWI	Myelin water imaging
NAA	N-acetyl-aspartate
NVC	Neurovascular coupling
O_2	Oxygen
PCA	Posterior cerebral artery
PCAv	Posterior cerebral artery blood velocity (cm/s)
PCO_2	Partial pressure of carbon dioxide (mmHg)
PCS	Post-concussion syndrome
$P_{ET}CO_2$	End-tidal partial pressure of carbon dioxide (mmHg)
PLA	Peak linear acceleration (g)
PRA	Peak rotational acceleration (rad/s^2)
RD	Radial diffusivity
SAC	Standardized Assessment of Concussion™
SCAT	Sport Concussion Assessment Tool™
SD	Standard deviation
SEM	Standard error of the mean
SRC	Sport-related concussion
TBI	Traumatic brain injury
TBSS	Tract-based spatial statistics
TCD	Transcranial Doppler ultrasound
TFA	Transfer function analysis

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Despite the fact that my name alone is listed on the title page, this thesis would not have been possible without many others. I offer my enduring gratitude to all members of the Sport Concussion Research Lab, with particular thanks to Jonathan Smirl, Mike Kennefick, and Kelsey Bryk for their contributions to this work and to my overall doctoral experience. Thank you to all of the teams with whom we have worked, especially Mike Bois and the West Kelowna Warriors. I am grateful to have received substantial financial support during my doctoral program from the Vanier Canada Scholarship Program, the Canadian Institutes for Health Research, the O'Brien Foundation, the UBC Faculty of Medicine, and the UBC MD/PhD Program. I must also thank Jane Lee, Virginia Grossman, Carri Folk, as well as Drs. L Raymond and V. Duronio for your practical and emotional support in navigating the challenges of being the first student completing the MD/PhD program outside of Vancouver. I owe many thanks to my supervisory committee – Drs. C. Wellington, A. Rauscher, P. Ainslie, and P. van Donkelaar – for your mentorship and support, for providing coherent answers to my oft-incoherent questions, and for enlarging my vision of science and academic medicine. My success thus far is a function of the love, guidance, and emotional and financial support provided by my family. My mother, father, and sister have always supported my pursuits, academic and otherwise, without hesitation or question. You often tell me of how proud you are, but I too am so proud to be able to call you family. To my daughter, Ella, your kind soul and adventurous spirit are contagious; coming home to you each day fills my world in ways you cannot yet understand. I am so excited to watch you grow into a strong and beautiful woman. To my wife, Kari, I owe you endless gratitude for your love, support, and patience over the past 8 years. Amazingly, we are still but at the beginning of our exciting journey together, *Gallivanting* through life as partners-in-crime. I love you.

For Ella

Be a free thinker and don't accept everything you hear as truth – be critical and evaluate what you believe in.

- Aristotle

Chapter 1: Introduction to sport-related concussion

Concussion has existed for centuries as a clinical entity, but scientific understanding and public awareness around this complex traumatic brain injury (TBI) has dramatically accelerated over the last decade. Sport-related head injury remains one of the most poorly understood injuries in the fields of sports medicine and neuroscience (1,2). Only recently has the perfect storm been set: the combination of intense public interest, advanced clinical techniques, and more comprehensive research approaches have shaped today's "collective cultural focus on sports concussion" (3). Despite this widespread attention, many questions remain; the research question that guides this thesis is: what are the roles for disruptions to brain myelination and to control of cerebral blood flow towards the symptoms and deficits in neurocognitive function observed with exposure to sport-related head trauma?

1.1 Scope of the problem

Sport-induced head trauma occurs at an alarmingly high rate – recent reports estimate the annual incidence of sport-related concussion at 1.1-1.8 million in US youth alone (4). According to the Centers for Disease Control and Prevention, the concussion problem has reached epidemic levels (5). Estimates of the prevalence of sport-related concussion in adolescent contact sport athletes range from 10-25% (6,7). Concerningly, these estimates are thought to drastically underrepresent the true injury burden as the majority of concussed individuals do not report for medical attention; for example, it has been estimated that up to 53% of high school football players do not report concussion symptoms (8) and consequently go undocumented.

Management of concussion is based primarily on resolution of athlete-reported symptoms (9). The Canadian Paediatric Society has outlined the need for more objective tools to improve initial diagnoses, guide management, and inform decisions regarding appropriate timing for athletes to return-to-learn and return-to-play (10). Intentional underreporting of symptoms in order to expedite return-to-play increases the risk for further concussions and longer-term consequences associated with repetitive head trauma (11-14). While 80-90% of concussions resolve in 7-10 days, a subset of individuals battle persistent disability for months to years (9). Emerging evidence also suggests a link between clinically-manifest concussions, repetitive sub-clinical head impacts, and the development of long-term neurodegeneration termed chronic traumatic encephalopathy (15). Furthermore, there is a growing evidence base supporting a link between repetitive subconcussive trauma specifically, and impairments in various aspects of brain structure and function including learning (16), cerebral blood flow regulation (17-19), functional brain connectivity (20-22), gray matter density and volume (23), and diffusion properties in white matter tracts (24-26). Long-lasting effects of sport-related head trauma may include problems with affect regulation, attention, memory, and depression (15). Collectively, the persistent physiological effects of sport-related head trauma in combination with the heavy reliance on symptom reporting for patient management emphasize the pressing need to develop objective techniques to identify, treat, and manage dangerous levels of head trauma towards minimizing the burden of injury at an individual and population level. Despite the recent surge in clinical, academic, and public attention to concussion, the problem is a historical one.

1.2 Historical perspective

1.2.1 Earliest descriptions of concussive injury

The earliest clinical description of concussion as a mild brain injury characterized by an alteration in physiologic state rather than more severe structural brain injury was proposed by the Persian physician Rhazes (AD 850-923) in the 10th century, representing a "critical turning point in … the understanding of [concussion]" (27). Lanfrancus (d. 1306) made particular note of the transient nature of concussion symptoms, hypothesizing them to be a consequence of a functional paralysis caused by the brain being shaken. In doing so, he was the first to distinguish between *commotio cerebri* (shaking of the brain) and *contusio cerebri* (involving structural damage). In the early 1500s, Berengario da Carpi postulated *commotio cerebri* caused cortical tissue to be pressed against the solid skull (da Carpi 1535, Abbott 1961, and Flamm 1996 in (27)), an idea that was expanded upon by Ambroise Paré in his *Workes* in which he extensively described "the moving or concussion of the brain" and considered "commotion" to be a disorder of brain movement (Paré 1579 in (27)). Many clinical insights were documented over the subsequent ~100 years, including descriptions of lethargy, vertigo, tinnitus, as well as disturbances of balance, sleep, and emotions following concussive injury (27). Queyrat (17th century) furthered the *commotio cerebri* hypothesis, adding that concussion was not the result of a single brain movement, but rather from oscillatory movement of the brain within the skull (Queyrat 1657 in (27)). The invention of the microscope in 1694 permitted the earliest hypotheses into the pathological consequences of concussion, which ranged from vascular causes to neuronal shock. A case study reported by Littre in 1705 generated the first hypothesis that concussion caused circulatory failure and cerebral venous congestion, ultimately irritating the brain. His observations led to the theory that head impacts causing deformation of the skull could

redistribute blood and lead to an underperfusion ("anemia") in the underlying gray matter (Littre 1705 in (27)). Despite many theories, the prevailing view on concussions by the late 1700s entailed that such injuries were not accompanied by structural brain injury – an idea that has only been challenged in the 21st century.

1.2.2 Defining and classifying concussion – evolution of the grading system era

The term concussion remains poorly defined in both clinical and research realms, and debate continues as to whether the term can and / or should be used interchangeably with mild traumatic brain injury (mTBI). The earliest proposal for a consensus definition of concussion or mTBI came in 1966, when the Congress of Neurological Surgeons defined concussion as "a clinical syndrome characterized by immediate and transient impairment of neural functions, such as alteration of consciousness, disturbance of vision, equilibrium, etc. due to mechanical forces." (28). Four definitions of mTBI have subsequently been adopted by groups including the American Congress of Rehabilitation Medicine (ACRM) (29), the American Academy of Pediatrics (AAP) (30), the Centers for Disease Control and Prevention (CDC) (31), and the World Health Organization (WHO) (32). Each of these definitions places emphasis – to differing degrees – on loss of consciousness (LOC), post-traumatic amnesia (PTA), mental status, and neurological signs (Table 1-1).

Table 1-1 Comparison of four main definitions of mild traumatic brain injury.

Source	Etiology	LOC	PTA	Mental Status	Neurological Signs	Other
ACRM	A traumatically induced physiological disruption of brain function	≤ 30 mins	≤ 24 h	Any alteration in mental status at time of injury	May or may not be transient	Initial GCS of 13-15
CDC	Injury to the head resulting from blunt trauma or acceleration / deceleration	≤ 30 mins	Observed / self-reported dysfunction of memory around the time of injury	Observed / self-reported transient confusion, disorientation, or impaired consciousness	Observed signs of other neurological or neuropsychological dysfunction (seizures, irritability, lethargy, nausea / vomiting)	Not specified
WHO	An acute brain injury resulting from mechanical energy to the head from external physical forces	≤ 30 mins	≤ 24 h	Confusion and disorientation	Transient neurological abnormalities (focal signs, seizure, intracranial lesion not requiring surgery)	GCS 13-15 after 30 minutes must not be due to drugs / alcohol / medications / other injuries
AAP	Not specified	≤ 1 min	Not specified	Normal mental status at time of initial evaluation	None at exam, but may have seizures, emesis, headache, lethargy immediately following injury	No evidence of skull fracture

ACRM = American Congress of Rehabilitation Medicine; CDC = Center for Disease Control and Prevention; WHO = World Health Organization; AAP = American Academy of Pediatrics; LOC = loss of consciousness; PTA = post-traumatic amnesia; GCS = Glasgow coma scale

As a diagnostic term, “concussion” is unsurprisingly used more frequently in the sports medicine community, while “mTBI” may be preferred by other medical specialties (33). For the purposes of this thesis, concussion will be as assumed to represent a variant at the mildest end of the mTBI spectrum.

Within the sports medicine setting, three prominent grading systems evolved during the late 20th century in an effort to stratify concussions based on acute injury characteristics. These scales – the Cantu Evidence-based Grading System (34), the Colorado Medical Society Guidelines (35), and the American Academy of Neurology Guidelines (36) – all described concussion as a transient abnormality of neurological function and relied heavily on LOC and PTA to classify injury severity (Table 1-2). Each of these scales also permitted concussed athletes to return-to-play on the same day in certain situations.

Table 1-2. Summary of three primary concussion-grading scales

Severity	Colorado Medical Society (1991)	Cantu Grading System (2001)	American Academy of Neurology (1999)
Grade 1 (mild)	• Transient mental confusion • No PTA • No LOC	• No LOC • Either PTA or post-concussion SSx clear in ≤ 30 mins	• NO LOC • Transient confusion • Post-concussion SSx clear in ≤ 15 mins
Grade 2 (moderate)	• No LOC • Confusion with PTA	• LOC lasting ≤ 1min and PTA or • Post-concussions SSx > 30 mins but ≤ 24 hrs	• No LOC • Post-concussion SSx > 15 mins
Grade 3 (severe)	• Any LOC, however brief	• LOC > 1 min • PTA > 24 hrs • Post-concussion SSx > 7 days	• Any LOC, either brief or prolonged

PTA = post-traumatic amnesia; LOC = loss of consciousness; SSx = signs and symptoms

1.2.3 Concussion in Sport Group meetings

In 2001, the first meeting of the International Consensus Group on Concussion in Sport (CISG) was held in Vienna. This meeting brought together an international panel of experts in basic and clinical science, epidemiology, cognitive assessment, protective equipment, research methods, and injury management and prevention, with the expressed aim of providing recommendations for improving the health and safety of athletes who suffer concussions (37). This landmark

meeting led to a new definition of concussion as well as the first widely accepted concussion protocol to be followed by those caring for concussed athletes on the basis of tolerance to graded physical exertion guided by symptom exacerbation. Existing concussion grading scales were acknowledged and abandoned based on limited evidence relating injury severity with the number / duration of acute symptoms and / or degree of impairment on neuropsychological testing (37). Importantly, the CISG recommended an individualized management approach to assess injury severity and tailor return-to-play decisions based on the specific patient profile, with an emphasis on the importance of neuropsychological testing as a cornerstone of concussion evaluation (37). An important idea that was raised included the potential that concussion does not follow a linear spectrum of injury severity, but rather there may exist multiple subtypes of concussion, including differences in clinical manifestation, anatomical localization, biomechanical impact profile, genetic phenotype, and structural versus no structural injury (37).

The second CISG meeting was held in Prague in 2004 and led to the development of the Sport Concussion Assessment Tool (SCAT), a standardized form to be used for patient education and sideline injury screening (38). The concussion protocol was modified to include "cognitive rest" to limit mental exertion while symptomatic. The major change evolving from this meeting entailed the classification of "simple" versus "complex" concussions for management purposes, distinguished primarily by the presence of persistent symptoms, prolonged LOC, or prolonged cognitive impairment. It was suggested that management of complex concussions include formal neuropsychological testing in a multidisciplinary setting, whereas it was not regarded as important in evaluating simple concussions. This meeting also marked the first formal

recommendation that baseline cognitive and symptom evaluation be performed prior to participation, regardless of age of performance level.

Major changes in recommendations from the 3rd meeting of the CISG, held in Zurich in 2008, included abandoning the complex versus simple classification scheme, after it was found that such terminology was not adequately descriptive (39). For the first time, a range of modifying factors were formally identified that may influence management and prognostic expectations for concussed patients, including the frequency and timing of multiple concussive injuries, decreasing impact threshold required to cause additional concussions, progressively slower recovery from additional injury, age, prolonged LOC, and psychiatric pre-/co-morbidities. The SCAT was amended to include an assessment of balance using the modified Balance Error Scoring System (BESS), as well as an upper limb motor coordination task.

The first unanimous agreement that return-to-play should not occur on the same day as concussive injury arose at the 4th CISG meeting in 2012. Additional attention was dedicated to concussion in children, with the first suggestion that the child/adolescent athlete must first successfully return-to-school before being allowed to return-to-play. Furthermore, the first child SCAT was introduced for use in individuals aged 5-12.

A noticeable trend that has developed across the various CISG meetings entails recommendations that are gradually more cautious and conservative towards the management of concussion at all levels, based on the growing understanding that structural and physiological deficits may occur with subconcussive trauma and may persist even after symptoms and

cognitive deficits have resolved clinically (40-45). At the most recent CISG meeting – held in October 2016 (statement not yet published) – an entire session was devoted to discussing the importance and timeline of physiological recovery. Indeed, the field is calling for a shift away from symptom-based concussion assessments towards structured physical examinations rooted in evaluations of physiologic function (46).

1.2.4 Current working definition of sport-related concussion

The operational definition of sport-related concussion used in this thesis was adopted from the 4th International Consensus Statement on Concussion in Sport, which defined concussion as a brain injury representing a "complex pathophysiological process affecting the brain, induced by biomechanical forces", and describes several common features (9):

"1. Concussion may be caused either by a direct blow to the head, face, neck or elsewhere on the body with an 'impulsive' force transmitted to the head.

2. Concussion typically results in the rapid onset of short-lived impairment of neurological function that resolves spontaneously. However, in some cases, symptoms and signs may evolve over a number of minutes to hours.

3. Concussion may result in neuropathological changes, but the acute clinical symptoms largely reflect a functional disturbance rather than a structural injury and, as such, no abnormality is seen on standard structural neuroimaging studies.

4. Concussion results in a graded set of clinical symptoms that may or may not involve loss of consciousness. Resolution of the clinical and cognitive symptoms

typically follows a sequential course. However, it is important to note that in some cases symptoms may be prolonged.

The majority of concussions are thought to resolve clinically in a 7-10 day period, though a subset of individuals may experience symptoms and dysfunction for months to years."

1.3 Pathophysiology of sport-related concussion

Concussions and other forms of TBI are thought to involve primary, secondary, and tertiary injury phases. The primary injury phase is characterized by the translation of kinetic energy to the head, either directly or indirectly, at the moment of impact. The secondary injury phase describes the subsequent pathophysiological processes occurring within the brain and includes immediate and delayed cellular events. The tertiary injury phase entails the growing appreciation for the persistent abnormalities in cerebral structure and function that may accompany sport-related head trauma (40).

1.3.1 Biomechanical insult – the inciting force

As outlined in the working definition in Section 1.2.4, concussions are the result of a direct or indirect biomechanical insult to the head, and myriad studies have attempted to delineate a relationship between the characteristics of concussion-causing head impacts and clinical outcomes. Though the exact pathway between mechanical insult and subsequent pathology is not definitively known, forces imparted on the head cause acceleration-deceleration of the brain within the rigid cranium, creating diffuse tensile, compressive, and shear strains in the brain tissue. Finite element modeling studies have shown peak strains during concussive impacts to

occur at the level of the brainstem, with suggestions that forces imparted to the mesencephalon, corpus callosum, and fornix may be responsible for concussion symptoms (47). Much of the biomechanical research on concussion has focused on defining thresholds for predicting injury diagnosis on the basis of the severity of concussive impacts, often characterized by peak linear and rotational acceleration of the head following impact (47-50). However, these approaches have proven unfruitful, as concussions frequently occur well below proposed thresholds (51,52), and repetitive lower severity, subconcussive head trauma can also lead to progressive impairments in brain function (further described in Section 1.3.1.1). In a prospective study of 88 collegiate American football players over 5 seasons, no relationships were observed between impact magnitude or location and diagnosed concussion, changes in postural stability, cognitive performance, or symptom-reporting (52). Declines in performance on neuropsychological tests acutely post-injury were not correlated with any biomechanical variables describing impact exposure, including the total number of impacts, peak or cumulative linear acceleration, peak or cumulative rotational acceleration, time from game start until injury, or time from previous impact (51). Overall, it is clear that the role of biomechanical parameters describing head impact exposure towards predicting concussion diagnosis is not straightforward, and may be influenced by exposure to repetitive lower severity trauma (48).

1.3.1.1 Repetitive head trauma and subconcussion

Recently, increasing concern has been raised over the potential for repetitive concussive and subconcussive head trauma to impart long-term neurological consequences including chronic traumatic encephalopathy (CTE) (15,40,53-55). As many as 16% of pathologically confirmed cases of CTE occurred in individuals with no reported history of concussion (15,53). In a recent

study of deceased amateur athletes and controls, a history of involvement in contact sports was reported as the greatest risk factor for CTE neuropathology (54). While a direct cause-and-effect relationship between repetitive head trauma and CTE has not yet been established, these findings nevertheless highlight the reason for growing concern over the potential long-term risks of subconcussive trauma.

Subconcussive head impacts are defined as trauma not resulting in signs or symptoms typical of a concussion (40,56), although their effects on the vulnerability of the brain to concussive injury and long-term neurodegenerative changes is unclear. Clinically, various metrics describing head impact exposure have been linked to later-life disturbances in cognitive and neurobehavioural function, including age of first exposure to American football (57,58), duration of participation in contact sports (15,59,60), and concussion history (61-64). Multiple studies have shown repetitive subconcussive trauma can induce deficits in brain structure and function that are associated with the degree of impact exposure (20,22,25,26,65-72). Human and animal studies have reported cumulative subconcussive impacts to be associated with axonal injury and damage to the blood-brain barrier (22,59,66,73-77).

Limited evidence exists to suggest head impact thresholds may exist for elevated risk of long-term changes in brain structure and function (25,72). A recent study demonstrated a threshold dose-response relationship between estimated cumulative head impact exposure from playing high school or collegiate American football and risk for later-life deficits in cognition, executive function, depression, anxiety, apathy, and behavioral dysregulation (72). Clinically meaningful impairments across all domains were elevated after a threshold of ~2000 hits, and increased

linearly with every ~1000 hits thereafter – equating to roughly two seasons of participation. Lipton and colleagues similarly estimated a threshold of 1800 head impacts in the context of soccer heading toward elevated risk of memory impairment (25). However, the threshold for alterations in white matter microstructure were much lower, ranging from 885-1550 hits depending on the brain region, suggesting structural changes may accrue prior to overtly manifest cognitive and behavioural impairments. It is clear that repetitive subconcussive head impacts have the potential to detrimentally affect the brain. The pathophysiological mechanisms underlying such changes are not clear, but likely are similar to those involved in clinically manifest concussion.

1.3.2 Neurometabolic cascade of concussion

Current paradigms describing the underlying pathophysiology of concussion depict a complex post-injury series of events contributing to secondary and tertiary injury mechanisms. This "neurometabolic cascade" involves indiscriminate glutamate release and ionic flux, leading to a state of elevated glycolytic demand in an attempt to preserve ionic gradients (Figure 1-1). The ensuing hypermetabolic state is thought to lead to an energy crisis, involving failure of ATP pumps, mitochondrial dysfunction, and sequestration of calcium, ultimately leading to cytoskeletal damage (78,79). While primary axotomy is often observed following high magnitude head impacts associated with more severe traumatic brain injuries, the axonal pathology observed following concussion is thought to develop over days to weeks following the initial insult (78,80,81).

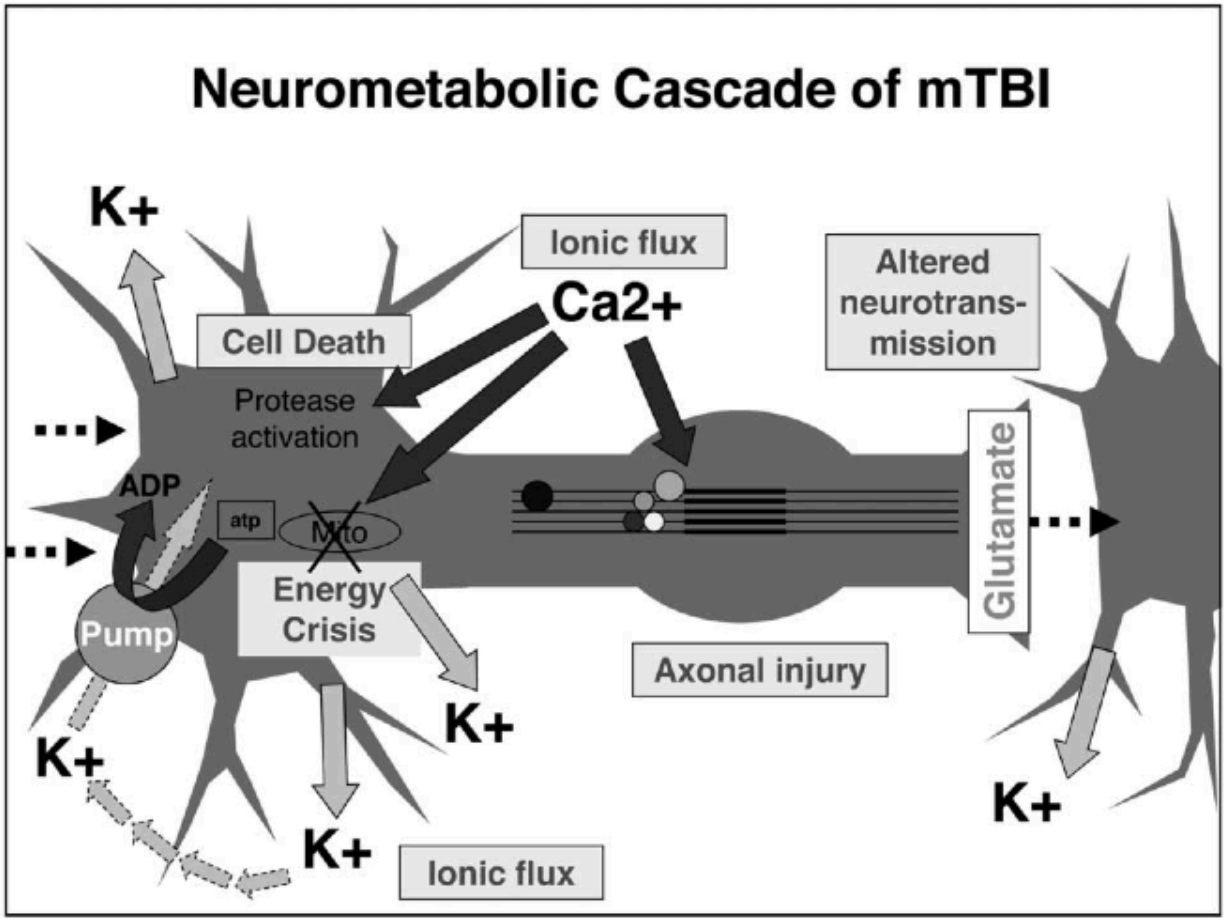

Figure 1-1. Diagram outlining the acute cellular processes proposed to occur during the neurometabolic cascade of concussion. Fig 1 in (79). Image reproduced with permission from Neurosurgery.

1.3.3 White matter alterations following sport-related head trauma

Although concussions have long been regarded as a strictly functional disturbance of the brain without any concomitant structural brain damage (Section 1.2.1), recent evidence has challenged this assumption. Myelin is becoming recognized as an important player in the pathophysiology of TBI, though its role is poorly understood (82-84). Myelin plays a fundamental role in the health and function of axons, serving as a primary determinant of conduction velocity during propagation of action potentials. It has been estimated myelinated axons are up to 70 times more

metabolically efficient than unmyelinated axons (85), highlighting an important role for myelin in neuronal metabolism. Furthermore, recent work has implicated an important role for oligodendrocyte precursor cells in promoting blood-brain barrier integrity (86). Glutamate excitotoxicity, intracellular calcium overload, mitochondrial dysfunction, oxidative stress, and inflammation –all components of the post-concussion neurometabolic cascade (Section 1.3.2) – have all been recognized as significant causes of damage to both myelin integrity and oligodendrocytes (84,87).

Increasingly, evidence suggests damage to either myelin or the axon can lead to subsequent damage to the other (84,88). Impaired axonal transport after concussive injury leads to swellings within the axon containing organelles and other transport materials, creating a ripe environment for secondary axonal disconnection and Wallerian degeneration (78,89). Histopathologically, these processes have been documented in brain slices as axonal bulbs, irregular tortuous axonal varicosities, and small globoids of degraded myelin sheath (82,90,91). Experimental work has demonstrated that accumulative oxidative stress in the context prolonged cerebral hypoperfusion – which evidence suggests may occur following concussion (Section 1.3.4) – suppresses the differentiation of oligodendrocyte precursor cells to oligodendrocytes and leads to reduced myelin content (92). Thus, loss of oligodendrocytes and corresponding demyelination of affected axons is anticipated following traumatic injury (83,84,93). Whether the result of direct, multifocal primary traumatic axonal injury or secondary axotomy, subsequent axonal degeneration is a possible outcome. However, axonal damage and myelin disruption may be reversible (82,83). In contrast to situations where strain is focally applied (e.g. at anatomic boundaries), strains distributed more diffusely along the length of an axon create an elongated

pattern of axonal swelling, which may be more amenable to intrinsic repair mechanisms (78). Finite element models have implicated tensile elongation as a primary mechanism of traumatic axonal injury (94). While various studies in animal models have outlined myelin fragmentation and degradation following traumatic axonal injury (82,95), dynamic in-vivo myelin changes have not been directly observed in the concussed human brain.

Advanced MRI-based neuroimaging techniques like diffusion tensor imaging (DTI) have provided valuable insight into the effects of sport-related head trauma on white matter microstructure. DTI is sensitive to diffusion properties of the various tissues within the brain, and has frequently been used as an indirect marker of myelin integrity in concussion research. Within cortical white matter, the highly ordered nature of neurofibrils, axonal membrane, and myelin sheath cause water to preferentially diffuse along the length of the axons as opposed to perpendicularly. This asymmetrical pattern of water movement is termed anisotropic diffusion (96) and is quantifiable on a voxel-wise basis using DTI as fractional anisotropy (FA). While a multitude of reports have demonstrated diffusivity changes following sport-related head trauma (reviewed in (97-99)), results have been equivocal and difficult to interpret physiologically. Many studies have reported decreases in FA following mTBI (100-102) indicative of less restricted diffusion and interpreted to reflect axonal / myelin damage. However, numerous other studies have also demonstrated increases in FA post-injury (103-105), perhaps illustrating inflammation and / or edema, while others have observed no changes in FA (106,107) or changes in both directions (24,105). Despite these discrepancies, a number of cortical structures are consistently implicated and show alterations in white matter microstructure following

concussion, including the cingulum, anterior corona radiata, uncinate fasciculus, corpus callosum, posterior limb of the internal capsule, and superior longitudinal fasciculus (97-99).

1.3.3.1 Limitations of diffusion tensor imaging

While at least part of the inconsistencies observed across DTI studies in concussion may be related to inconsistent injury-to-scan intervals, methodological constraints of this imaging technique permit limited insight into the physiological relevance of these findings. Many authors have reported changes in DTI metrics following concussion to reflect alterations in white matter and, more specifically, myelin integrity (108). However, such interpretations must be made cautiously, as evidence shows myelin changes are neither necessary nor sufficient to effect changes in metrics of anisotropic diffusion (96,109). This highlights the fundamental limitation of using DTI to gain insight into pathophysiological mechanisms: that is, its inability to distinguish between multiple factors that influence water diffusion within white matter tracts, including fiber diameter, fiber density, fiber orientation, membrane permeability, edema, and demyelination (Figure 1-2) (110). DTI metrics reflect fibre coherence; therefore, factors such as inflammation, gliosis, and axonal loss can quickly confound interpretation (109).

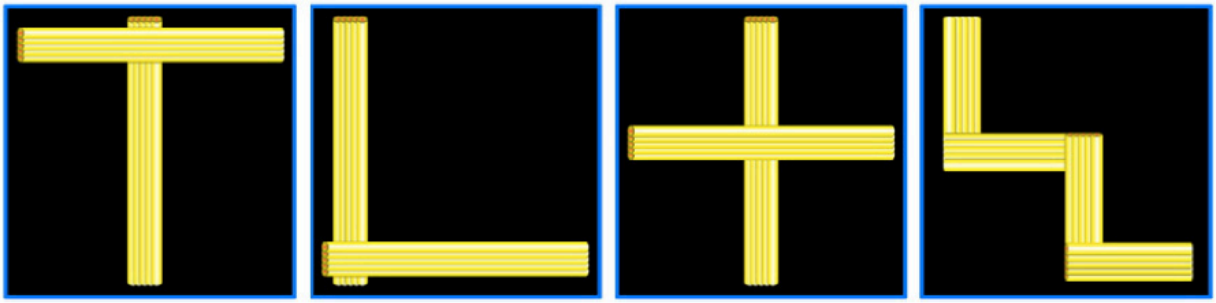

Figure 1-2. Schematic representation of fiber orientation in four hypothetical voxels. Despite dramatically different configurations, the fiber orientational density function obtained using diffusion tensor imaging would be identical. Fig 1 in (110). Image reproduced with permission from NeuroImage.

Consequently, DTI lacks the specificity required for accurate interpretation of the underlying physiological mechanisms at play following sport-related head trauma. In contrast, newer MRI-based techniques using decomposition of T_2-weighted decay curves – termed myelin water imaging – directly evaluate myelin content in the brain, and have been validated histopathologically (111-114).

1.3.3.2 Myelin water imaging

The first study in this thesis (Chapter 2:) uses T_2 relaxation-based MWI to provide insight into tissue characteristics that are not observable using DTI or standard MR imaging techniques (114). Although it is often assumed that each voxel in a MRI-generated image (~1-5 mm^3) represents homogeneous tissue, this notion is inaccurate, especially in the brain. Structural features of myelinated tissue cause the protons on water molecules – which provide the source for H^1-MRI signals – to experience different local environments that affect T_2 decay time. Consequently, decomposition of the T_2 relaxation curve within each voxel reveals multiple

components, the shortest of which is specific to water molecules trapped with myelin bilayers (Figure 1-3). The myelin water fraction (MWF) can be calculated voxel-wise as the ratio of the area in the T_2 distribution arising from myelin water (T_2 time = ~10-40 ms) to the entire area of the T_2 distribution (114). With recent technological advancements, whole-brain MWF images can be acquired in less than 10 minutes (Figure 1-4) (114,115).

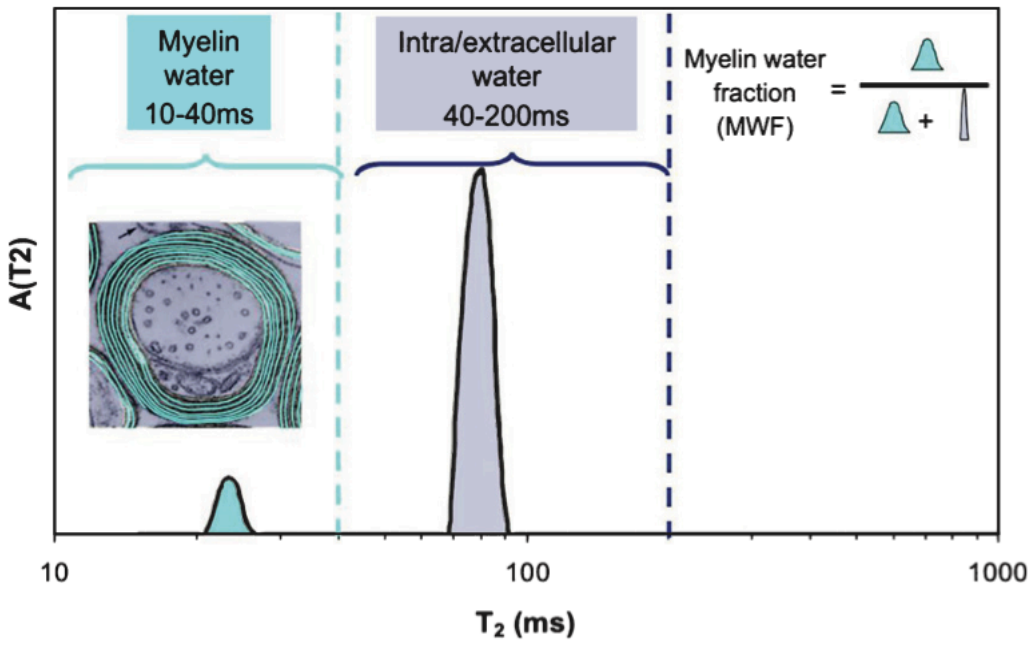

Figure 1-3. T_2 distribution from human white matter. Insert shows locations of myelin water versus intra/extracellular water on an electron micrograph of myelinated tissue (adapted from Fig 2 in (114)).

It has been suggested that myelin water imaging may be the best imaging technique to distinguish between myelination and inflammation *in vivo* (112,116-118). Post-mortem studies correlating MRI and histopathological findings in both central (111) and peripheral nervous tissue (119) have demonstrated strong relationships between T_2 relaxation-derived MWF and histological staining for myelin. Myelin water imaging was shown to have high reproducibility in

healthy brains, both longitudinally and between imaging sites using the same scanner type and the same protocol (120,121). Moreover, MWF in the normal appearing white matter of people with multiple sclerosis was also shown to be stable over at least six months (122). In a guinea pig model of demyelination (experimental allergic encephalomyelitis), the observed decrease in the short T_2 component (i.e. myelin water) was consistent with histological myelin loss (116). While other imaging methods are sensitive to myelin content, such as magnetization transfer, T_2-relaxation based MWI has been suggested as the technique of choice for evaluating myelin dynamics (114). Indeed, investigations have used MWI to further our understanding of many clinical conditions, including multiple sclerosis (123-128), autism (129), and stroke (130). As such, this approach was chosen to evaluate the influence of sport-related head trauma on myelin, as presented in Chapter 2:.

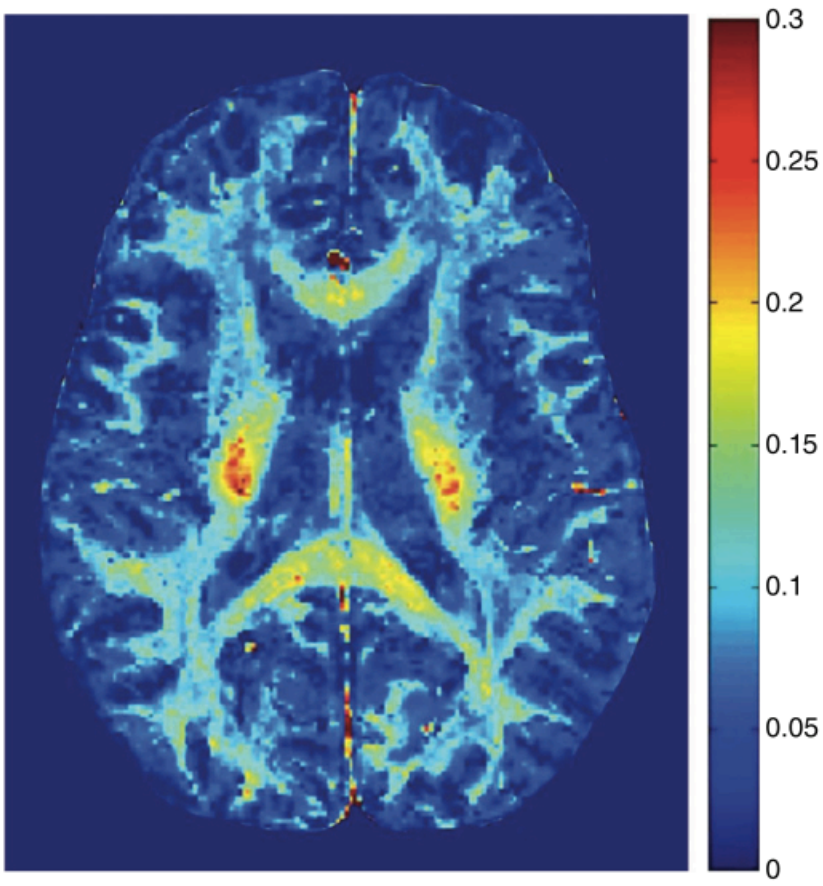

Figure 1-4. Example of a myelin water fraction map in a healthy brain obtained using T_2-relaxation based myelin water imaging.

1.3.4 Cerebral blood flow

In addition to structural damage to white matter, recent research has also implicated an important role for compromised cerebrovascular function in the disturbances associated with sport-related head trauma (131-133). Despite comprising only 2% of total body weight, the brain accounts for ~15% of total cardiac output and ~20% of total oxygen consumption at rest, illustrating its high metabolic demands. The brain is highly reliant on glucose as a substrate for energy production – almost all adenosine triphosphate production in the brain occurs via oxidative glucose

metabolism (134) – yet has a remarkably low capacity for energy storage (135), necessitating effective and tightly controlled mechanisms to maintain blood flow.

Metabolic disruptions resulting from the post-concussion neurometabolic cascade (Section 1.3.2) occur during periods of altered cerebral blood flow. Transient disruptions in CBF following concussion have repeatedly been demonstrated (2,43,136,137). In the earliest post-mTBI phase (<48 hours), a period of acute hyperemia occurs with CBF peaking at 24-hours (138), although this may be age-dependent (139,140). Subsequently, evidence shows global and regional reductions in CBF, both sub-acutely (2,43,141) and chronically (142,143). This has been demonstrated in both paediatric (2) and young adult (43,141) athlete populations. Brain blood flow recovers to near-healthy athlete levels by ~30 days, although this may take longer in adolescents (2), and may be related to symptom resolution (43,137). Despite this finding, when compared to athletes whom had never sustained prior concussions, arterial spin labeling studies have shown those with a history of concussion display chronically reduced fronto-temporal CBF, influenced by the number of previous concussions (141,144). Patients often report symptom exacerbation with increased physical and cognitive demand, suggesting a potential mismatch between cerebral metabolic demand and CBF delivery may partly explain the clinical manifestations of concussive sequelae (132).

To understand the underlying reasons for post-concussion CBF disturbances, it is pertinent to consider the effects of sport-related head trauma on the mechanisms controlling CBF. Regulation of CBF involves a spectrum of mechanisms that work complementarily to alter resistance within the cerebral blood vessels, adjusting CBF to provide an adequate supply of oxygen and nutrients

based on situational demands (145). The cerebrovasculature adapts to regional metabolic requirements associated with neural activity (neurovascular coupling), to changes in perfusion pressure (cerebral autoregulation), and to changes in arterial blood gases (cerebrovascular reactivity) with particular sensitivity to carbon dioxide (145). Although recent studies using MRI-derived parameters of CBF – including arterial spin labeling (43,137,142,144,146,147), phase contrast angiography (2,143), and functional MRI (45,148,149) – have provided valuable insight into the effects of concussion on CBF, these techniques lack the temporal resolution required to optimally evaluate the dynamics of CBF control mechanisms (150). Conversely, transcranial Doppler ultrasound (TCD) has been suggested as an ideal modality for evaluating cerebrovascular function following concussion due to its high temporal resolution, portability, relative affordability, lack of radiation, and non-invasive nature (151). However, accurate use of TCD requires an understanding of cerebrovascular anatomy (150).

1.3.4.1 Anatomy of the cerebrovasculature

Blood flow is supplied to the brain via two primary routes: the internal carotid artery (ICA) supplying ~70% of total CBF (anterior circulatory system), and the vertebrobasilar system supplying the remainder (posterior circulatory system).

The anterior circulatory system supplies the majority of the frontal, temporal, and parietal lobes, and originates from the common carotid arteries at the aortic arch / brachiocephalic trunk. The ICA originates at the bifurcation of the common carotid artery, enters the skull at the foramen lacerum, and courses through the carotid canal before entering the cranial cavity in the cavernous

sinus. The cavernous segment of the ICA abruptly bends superiorly and posteriorly to penetrate the dura matter at the proximal dural ring (Figure 1-5).

Figure 1-5. Three-dimensional cutaway schematic showing a cervical ICA loop and the intracranial course of the ICA. Adapted from (152). Image reproduced with permission from *Neurosurg Clin N Am*.

After giving rise to the ophthalmic artery at the distal dural ring, the posterior communicating artery branches from the ICA and passes posteromedially to join the posterior cerebral artery (PCA) as part of the Circle of Willis. The ICA bifurcates into the middle cerebral artery (MCA) and anterior cerebral artery (ACA). The MCA courses antero-laterally within the Sylvian fissure before turning sharply in the postero-superior direction (the genu), reaching the surface of the insula and giving rise to many perforating branches. The anterior cerebral artery (ACA) arises as the medial terminal branch of the ICA, and connects with the contralateral ACA in the interhemispheric fissure via the anterior communicating artery (Figure 1-6).

In the majority of healthy individuals, the posterior circulatory system arises from the vertebral arteries that branch from the subclavian arteries. After giving rise the branches that serve the cervical spinal cord, the vertebral arteries converge to form the basilar artery at the level of the pontomedually junction. A number of arteries branch from the basilar artery to supply the cerebellum and pons before it terminates as a bifurcation, giving rise to two posterior cerebral arteries that course laterally and then loop posteriorly to supply the occipital lobes (Figure 1-6).

1.3.4.1.1 Principles of transcranial Doppler ultrasound

Transcranial Doppler provides a real-time index of CBF by using ultrasound probes to emit 1-2-MHz sound waves. Insonation of intracerebral arteries is possible through the thin regions of the temporal bone, referred to as acoustic windows (150). Using a transtemporal approach, three acoustic windows may be observed, including the posterior window (directly anterior to the ear, above the zygomatic arch), anterior window (superior to the anterior process of the zygomatic arch), and middle window (between anterior and posterior windows). Emitted sound waves reflect off erythrocytes travelling within the insonated vessel and are detected by the transducer (Figure 1-7). The Doppler-shift between emitted and received signals is proportional to the velocity of the moving blood cells:

Equation 1.1: $$\text{Doppler Shift} = 2 \bullet f_t \bullet v \bullet \cos(\theta) / C$$

Where f_t is the transmitted ultrasound frequency (2 MHz); v is the velocity of the reflector (erythrocytes); θ is the angle of insonation; and C is the speed of sound in the blood (1540 m/s)

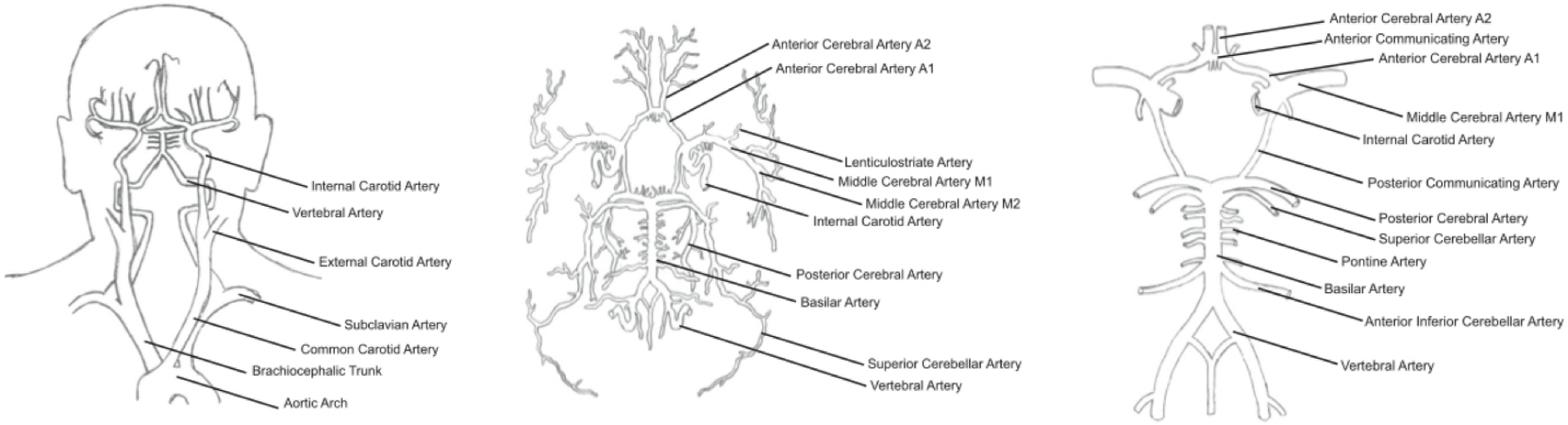

Figure 1-6. Schematic of the cerebral arterial system. (Left) Coronal section through the head and neck; (Middle) Looking up through the base of the brain; (Right) Vessels of the vertebrobasilar system feeding into the circle of Willis. Adapted from (153). Reproduced with permission from NMR in Biomedicine.

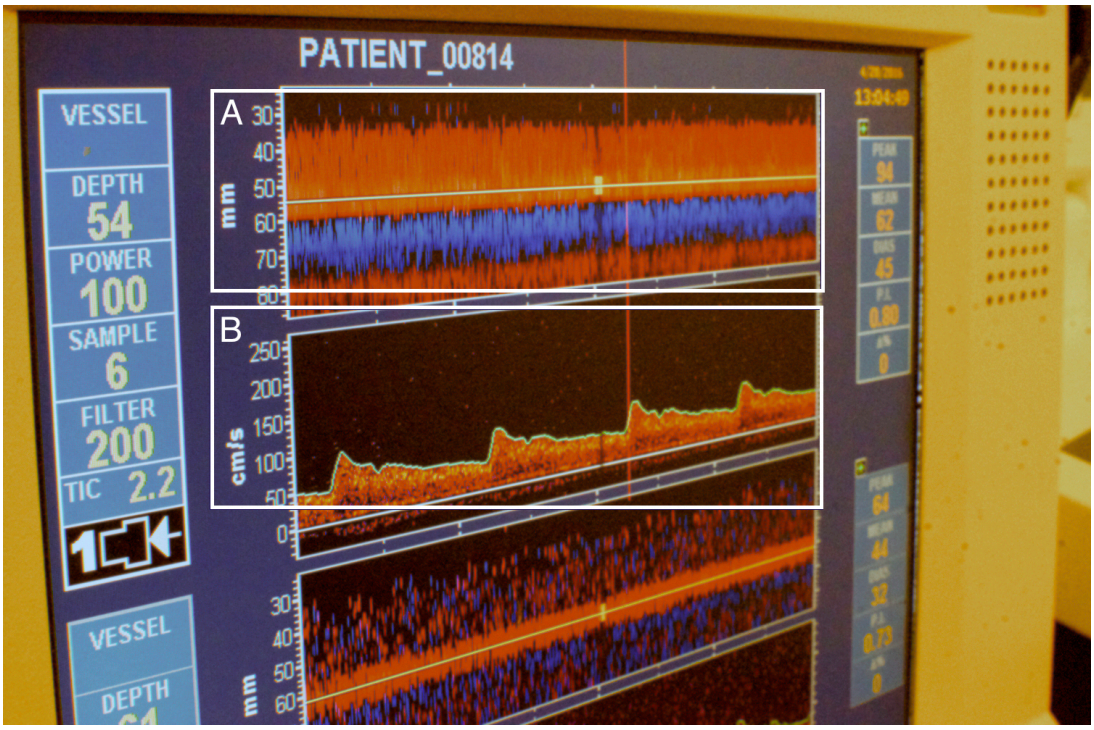

Figure 1-7. Picture of the TCD interface. A) Depth of blood velocity signals oriented towards the probe (red) and away from the probe (blue) – note the bifurcation of the ICA visible at ~60 mm as a demarcation between blood flowing in the MCA (red) and ACA (blue); B) Velocity profile at selected depth (54 mm).

It is important to note that the diameter of the insonated vessel cannot be determined using TCD. As such, the main limitation of this technique is its inability to measure volumetric flow; rather TCD provides estimates of blood flow velocity under the assumption that vessel diameter remains constant.

Using TCD, the bifurcation of the ICA can typically be insonated through the anterior or middle temporal window at a depth of 55-65 mm, providing an important landmark for locating other vessels and standardizing insonation. The anatomical distribution of the MCA typically allows for direct insonation of the M-1 segment through the anterior temporal window – with near-zero

insonation angle – at depths between 20-60 mm. The P-1 segment of the PCA can be found posterior and deep to the ICA bifurcation at depths of ~60-70 mm (Figure 1-8).

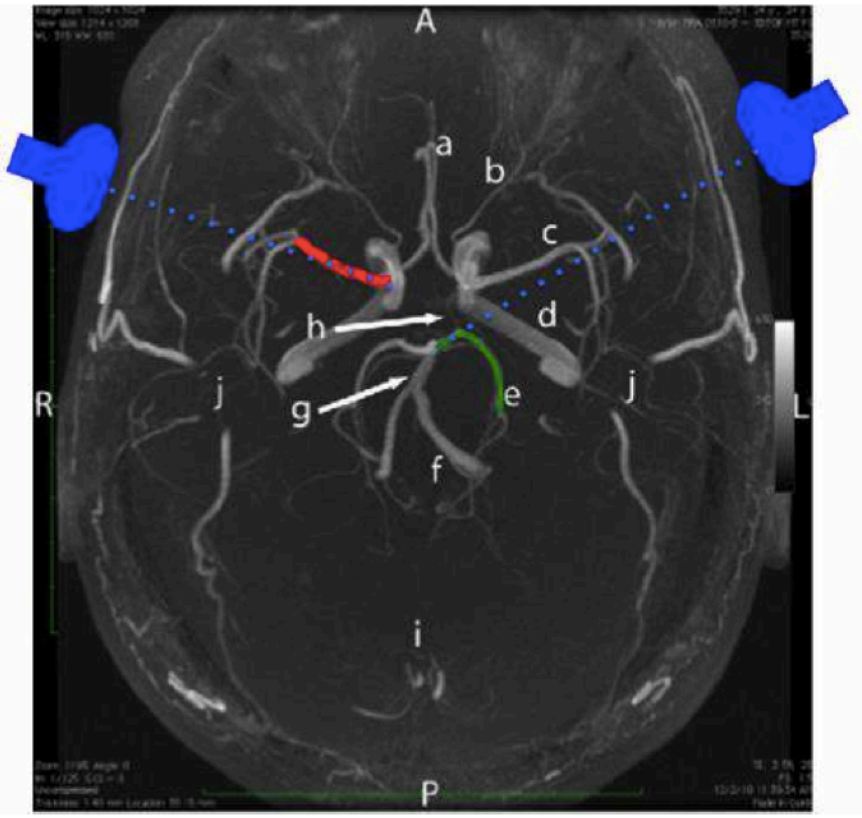

Figure 1-8. Schematic demonstrating insonation of left posterior cerebral artery (right side of picture) and right middle cerebral artery (left side of picture). Image modified from Fig 3 in (150). Image reproduced with permission from Journal of Neuroscience Methods.

A recent review has highlighted the "considerable utility" of TCD in providing an efficient, cost-effective tool towards the comprehensive assessment of cerebrovascular function in physiological and pathological states for both research and clinical purposes (150). Indeed, TCD has been used to gain insights into a variety of clinical populations including vasospasm (154), spinal cord injury (155,156), Alzheimer's diesease (157), ageing (158), stroke (159), and severe

TBI (160). Specifically, TCD represents an excellent tool to assess the integrity of the primary mechanisms controlling cerebral blood flow: CO2 reactivity, cerebral autoregulation, and neurovascular coupling (150).

1.3.4.2 Cerebrovascular reactivity to carbon dioxide

Across the entire length of the cerebrovascular tree, cerebral blood vessels are highly sensitive to the arterial partial pressure of carbon dioxide (P_aCO_2), although resistance modulation is thought to occur primarily at the level of pial arterioles (145,161-164). While changes in CBF are linked to alterations in P_aCO_2, the true vasoactive stimulus is a change in extravascular pH, resulting from the diffusion of non-polar CO_2 molecules across the blood-brain barrier into the extravascular environment (165-167). Once in the extracellular fluid, CO_2 dissociates via the bicarbonate buffer system into H^+ and HCO_3^-, generating substantial changes in pH. Increased P_aCO_2 leads to a decrease in pH and induces relaxation of vascular smooth muscle cells, leading to vessel dilation (up to 40% in pial arteries) and increased CBF (167). Conversely, hypocapnia yields an increase in pH, enhancing vascular tone and decreasing flow (168). The cerebrovascular response to altered CO_2 occurs with a ~6 second delay and is critical to regulate and maintain central pH (168). Sensitivity to CO_2 appears to differ in hyper- versus hypocapnic ranges (169), whereby hypercapnic stimuli result in CBF increases of ~2-5%/mmHg and hypocapnic stimuli elicit CBF reductions of ~1-2%/mmHg (162,170,171). Data suggests that CO_2 reactivity is greater in grey matter than in white matter, likely due to relative differences in vascularization (172,173). While CBF is also sensitive to oxygen content within the blood, particularly at very low levels, the effect is dependent on the prevailing P_aCO_2 (174).

1.3.4.2.1 Alterations in CO_2 reactivity following sport-related head trauma

Of the primary mechanisms controlling CBF, the one that has received the most research attention in the context of sport-related head trauma is CO_2 reactivity; multiple studies have used TCD or blood oxygen level dependent (BOLD) MRI in athletic populations and have shown trauma-induced alterations in the sensitivity of cerebral blood vessels to CO_2 (reviewed in (175) and (132)). In acutely concussed young adult athletes (primarily hockey players) and contact sport athlete controls, TCD has demonstrated concussion-induced impairments in CO_2 reactivity that resolved within 5 days post-injury (176,177). BOLD-based CO_2 reactivity impairments have been shown in a small sample of concussed adolescent athletes and persisted beyond clinical recovery (45). At a whole brain level, BOLD-based CO_2 reactivity was not different between concussed adults and normal controls, although patient-specific alterations were observed in both symptomatic and asymptomatic patients that were not present in controls (178). In a longitudinal case study of a single mTBI patient, Chan and colleagues (148) reported asymmetries in whole brain CO_2 reactivity 2-months following injury that normalized on follow-up assessment 1-year later. Militana reported regional increases in CO_2 reactivity in acutely concussed patients (3-6 days post-injury) that were correlated with functional connectivity in the hippocampus (179). Chronically, adolescent patients suffering persistent post-concussion symptoms demonstrated reduced CO_2 reactivity compared to normal controls despite no differences in global mean CBF (149). Further work has been proposed towards generating an ultrasonographic index of CO_2 reactivity to assist in the diagnosis and management of concussion (151).

Impairments in CO_2 reactivity have also been demonstrated in the context of repetitive subconcussive trauma. In one TCD study, professional boxers exhibited substantial alterations in

CO2 reactivity when compared to controls that were negatively correlated with number of rounds fought (17). Impairments in CO_2 reactivity have been reported in high school American football players compared to non-contact sport controls during the first half of the season, but resolved by end-of-season (18). Female high school soccer players exhibited CO_2 reactivity impairments that were associated with cumulative impact exposure and persisted for up to 4-5 months post-season (19). It is evident that sport-related head trauma induces alterations in cerebrovascular sensitivity to CO_2; however, much less is known regarding the effects on other mechanisms controlling CBF, including cerebral autoregulation and neurovascular coupling.

1.3.4.3 Cerebral perfusion pressure and cerebral autoregulation

Cerebral perfusion pressure is the driving force for blood flow to the brain – equal to the difference between mean arterial pressure (MAP) and intracranial pressure (ICP). The importance of perfusion pressure in the regulation of CBF has been recognized for over a century: in 1895 Bayliss, Hill, and Gulland reported that "*In all physiological conditions a rise in arterial pressure accelerates the flow of blood through the brain, and a fall slackens it*" (180). This concept evolved in the late 1950s when Lassen plotted data from seven studies in 11 patient groups of average steady-state blood pressure and total brain blood flow, and revealed a range of MAP (~60 – 150 mmHg) across which CBF appeared to be stable (181). Coined static cerebral autoregulation (CA), this observation indicates reflexive adjustments in cerebrovascular resistance to changes in blood pressure. As perfusion pressure falls there is a consequent vasodilation and reduction in cerebrovascular resistance to support CBF; the reverse occurs in response to elevations in perfusion pressure. It is accepted that CA acts to adjust resistance at the level of the cerebral arterioles and pial vessels (182), though animal data exists to support

potential contributions from large intracranial and extracranial arteries (183-185). Subsequent human research has determined that the within-subject steady-state MAP-CBF relationship is not flat through the broad range of blood pressures originally described by Lassen, but encompasses a ~10 mmHg window (186). Furthermore, the slope of the pressure-passive regions of the CA curve differs in hypertensive versus hypotensive ranges, suggesting a greater buffering capacity against surges in pressure relative to reductions in pressure (145).

While classical definitions of CA were derived from evaluations of the pressure-flow relationship during steady-state conditions (static CA), research has increasingly focused on the influence of dynamic changes in MAP – for example, during changes in posture – on CBF (dynamic CA). The advent of TCD allowed for improved temporal resolution, indexing CBF in major intracranial blood vessels on a beat-by-beat basis. Following rapid transient hypotension induced by the release of inflated thigh occlusion cuffs, the earliest study of dynamic CA demonstrated a similar drop between MAP and CBFv, but a quicker recovery of the CBFv, suggesting relative rather than absolute buffering (187). Contemporary views of CA hold that the cerebrovasculature behaves as a high-pass filter, whereby higher frequency (> 0.20 Hz) changes in blood pressure are passed linearly into the cerebral blood vessels, and lower frequency changes are more extensively buffered (188,189). The frequency-dependent behaviour of CA is known to involve autonomic and myogenic contributions, which appear to operate at different frequencies (190-194). Characterization of dynamic CA has provided valuable clinical information, allowing differentiation of cerebrovascular responses to fluctuations in blood pressure occurring at different durations and amplitudes observed during everyday life (195,196).

1.3.4.3.1 CA disruptions in acquired brain injury / moderate-severe TBI

Autoregulatory mechanisms appear sensitive to trauma, as disruptions to the cerebral-pressure flow relationship are well documented following moderate and severe TBI. Across multiple studies, 49-87% of severe TBI patients exhibit absent or impaired CA (197-199), while recovery of CA is delayed in some cases beyond 2 weeks (199). Disruptions in CA are predictive of outcome in acquired brain injury populations: in severe TBI, acutely compromised CA is a significant predictor or poor outcome (200,201); in patients with Fabry disease, impaired CA is thought to increase risk of stroke (202); following subarachnoid haemorrhage, CA dysfunction is among the primary factors predisposing patients to delayed cerebral ischemia and vasospasm at an individual level (203-205); impaired CA is also an established independent risk factor for stroke (159). However, evidence for CA impairments in cases of mild head trauma is not as robust.

1.3.4.3.2 Alterations in CA following sport-related head trauma

Despite widespread postulation that disruptions in CA play an important role in the pathophysiology of sport-related concussion (9,131,206,207) – including an assertion of responsibility for persistent post-concussion symptoms (206) – no studies to-date have explicitly evaluated autoregulatory function in the context of sport-related concussion. In one small study, a subset of hospitalized mTBI patients – eight of 29, the majority of whom also exhibited structural damage on CT – demonstrated impaired CA (208). One other case study reports impaired CA in a hospitalized mTBI patient (209). In the only study evaluating CA in the context of sport-related head trauma, Bailey and colleagues reported impairments in an index of

CA relative to control subjects in a cohort of professional boxers that correlated with combat volume (17). Apart from this study, very little is known to what extent the dynamic, frequency-dependent relationship between BP and CBF is affected by sport-related concussion and repetitive subconcussive trauma (99). As such, Chapter 3: and Chapter 4: of this thesis entail the first prospective evaluations of CA function via driven MAP oscillations and a widely-used technique known as transfer function analysis (TFA)(196), recently suggested as a gold-standard for evaluating the cerebral pressure-flow relationship (210).

1.3.4.3.3 Transfer function analysis for characterizing dynamic CA

Whereas classical methods of indexing CA (namely, the rate of regulation (RoR) (187), autoregulatory index (ARI) (195), autoregressive-moving average ARI (211)) evaluate information in the time domain of CBF velocity and blood pressure signals, TFA also considers data contained within the frequency domain. This is pertinent to the evaluation of CA, as the pressure-flow relationship is frequency dependent (described in Section 1.3.4.3). The TFA approach models CA as a simplified linear control system, using a Fourier decomposition to describe the relationship between oscillations in input (MAP) and output (CBFv) signals at different frequencies (212). The frequency-dependent transfer function H(f) between MAP and CBFv signals is calculated as:

Equation 1.2 $$H(f) = S_{MAP\text{-}CBFv}(f)/S_{MAP\text{-}MAP}(f)$$

where $S_{MAP\text{-}MAP}(f)$ is the autospectrum of the BP signal and $S_{MAP\text{-}CBFv}(f)$ is the cross-spectrum between the MAP and CBFv signals. The squared *Coherence* function is estimated as:

Equation 1.3 $MSC(f) = | S_{MAP\text{-}CBFv}(f) |^2 / \cdot \{SMAP\text{-}MAP(f)\ \ SCBFv\text{-}CBFv(f)\}$

Similar to a correlation coefficient, *Coherence* describes the proportion of variance in the output signal (CBFv) explained by the input signal (MAP), and may have values between 0 and 1. A high coherence improves within-subject reliability of *Gain* $| H(f) |$ and *Phase* $| \phi(f) |$(212,213), which are calculated using the real $H_R(f)$ and imaginary $H_I(f)$ components of the complex transfer function:

Equation 1.4 $| H(f) | = \{HR(f)2 + HI(f)2\}$

Equation 1.5 $\phi(f) = \tan^{-1}\{HI(f) / HR(f)\}$

Phase represents the timing offset between input and output oscillations and is indicative of the time to change in cerebrovascular resistance following a change in MAP. *Gain* provides a ratio of output amplitude to input amplitude. In the setting of intact CA, higher *Phase* indicates more rapid adjustment of cerebrovascular resistance to changing MAP. Low gain indicates low magnitudes of MAP oscillation transferred to the cerebrovasculature (i.e. greater buffering) (210). Dynamic CA function has been evaluated using TFA in a variety of clinical conditions, including autonomic failure (214), Alzheimer's disease (215); heart transplantation (216), and schizophrenia (217), and has shown clinical value; a recent study demonstrated that less effective CA acutely post-ischemic stroke (in the form of reduced TFA phase) was associated with elevated risk of hemorrhagic transformation and cerebral edema (218). No published studies to date have used TFA to evaluate the influence of sport-related head trauma on CA function.

1.3.4.4 Neurovascular coupling

Regional increases in neural activity – and the associated elevation in metabolism – require appropriate and coordinated responses by the neurovascular unit to modulate local cerebrovascular resistance and direct nutrient-rich blood to active cortical regions. For over 100 years, it has been appreciated that local neural and metabolic activity within the cortex are tightly coupled with regional perfusion (219), though the exact mechanisms underlying this relationship in humans are still debated. The coupling between cortical activity and CBF is a characteristic of the neurovascular unit, a term describing the functional and anatomical relationship between neurons, supporting glial cells (astrocytes, pericytes, oligodendroglia), vascular smooth muscle cells, and endothelial cells (Figure 1-9). Multiple pathways have been proposed to contribute to the NVC response, including local accumulation of vasoactive metabolic by-products (e.g. CO_2, nitric oxide (NO), adenosine, arachidonic acid metabolites), direct neural control of the cerebrovasculature, pericyte-mediated changes in capillary tone, and astrocyte-mediated vasosignaling (reviewed in: (145,220)). Ultimately, gap junctions between adjacent vascular smooth muscle cells are thought to facilitate intramural propagation of vascular signals to yield vasoactivity in remote pial arterioles up-stream of the signal origin (221-223).

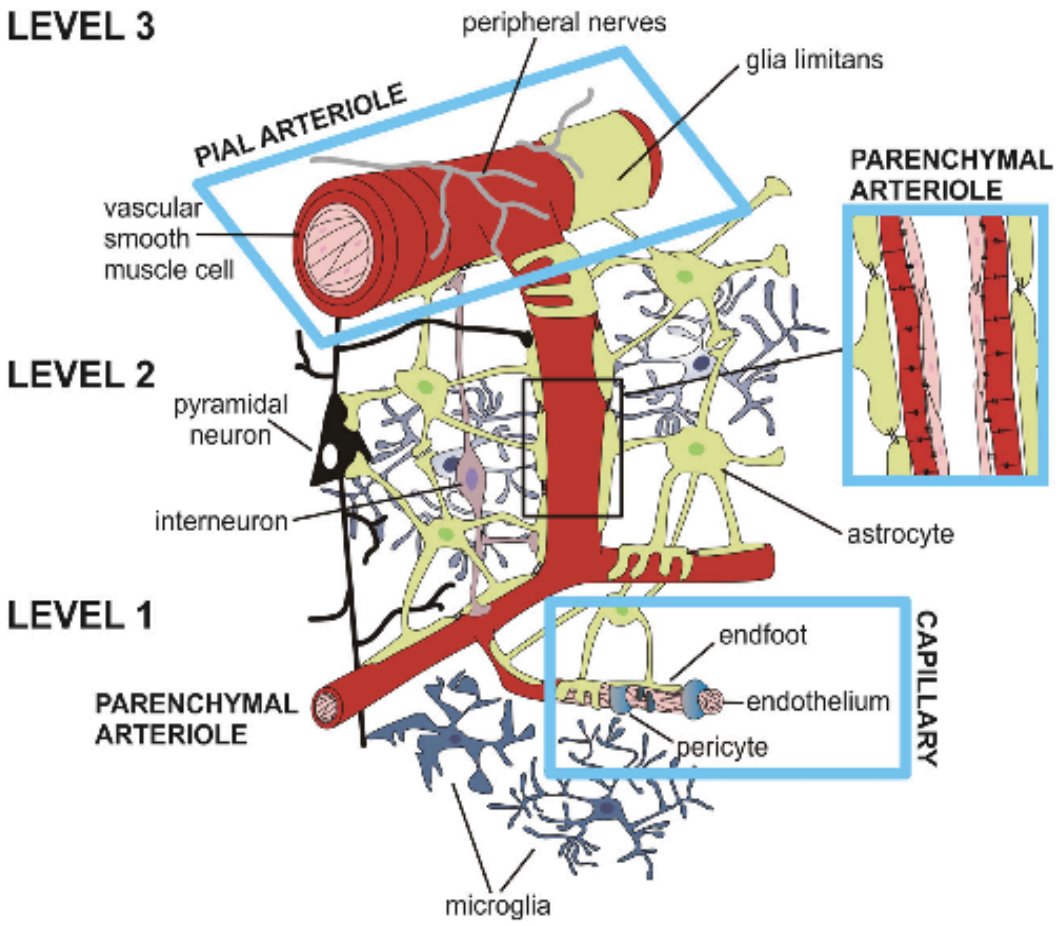

Figure 1-9. Schematic illustration of the components of the neurovascular unit from the level of capillaries to pial arterioles. At the capillary level (Level 1), the neurovascular unit is comprised of endothelial cells, pericytes, astrocytes and neurons. At the parenchymal arteriole level (Level 2), the neurovascular unit consists of a single layer of vascular smooth muscle cells, pericytes (not illustrated) astrocytic end-feet, and neuron processes. At the pial arteriole level (Level 3), the endothelium is surrounded by multiple layers of smooth muscle, astrocyte processes, and perivascular nerve endings originating from peripheral ganglia. Fig 1 in (224). Image reproduced with permission from Elsevier.

1.3.4.4.1 Multiple mechanisms contribute to the NVC response

Neural control of the cerebral microvasculature plays an important role in the NVC response. The entire cerebrovascular tree is innervated with sympathetic and parasympathetic fibres and growing evidence supports a role for autonomic activity in modulating CBF via its effects on cerebrovascular resistance (145,225,226). Indeed, the fast initial rise in CBF following cortical activation is thought to result from sympathetic activation within the cortex (145,213,220). Furthermore, increased extracellular glutamate activates N-methyl-D-aspartate (NMDA) receptors to stimulate neuronal NO synthase and the subsequent release of NO onto nearby parenchymal arterioles (227). When ATP levels are low in neurons, adenosine is also released locally to exert a vasodilatory effect (228).

Mounting evidence supports a role for pericytes in initiating the NVC response, wherein neuronal activation may modulate pericyte / capillary resistance with subsequent upstream signal propagation (229). In fact, models have estimated pericytes, and their effect on capillary tone, may contribute 16% to 70% of resistance within the parenchyma (230,231). Glutamate has been shown to induce dilation, whereas norepinephrine induces constriction of pericytes in brain slice preparations (230).

Astrocytes form a critical component of the neurovascular unit, acting as a bridge between neurons and cerebral blood vessels with end-feet that envelope penetrating arterioles. Excitatory and inhibitory neurons synapse on both astrocytes and GABAergic interneurons that are closely associated with astrocytic end-feet. Astrocytes are thought to contribute to a slow-onset (3-4 seconds) neurovascular coupling response, but may play a lesser role in the immediate

hyperemic effect (< 1 second) following neural activation (232). Glutamate released from active neurons triggers metabotropic receptor-mediated calcium uptake into neighbouring astrocytes (reviewed in: (232)). The resulting change in calcium concentration initiates a signaling cascade leading to the release of arachidonic acid (vasoconstrictory) and its metabolic derivatives including epoxyeicosatrienoic acid (EET) and prostaglandins (vasodilatory) onto arteriolar smooth muscle cells and vascular endothelial cells (233-236). Inhibitory interneurons communicating with neurons, astrocytes, and microvessels play a role in integrating local signals and releasing vasoactive substances (e.g. NO, acetylcholine, neuropeptide-Y, vasoactive intestinal peptide) to precisely regulate CBF (237,238). Interestingly, recent work has suggested reciprocal communication from blood vessels to astrocytes to neurons – a so-called vasculo-neuronal coupling (239).

Although our understanding of exact mechanisms governing neurovascular coupling – and certainly vasculo-neuronal coupling (239) – remains incomplete, it is clear that multiple overlapping pathways exist to ensure CBF is appropriately altered in response to changes in local neural activity (220).

1.3.4.4.2 Characterizing NVC dynamics using transcranial Doppler ultrasound

A robust NVC response can be observed *in-vivo* using TCD to record blood velocity in the PCA before and after activation of the occipital cortex, typically achieved by having patients close and open their eyes to a visual stimulus such as reading (156,213,240-244). After stimulus onset, the canonical NVC response exhibits a brief delay followed by an increase in PCAv to 15-20% above eyes-closed baseline velocities, dependent on stimulus intensity (145,220,240-246). Flow

velocity in the PCA then tends to plateau for the duration of visual stimulation, reflecting a matching of CBF to metabolic demand. Whereas this phenomenon is maintained at rest (156,240-242,244,245), during moderate exercise (242), and throughout healthy human aging (247,248), impairments in NVC have been revealed across a variety of clinical conditions. For example, patients with autonomic dysfunction have shown altered neurogenic regulation of NVC responses (156,249); in high-level spinal cord injury patients, NVC dysfunction persisted even after correcting blood pressure pharmacologically (156); oxidative stress consequent to ß-amyloid plaques in the brains of Alzheimer patients is thought to directly inhibit NVC responses (250,251). It has been proposed that neuronal damage and astrocytic scar formation precludes normal NVC responses in severe TBI patients, with some of these individuals exhibiting inverse NVC responses (252,253). Mechanistically, biophysical models of TBI have estimated increased heterogeneity in CBF transit time across capillaries – secondary to capillary compression by astrocytic end-feet – to cause relevant reductions in oxygen availability (254). However, effects of mild sport-related head trauma on NVC dynamics are currently unknown.

1.3.4.4.3 Alterations in NVC dynamics following sport-related head trauma

To-date, no studies have been conducted using techniques with sufficient temporal resolution to evaluate the dynamics of NVC responses in the context of sport-related head trauma. Insights may be gleaned from the various functional MRI studies that have been conducted, though the interpretation of results is not straightforward. The majority of task-based fMRI studies have used tasks probing frontal lobe/executive function. Typically, increases in cortical activation in mTBI patients relative to healthy controls have been reported across multiple brain regions (reviewed in (67)) and may be related to symptom severity, often despite no differences in task

performance between groups (255-257). However, other fMRI studies have concluded concussions cause *hypo*activation, particularly in individuals with persistent symptoms, though task performance was not always equal between groups (258-261). A recent prospective study demonstrated global hyperactivation that gradually declined in extent during the recovery process, with BOLD responses measured at 3-days, 2-weeks, and 2-months post-injury (257). One study has suggested an association between the degree of hyperactivation and prolonged recovery (262). Participants reporting more post-concussion syndrome symptoms (persisting >1 year post-injury) exhibited greater activation in attention-related areas but reduced activation in task-irrelevant areas as cognitive load was increased during a top-down attentional task (263). Elevation of task-based BOLD responses in mTBI patients have been interpreted to reflect recruitment of additional neural resources to compensate for cognitive and/or attentional deficits (257,264). Only one study has considered the potential for mTBI to alter the dynamics of hemodynamic responses, and reported greater activation in the visual cortex of mTBI patients presenting to the emergency department relative to controls, particularly in the 2-4 second window after stimulus onset, suggesting a shorter time-to-peak response (265). Although inconsistency across fMRI studies may be partly attributed to methodological differences and injury heterogeneity across samples (266), a recent meta-analysis of fMRI findings in mTBI observed hyperactivation during continuous tasks and hypoactivation during tasks requiring discrete periods of working memory (267).

It remains unclear if multiple concussions in combination with repetitive subconcussive trauma are detrimental to NVC over the course of a career. Multiple recent task-based fMRI studies in high-school American football athletes have demonstrated changes in hemodynamic response

patterns to cortical activation as a function of head impact exposure across a single season (20,22,69,76,268). During in-season assessments, athletes performing abnormally on tests of cognition showed reduced temporal and occipital cortex BOLD responses and experienced higher numbers of impacts to the top-front of the head relative to subjects performing normally (22). Relative to preseason scans, within-subject visual BOLD responses were much more variable during periods of the season with high contact volume, although these deficits tended to normalize with cessation of contact (69). Altered BOLD responses were more likely to persist after the end of the athletic season in athletes receiving 50^{+} hits per week during the season (268). Whereas a history of multiple concussions may not detrimentally affect hemodynamic responses to cortical activation in otherwise healthy young adult athletes (269,270), multiple concussions have been shown to negatively affect cortical recruitment patterns in the relational memory network of former professional football players (271). Thus, multiple studies have reported changes in BOLD responses following repetitive subconcussive trauma. However, the extent to which NVC dynamics are affected by sport-related head trauma may be better estimated by obtaining pre-injury data using techniques providing better temporal resolution, such as TCD.

1.3.4.5 Autonomic factors in cerebral blood flow regulation

The entire cerebrovascular tree is extensively innervated by autonomic fibers. Extrinsic perivascular neurons from both sympathetic and parasympathetic origins innervated extracranial arteries (ICA/VA), large arteries of the brain (MCA/PCA) and pial arteries on the brain surface, while intrinsic fibres innervate parenchymal arterioles and astrocytes (272). Vascular tone in pial arterioles is sensitive to neurotransmitters including norepinephrine, whereas parenchymal

arterioles do not respond as effectively (273). Rather, *in vitro* work suggests signaling from intrinsic neurons causes a release of vasoactive mediators that exert constrictory or dilatory effects on local endothelial cells, astrocytes, and upstream vascular smooth muscle (reviewed in: (226,272)). However, determining the functional role of intrinsic cerebrovascular innervation in humans has proved challenging (145).

Extrinsic postganglionic autonomic fibres arise from three sources, including the sphenopalatine ganglion, superior cervical ganglion, and the trigeminal ganglion (145,226). A role for sympathetic modulation of CBF is directly supported by observations in of increases in CBF following cervical ganglionectomy (274-277) and following pharmacological blockade of cervical ganglion (278-281). Indeed, it is thought that sympathetic activity plays a protective role in preventing cerebral hyper-perfusion by increasing vessel tone, particularly during changes in perfusion pressure (226,282-286). For a given rise in MAP, sympathetic ganglion blockade (287) and α-adrenoreceptor block (288) both led to larger increases in CBF and caused increases in transfer function gain and decreases in phase, indicative of impaired CA (191,289). The influence of parasympathetic activity is more poorly understood, though it is thought to counteract sympathetically-mediated vasoconstriction through activation of the trigeminal nerves (226). While the precise role of autonomic innervation in the regulation of CBF remains controversial (286), it is clear that the autonomic nervous system has the capacity to modulate cerebral hemodynamics.

1.3.4.5.1 Autonomic dysregulation following sport-related head trauma

Autonomic dysregulation has been repeatedly documented following sport-related concussion, most often in the form of altered heart rate variability. Generally, parasympathetic activation slows heart rate and increases heart rate variability, whereas sympathetic activation yields opposite effects. Reduced heart rate variability is thought to reflect an impaired ability of the autonomic nervous system to respond dynamically to the environment, and has been associated with poorer outcome in a variety of clinical scenarios; for example, decreased variance in R-R intervals as well as decreased power in the very low and low frequency ranges have been found to be independently predictive of mortality in myocardial infarction patients (290). In severe TBI, HRV metrics have shown acute cardiac autonomic dysfunction to be directly associated with high mortality rates in TBI patients (291). Some groups have suggested that central autonomic dysregulation may at least partly explain the observed increase in long-term mortality in persons with a history of mTBI (292-295). Associations reported in recent studies from Sung and colleagues suggest altered cardiac autonomic function may contribute to enduring emotional dysfunction following mTBI, particularly in female patients (296,297). Following sport-related concussion, various cross-sectional studies have outlined reductions in heart rate variability suggestive of elevated sympathetic and / or reduced parasympathetic outflow that may persist beyond clearance for clinical recovery, particularly during activity (298-306). Concussion-induced increases in sympathetic drive have been shown to induce a transient “stiffening” of peripheral blood vessels (300). Given the extensive autonomic innervation of the cerebrovasculature (272), alterations in autonomic drive could contribute to altered cerebral hemodynamics following sport-related head trauma, though no evidence currently exists to support this idea.

1.4 Thesis objectives and summary of studies

The specific objective of this proposal was to use a prospective, longitudinal cohort study design to test the hypothesis that sport-related head trauma leads to quantifiable changes in brain myelination, neurovascular coupling dynamics, and the MAP-CBF relationship. Secondary objectives were to evaluate relationships between changes in cerebrovascular function and biomechanical metrics of head impact exposure. The results of this work will be incorporated within a broader investigation of the physiological effects of head trauma in sport, towards an evidence base that will aid in the development of objective tools to be used to prevent, identify, and manage dangerous levels of exposure to sport-related head trauma.

1.4.1 Specific aims and hypotheses

1.4.1.1 Study 1 (Chapter 2)

Primary Aim: To investigate how myelin content in the *in-vivo* human brain is influenced by concussion, as well as exposure to repetitive subconcussive head trauma. **Hypothesis 1a**: Brain myelin content will be reduced following a concussion. **Hypothesis 1b**: Brain myelin content will be reduced following a season of exposure to repetitive subconcussive head trauma.

1.4.1.2 Study 2 (Chapter 3)

Primary Aim: To investigate how metrics of dynamic cerebral autoregulation are influenced by sport-related concussion, both acutely and as a function of multiple previous concussions. **Hypothesis 2a**: Dynamic cerebral autoregulation will be temporarily impaired following acute concussion. **Hypothesis 2b**: The dynamic cerebral pressure-flow relationship will be impaired in otherwise healthy athletes with a history of three or more concussions relative to those with no

history of concussion. **Hypothesis 2c**: Greater impairment in CA will correlate with higher symptom severity scores, as well as with degree of impairment in neurocognitive function.

1.4.1.3 Study 3 (Chapter 4)

Primary Aim: To investigate how metrics of dynamic cerebral autoregulation are influenced by exposure to repetitive subconcussive head trauma in elite athletes. **Hypothesis 3a**: Dynamic cerebral autoregulation will be impaired following one season of participation in contact sports. **Hypothesis 3b**: The dynamic cerebral pressure-flow relationship will be unaffected following one season of competition in elite athletes who experience little-to-no head trauma (i.e. non-contact sports). **Hypothesis 3c**: Higher degrees of head impact exposure will be associated with higher degrees of impairment in cerebral autoregulation.

1.4.1.4 Study 4 (Chapter 5)

Primary Aim: To investigate how the dynamics of the neurovascular coupling response are influenced by sport-related concussion, both acutely and as a function of multiple previous concussions. **Hypothesis 4a**: NVC dynamics will be temporarily altered following a single sport-related concussion. **Hypothesis 4b**: NVC dynamics will be altered in otherwise healthy athletes with a history of three or more concussions relative to those with no history of concussions. **Hypothesis 4c:** Larger alterations in NVC dynamics will be related to higher symptom severity scores, as well as the degree of impairment in neurocognitive function post-injury.

1.4.1.5 Study 5 (Chapter 6)

Primary Aim: To investigate how the dynamics of the NVC response are influenced by exposure to repetitive subconcussive head trauma in elite athletes. **Hypothesis 5a**: NVC dynamics will be altered following one season of participation in contact sports. **Hypothesis 5b**: NVC dynamics will be unaffected following one season of competition in elite athletes who experience little-to-no head trauma (i.e. non-contact sports). **Hypothesis 5c**: Higher degrees of head impact exposure will be associated with larger alterations in NVC dynamics.

1.4.2 Presentation

The current dissertation contains six additional chapters. Following this overview chapter, Chapter 2: entails an original research study into the effects of sport-related head trauma on the integrity of the myelin sheath using a novel MRI-based imaging technique. The fraction of total water content in the brain attributable to myelin was evaluated at preseason in collegiate ice hockey athletes, with repeat evaluations occurring at three post-concussion time-points as well as after completion of the competitive season. In a similar longitudinal design, Chapter 3: assessed the effects of acute and multiple prior concussions on indices of dynamic CA using a hemodynamic challenge commonly experienced in everyday life – shifts in posture from squatting to standing. Chapter 4: extends from the findings in Chapter 3 but with a focus on the effects of exposure to repetitive subconcussive head trauma through comparison of pre- and post-season CA function in contact and non-contact sport athletes. Chapter 5: presents an evaluation of the within-subject effects of sport-related concussion on dynamics of the NVC response in the PCA using a visual stimulation paradigm. Chapter 6:details the effects of repetitive subconcussive trauma on NVC dynamics in the PCA. Finally, Chapter 7: summarizes

the five studies in this thesis, provides an interpretation of their collective conclusions, and identifies important avenues for future research.

Chapter 2: Myelin water fraction is transiently reduced after a single mild traumatic brain injury – a prospective cohort study in collegiate hockey players

2.1 Summary

Impact-related mild traumatic brain injuries (mTBI) are a major public health concern, and remain as one of the most poorly understood injuries in the field of neuroscience. Currently, the diagnosis and management of such injuries are based largely on patient-reported symptoms. An improved understanding of the underlying pathophysiology of mTBI is urgently needed in order to develop better diagnostic and management protocols. Specifically, dynamic post-injury changes to the myelin sheath in the human brain have not been examined, despite "compromised white matter integrity" often being described as a consequence of mTBI.

In this preliminary cohort study, myelin water imaging was used to prospectively evaluate changes in myelin water fraction, derived from the T_2 decay signal, in two varsity hockey teams (45 players) over one season of athletic competition. 11 players sustained a concussion during competition, and were scanned at 72 hours, 2 weeks, and 2 months post-injury. Results demonstrated a reduction in myelin water fraction at 2 weeks post-injury in several brain areas relative to preseason scans, including the splenium of the corpus callosum, right posterior thalamic radiation, left superior corona radiata, left superior longitudinal fasciculus, and left posterior limb of the internal capsule. Myelin water fraction recovered to pre-season values by 2

months post-injury. These results may indicate transient myelin disruption following a single mTBI, with subsequent remyelination of affected neurons. Myelin disruption was not apparent in the athletes who did not experience a concussion, despite exposure to repetitive subconcussive trauma over a season of collegiate hockey. These findings may inform many of the metabolic and neurological deficits observed clinically following mTBI.

2.2 Introduction

Impact-related mTBI are a growing public health concern globally, with major causes including sports, motor vehicle accidents, falls, and assaults. The Centers for Disease Control and Prevention estimate that approximately 1.6 - 3.8 million mTBI occur annually in the United States alone (307). Symptoms of concussion, a form of mTBI, are thought to result from mild diffuse axonal injury (DAI) that is not detectable on conventional computed tomography or magnetic resonance imaging (MRI). Currently, the diagnosis and management of this broadly defined and poorly understood injury are based on clinical observation and patient-reported symptoms, despite numerous recent efforts towards the development of objective tools to link functional deficits with quantifiable structural changes. While the majority (80-90%) of individuals with mTBI symptoms recover in 7-10 days, a subset of individuals are left with persistent disability for months to years (9). Furthermore, emerging evidence has suggested a possible link between clinically manifest concussions, as well as repetitive sub-clinical head impacts, and the development of long-term neurodegenerative changes, termed chronic traumatic encephalopathy (15). The long-lasting effects of single and repetitive mTBI may include serious cognitive and behavioural deficits, such as problems with affect regulation, attention, memory, and depression (15). Given these concerns, it is imperative that techniques be developed to

provide a more thorough understanding of precisely how mild traumatic brain injuries affect the brain.

Myelin is thought to be an important player in the pathophysiology of TBI, though its role is poorly understood (82,83); increasingly, evidence implicates that damage to either myelin or the axon can lead to subsequent damage to the other (84,88). In the case of sport-related head trauma, biomechanical forces imparted on the head cause linear and rotational acceleration-deceleration of the brain within the rigid cranium, which creates diffuse shear strains in the brain tissue. Due to the viscoelastic nature of this tissue, the rate at which such strains are applied is an important factor towards the resultant tissue damage. While primary axotomy is generally observed following high magnitude impacts associated with more severe traumatic brain injuries, it is thought that the axonal pathology observed in mTBI develops over days to weeks following the initial insult (78,80,81). Current theories describe a post-traumatic neurometabolic cascade involving ionic flux and indiscriminate glutamate release, leading to mitochondrial dysfunction and calcium sequestration, and a subsequent energy crisis with cytoskeletal damage (78,79). Intracellular calcium overload has been recognized as a significant cause of damage to both myelin and oligodendrocytes (87). Impaired axonal transport leads to swellings within the axon containing organelles and other transport materials, creating the potential for a sequence of secondary axonal disconnection and Wallerian degeneration (78,89). Prolonged global and regional reductions in cerebral blood flow have also been reported following concussion, and were related to recovery duration (2,43); experimental work has demonstrated that accumulative oxidative stress in the context prolonged cerebral hypoperfusion suppresses both the differentiation of oligodendrocyte precursor cells to oligodendrocytes as well as myelin staining

(92). Thus, loss of oligodendrocytes and corresponding demyelination of affected axons is anticipated (83,84,93). Histopathologically, this has been observed as axonal bulbs, irregular tortuous axonal varicosities and small globoids of degraded myelin sheath (82,90,91). While various reports have implicated that myelin fragmentation and degradation occurs following axonal injury (82,90,95,308-310), dynamic *in vivo* myelin changes have not been directly observed in the human brain. Whether the result of direct, multifocal primary traumatic axonal injury or secondary axotomy, subsequent axonal degeneration is a possible outcome of mild DAI, but axon damage and myelin disruption may be reversible (82,83). In contrast to situations where strain is focally applied (e.g. at anatomic boundaries), strains distributed more diffusely along the length of an axon create an elongated pattern of axonal swelling, which may be more amenable to intrinsic repair mechanisms (78).

Our current understanding of the pathophysiology of mTBI is derived primarily from post-mortem studies and animal models. While post-mortem studies are useful for exploring histopathological changes in the brain following mTBI, by definition they are unable to provide information on the dynamic post-injury changes explained using animal models. However, it is recognized that the substantial mass and inertia of the human brain play an important role in the development of diffuse axonal injury (78), highlighting an inherent challenge in the development of valid rodent models of mTBI (311). A non-invasive method for objective, *in-vivo* evaluation of the pathophysiological changes associated with concussion is currently one of the primary goals in mTBI research. Specifically, the dynamic effects of acute mTBI on myelin in the human brain represent a major gap in our understanding of these injuries.

Here, we use myelin water imaging (MWI (115)) to quantify metrics associated with changes in myelin after mTBI. It has previously been identified that decomposing the T_2 decay signal results in three components. They include a very long component (~2 seconds) that corresponds to cerebrospinal fluid, an intermediate component (~80 ms) derived from intracellular and extracellular water, as well as a short component (~20 ms) from water that is within the myelin bilayers (111,312). As such, the fraction of total brain water attributable to myelin can be estimated, and is termed the myelin water fraction (MWF). Recent advances in myelin water imaging have enabled rapid exploration of myelin damage across the whole brain (115). Previous work has demonstrated a good quantitative relationship between MWF, as derived from MR images, and histological staining for myelin in tissue from both the central nervous system (111) and peripheral nervous system (119). Being the only technique that has been validated by histopathology, T_2-relaxation based myelin water imaging has provided novel insights in demyelinating diseases, such as multiple sclerosis research (313), but has never been used in the context of mTBI to evaluate post-injury myelin dynamics. In this prospective study, we followed a group of individuals at high risk of sustaining a concussion, comprised of two varsity hockey teams, for one athletic season. This is the first study to directly evaluate myelin changes following mTBI in the *in vivo* human brain, assessing myelin water fraction in the context of sport-related head trauma.

Accordingly, our objectives in the current study were to observe changes in myelin water fraction, relative to baseline, at acute, sub-acute, and chronic post-mTBI time points in a group of individuals at high-risk of sustaining a mTBI. We hypothesized that a reduction in myelin water fraction would be observed following concussion, with recovery to near-baseline values by

two months post-injury.

2.3 Methods

2.3.1 Study design

25 male and 20 female college-aged (mean age 21.2 ± 3.1 years) amateur hockey players from two ice hockey teams participated. All players underwent baseline MRI scanning and neuropsychological testing (Sport Concussion Assessment Tool version 2 (SCAT2, (39)) before the beginning of the hockey season. Players who were diagnosed with a concussion by an independent physician, based on criteria outlined in the 3rd Consensus Statement on Concussion in Sport (39), underwent additional scans and testing at 72-hours, 2-weeks, and 2-months after injury. End of season scans were also completed for the non-concussed cohort. The sample size for this preliminary study was determined from previous cross-sectional literature reports on expected incidence of concussions. Cross-sectional imaging studies have found effects of concussion with less than 15 subjects each (103). The incidence of concussion in competitive contact sports such as Varsity ice hockey was estimated at 25% or higher (6). The rationale was then to work with two varsity ice hockey teams where one could expect eight to 12 concussions within a season, sufficient for a preliminary prospective study with pre-injury data from the same brains. Exclusion criteria included a history of severe cognitive impairment, psychiatric or other central neurological disorder, pacemaker use, previous eye surgery, or having worked in an environment likely to expose the participants to the risk of metal fragments being embedded in their eyes. No participants were excluded from this study on these grounds. All participants provided written informed consent prior to participation in the study, which was approved by the University of British Columbia Clinical Research Ethics Board (H11-00423).

2.3.2 Myelin water imaging and processing

MRI data were acquired on a Philips Achieva 3T scanner equipped with Quasar Dual Gradients and an eight-channel SENSE head coil. A 32 echo T_2 scan (14 minutes, 22 seconds) was acquired for myelin assessment (TR=1000 ms, TE=10, 20, … , 310, 320 ms, flip angle=90°, acquisition matrix=232x192, acquired voxel size 0.99 x0.99 x 5mm, reconstructed voxel size=0.96 x 0.95 x 2.5mm). The T_2 decay was decomposed using a non-negative least squares fit with an extended phase graph algorithm and flip angle optimization (115). MWF was calculated as T_2 signal from 0-40ms divided by the total T_2 signal. Other scans acquired during the scanning session included: (a) sagittal 3D T1-weighted scan (TR=8.1 ms, TE=3.7 ms, flip angle=6 degrees, acquisition matrix= 256 x 256 x 160, field of view=256 x 256 x 160 mm^3, acquired voxel size=1 x 1 x 1 mm^3, reconstructed voxel size=1 x 1 x 1 mm^3, SENSE factor of 2 along the left-right direction), (b) sagittal 3D fluid attenuated inversion recovery (FLAIR) (TR= 8000 ms, TI = 2400 ms, TE=337 ms, flip angle=6 degrees, acquisition matrix= 256 x 256 x 160 , field of view=256 x 256 x 160 mm^3, acquired voxel size=1 x 1 x 1 mm^3 , reconstructed voxel size=1 x 1 x 1 mm^3, SENSE factor of 2 along the left-right direction and 2.5 along the anterior-posterior direction), (c) multi echo susceptibility weighted imaging (SWI) using an axial 3D gradient echo scan (TR=36 ms, TE=6/12/18/24/30 ms, flip angle= 17 degrees, acquisition matrix= 440 x 222 x 64 , field of view=220 x 166 x 128 mm^3, acquired voxel size=0.5 x 0.5 x 2 mm^3 , reconstructed voxel size=0.5 x 0.5 x 1 mm^3, SENSE factor of 1.2 along the left-right direction), and (d) diffusion tensor imaging (DTI) scan (TR=7015 ms, TE=60 ms, flip angle=90 degrees, acquisition matrix=100 x 99, field of view=224 x 224 x 154 mm^3, acquired voxel size=2.2 x 2.2 x 2.2 mm^3, reconstructed voxel size=2 x 2 x 2.2 mm^3, SENSE factor of 2.1 along the anterior-

posterior direction, $b_0 = 0$, $b_1 = 700$ sec/mm^2, 60 noncolinear directions). Total data acquisition time was 48 minutes. Results from the conventional scans are reported elsewhere (314).

2.3.3 Statistical analysis

Myelin water fraction changes were evaluated through comparison of concussed athletes' baseline scans to those acquired at 72 hours, 2 weeks, and 2 months post-injury. Impact-related mTBI is a very heterogeneous injury (266). However, in the current preliminary study, the intent was to prospectively investigate myelin dynamics after mTBI without making any clinical predictions on an individual basis, let alone correlate clinical measures with imaging measures. Voxelwise statistical analysis of the data was performed using tract-based spatial statistics (TBSS) (315), part of the Functional MRI of the Brain Software Library (FSL (316)), which creates a white matter skeleton for each participant's brain using fractional anisotropy (FA) maps obtained from diffusion tensor images. First, FA images were created by fitting a tensor model to the raw diffusion data using FDT, and then brain-extracted using BET. All subjects' FA data were then aligned into a common space using the nonlinear registration tool FNIRT, which uses a b-spline representation of the registration warp field. Next, the mean FA image was created and thinned to create a mean white matter skeleton that represents the centres of all tracts common to the group. MWF maps were then registered into DTI space and projected onto the white matter skeleton. Voxelwise statistics were carried out using the randomise tool in FSL for 5000 permutations with threshold-free cluster enhancement to assess differences in myelin water fraction at each post-injury time point compared to baseline, while controlling for, as explanatory variables, age, gender, SCAT2 results, and whether scans were acquired before or after an upgrade to the scanner's gradient system occurred. The locations of significant voxels were

identified anatomically using the John's Hopkins University standard white matter atlas. No outliers were excluded from analysis, as possible "outliers" may well be within the normal spectrum of mTBI. Corrected level of significance was set at $\alpha = 0.05$. These methods were chosen explicitly to examine diffuse changes in brain structure, without *a priori* assumptions as to where such changes would occur, while compensating for multiple comparisons. This approach effectively restricts analysis to areas that are injured in the majority of subjects, and follows the myelin signal in these brain areas. Analysis of the data was performed by non-supervised algorithms. The study had a conventional imaging component as well, which required the reading of MRI data by radiologists. These data will be reported elsewhere.

2.4 Results

11 players (six female) sustained a concussion during the ice hockey season (Table 2-1). When comparing baseline scans between concussed and non-concussed athletes, voxelwise TBSS revealed no significant differences in myelin water fraction. Within the non-concussed cohort, no significant differences were identified between pre-season and post-seasons scans. Eight out of 11 concussed subjects were able to complete scanning at 72 hours post-injury, 10 out of 11 athletes were scanned at 2 weeks, and nine out of 11 were scanned at 2 months post-injury (Figure 2-1).

Table 2-1: Demographic characteristics of all athletes

Subject	Concussed (Y/N)	Sex	Age
1	Y	M	22
2	Y	M	21
3	Y	F	21
4	Y	F	19
5	Y	F	22
6	Y	F	21
7	Y	M	22
8	Y	M	24
9	Y	F	19
10	Y	F	19
11	Y	M	23
12	N	F	23
13	N	M	21
14	N	F	18
15	N	F	20
16	N	F	18
17	N	F	18
18	N	M	21
19	N	M	24
20	N	F	21
21	N	M	22
22	N	M	25
23	N	M	22
24	N	M	21
25	N	F	19
26	N	F	18
27	N	M	22
28	N	M	20
29	N	M	21
30	N	M	21
31	N	F	20
32	N	M	22
33	N	F	18
34	N	M	23
35	N	F	17
36	N	M	20
37	N	F	19
38	N	F	17
39	N	M	21
40	N	M	23
41	N	F	36
42	N	M	18
43	N	M	22
44	N	M	23
45	N	M	26

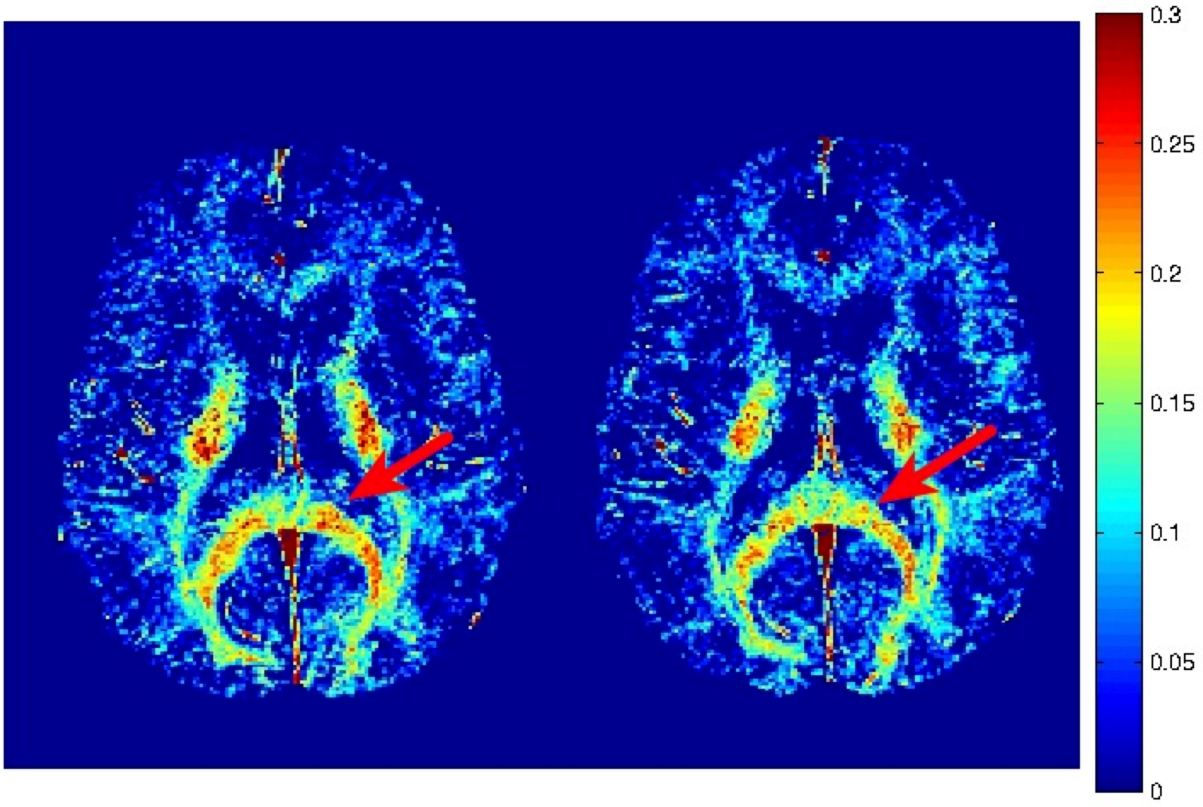

Figure 2-1: Representative myelin water fraction maps. Myelin water fraction maps from a concussed athlete at baseline (left) and two weeks post-injury (right). Myelin water fraction is measured as the T_2 signal from 0-40 ms divided by the total T_2 signal. A region of the corpus callosum with a visible reduction in MWF post-injury is highlighted by the red arrow. Fig 1 from (317). Image reproduced with permission from PLoS ONE.

Within the concussed cohort, TBSS showed clusters of voxels with significantly reduced myelin water fraction at two weeks post-injury, relative to baseline (Figure 2-2). Across all significant voxels, this represented a 5.9% ± 1.2% (mean ± standard error) reduction in MWF from baseline to 2 weeks. A decrease in MWF was observed at 72 hours post-injury in these voxels, but this did not achieve statistical significance (p=0.076). No significant MWF changes were observed between baseline and 2 months post-injury.

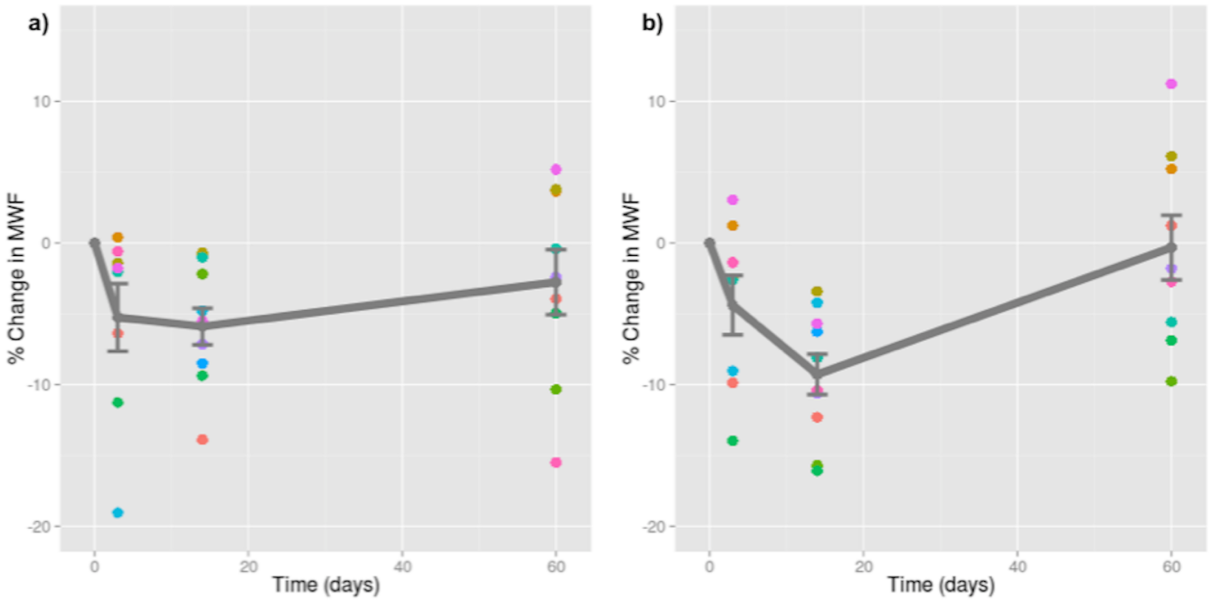

Figure 2-2. Relative myelin water fraction change post-injury. Change scores for myelin water fraction, relative to baseline, plotted against time for each subject with a mild traumatic brain injury in all significant voxels A) across the whole brain; B) in the splenium of the corpus callosum (a structure most commonly affected in mild TBI). Dots represent data points for each injured athlete (mean ± standard error plotted in grey). Note: time zero refers to baseline. Fig 2 in (317). Image reproduced with permission from PLoS ONE.

These voxel clusters were located in the splenium of the corpus callosum, right posterior thalamic radiation, left superior corona radiata, left superior longitudinal fasciculus, and left posterior limb of the internal capsule (as shown in Figure 2-3).

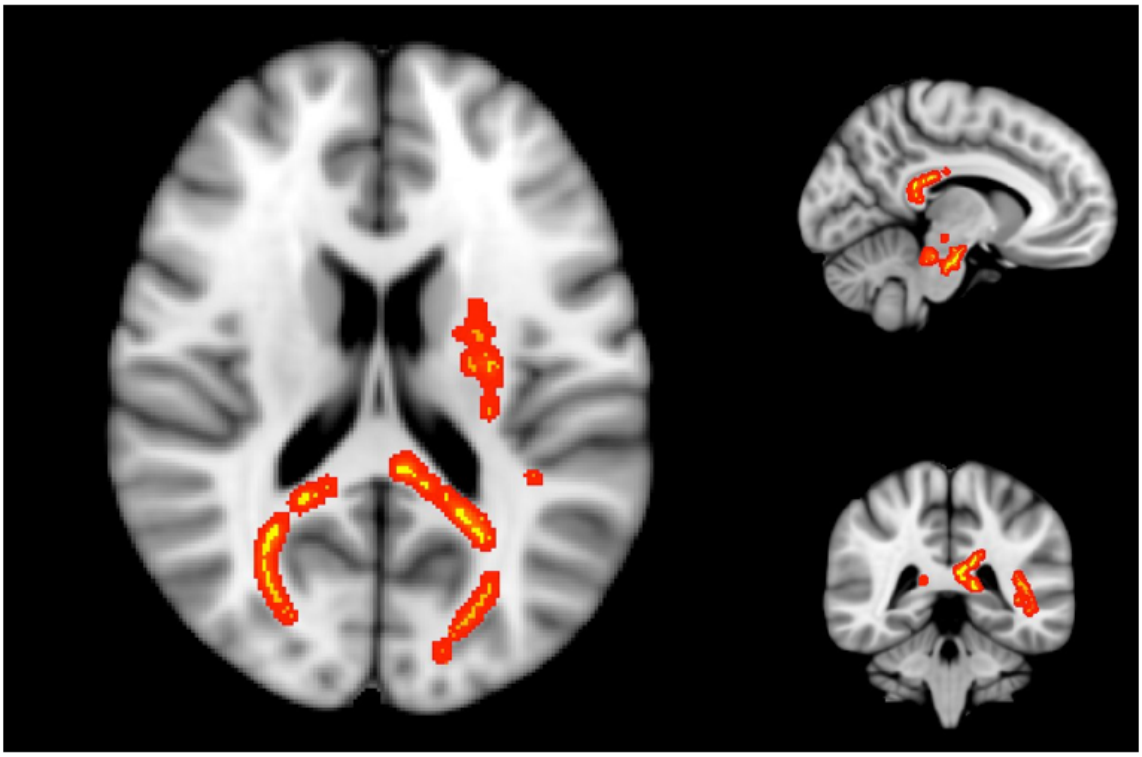

Figure 2-3. Brain areas with significantly reduced myelin water fraction. Areas of significantly reduced myelin water fraction in athletes with concussion at two weeks post-injury, superimposed on a standard brain. These areas include the splenium of the corpus callosum, right posterior thalamic radiation, left superior corona radiata, left superior longitudinal fasciculus, and left posterior limb of the internal capsule. Fig 3 from (317). Image reproduced with permission from PLoS ONE.

2.5 Discussion

In the current preliminary report, we present the first prospective study on myelin water fraction in the context of mTBI in the human brain, and demonstrate significant post-injury reductions. In contrast, tract-based spatial statistics of post-season MWF maps demonstrated no differences from pre-season scans in athletes who did not sustain a concussion. Myelin water fraction, derived from the T_2 decay signal, is a direct marker of myelin content in the brain. These data may indicate a process of transient myelin disruption following mTBI in areas of the brain

previously shown to exhibit diffusivity changes (reviewed in (97)). Furthermore, MWF recovered to near-baseline values by 2 months post-injury. Although significantly reduced MWF values were observed at the 2-week time point only, there was a trend towards decreased MWF at 72 hours post-injury ($p = 0.076$). That no differences were seen between pre- and post-season for non-concussed athletes is encouraging, given recent concern over the effect of exposure to repetitive sub-clinical trauma (318).

The exact time course of myelin alterations following sport-related concussion cannot be determined from this exploratory study. As the subjects were acutely concussed and enduring the worst phase of their symptoms, only eight of the 11 subjects were able to complete the 72 hour scan; it is possible that the reduced sample size at this time point may have obviated statistical significance from being achieved. Previous work has demonstrated that peak concentrations of myelin basic protein in the cerebrospinal fluid (CSF) occur at 48-72 hours following severe TBI, where primary axotomy likely occurred (319). In milder forms of TBI such as concussion, where primary axotomy does not occur, myelin degradation (as a downstream effect of the post-injury neurometabolic cascade and subsequent neuroinflammation) may take longer to occur, which may also explain the why the MWF reduction at 72 hours post-injury did not achieve statistical significance. It is important to point out that, although we observed a significant reduction in MWF at 2 weeks post-injury, peak reductions in myelin content may have occurred earlier or later. Indeed, significant loss of myelin for up to 21 days following injury has been reported following fluid percussion injury in rats (93), although this injury model is recognized to be of greater severity than sport-related concussion. Maxwell and colleagues demonstrated that optic nerve fibre degeneration may be initiated days to weeks after a mechanical stretch injury (81).

Using closed-skull impacts in mice (an injury model more similar to concussion), Mierzwa et al. (82) reported markers of neuroinflammation localized to areas of axon and myelin pathology for up to 6 weeks. Similarly, true recovery of MWF to baseline values may have occurred earlier or later than two months following injury. Myelin debris is known to stimulate neuroinflammatory processes (82,95). In the context of Wallerian degeneration, myelin debris has been reported to remain for several months before full clearance by macrophages (320), while previous work has shown myelin fragments contained within activated microglia for up to 4 weeks after fluid percussion injury (321). Remyelination of multiple sclerosis lesions has also been observed within 2 months (322). The exact time course of myelination dynamics following mTBI in humans is not currently known. Nevertheless, the authors of the current preliminary article postulate that myelin fragmentation and degeneration led to the observed reductions in MWF, with the recovery of MWF by two months post-injury likely due to remyelination of the affected axons by oligodendrocytes.

We are inferring that changes in myelin water fraction are indicative of true changes in myelination within the brain. T_2 relaxation-based MWI provides insight into tissue characteristics that are not observed using standard MR imaging techniques. Numerous studies have supported that the short component of the multicomponent T_2 relaxation distribution is specific to myelin, and have suggested this as the best imaging technique to distinguish between myelination and inflammation (112,116-118). Post-mortem MRI-pathology correlation studies in both CNS (111) and animal peripheral nerve (119) have demonstrated good quantitative and qualitative relationships between MR-derived MWF and histological staining for myelin. Myelin water imaging was shown to have high reproducibility in healthy brains, both longitudinally and

between imaging sites using the same scanner type and the same protocol (120,121). Moreover, MWF in the normal appearing white matter of people with multiple sclerosis was also shown to be stable over at least six months (122). In a guinea pig model of demyelination (experimental allergic encephalomyelitis), a decrease in the short T_2 component was observed, which was consistent with histological myelin loss (116). As such, it is reasonable to conclude that the MWF changes observed in the current study are indeed reflective of transient reductions in myelin content following mTBI.

The current results provide the first direct, *in-vivo* demonstration of myelin damage following sport-related mTBI in humans, a notion that is supported by previous work in different models. Experimental models have implicated myelin disruption and subsequent remyelination to be major components of the degeneration and recovery process in white matter following mTBI (82,83). Multiple mTBI studies using closed-skull impacts in mice have shown significant demyelination distributed across intact axons using electron microscropy and histological staining techniques. Donovan and colleagues observed structural abnormalities in rats that experienced controlled cortical impacts, including separation of myelin from the axon, as well as decompaction and fragmentation of the myelin sheath following repetitive mTBI, compared to sham injury (323). Both single and repetitive injuries have been shown to lead to significant reductions in the thickness of myelin surrounding axons (82,323). Observations of increased Luxol fast blue staining (a histological stain for myelin) within the cytoplasmic compartment of cells following traumatic brain injury has supported the role of myelin degeneration and subsequent active phagocytosis of myelin fragments (308). Reports of acute decreases in axial diffusivity following experimental closed-skull mTBI, measured using diffusion tensor imaging,

followed by delayed increases in radial diffusivity also imply that axonal damage may be followed by myelin damage (323,324). Myelin disruption slows signal conduction, thereby desynchronizing circuitry within the brain (325) that may contribute to the observed neurological deficits that accompany mTBI, such as reduced information-processing speed. Accordingly, demyelination has been proposed to explain significantly slower interhemispheric transfer times (a measure of conduction speed through the corpus callosum) in paediatric human TBI patients, who also exhibited lower fractional anisotropy and higher mean diffusivity values in at least 13 out of 18 white matter tracts that were evaluated (108).

Transient reductions in the myelination status of axons following mTBI, as observed in the current study, may also help to explain the observation of a 'temporal window of vulnerability' following concussion (42), by which the brain is more sensitive to additional trauma during the recovery period. Magnetic resonance spectroscopy findings have demonstrated significant reductions in N-acetyl-aspartate (NAA) in the brain (42,326), a marker recently shown to be localized in the myelin of adult brains (327). Such NAA changes largely resolved within 30 days, but they did not coincide with clinical resolution of symptoms, implying a period of acute metabolic imbalance during the post-mTBI recovery process (42,326). Interestingly, MR spectroscopy studies in multiple sclerosis patients have also detected significant NAA reductions in the absence of any visible lesions (328). The integrity of axonal neurofilaments is dynamically regulated by the influence of myelin on kinase and phosphatase activity. From a developmental perspective, myelin increases phosphorylation of neurofilaments, enabling lateral extension of these structural proteins, which correspondingly increases axonal calibre and conduction velocity (329). Animal studies have shown that demyelination leads to a reduction in neurofilament

phosphorylation and consequent axon calibre (330,331). While the role of the myelin sheath in facilitating propagation of electrical impulses is well known, its pivotal role in neuronal metabolism is highlighted by estimates that myelinated axons are up to 70 times more metabolically efficient than unmyelinated axons (85). Myelin may also modulate the activity of the enzyme that hydrolyzes NAA into aspartate and acetate (involved in the production of coenzyme A) (332). As such, post-mTBI metabolic disturbances may at least partly be explained by changes in myelination (84). Moreover, previous research has suggested that unmyelinated axons may be more vulnerable to trauma, demonstrating greater electrophysiological impairment than their myelinated counterparts (333,334). It is possible that myelination changes after concussion may sensitize axons to further damage if exposed to additional trauma (309).

In evaluating changes in myelination within the brain, neuroimaging techniques that enable us to reliably observe pathophysiological changes behind an intact blood-brain barrier, such as myelin water imaging, are more appropriate than measurement of blood and CSF biomarkers. An important clinical limitation of using CSF for biomarker analysis is the invasive nature of acquiring such samples, which requires a lumbar puncture. While acquiring blood samples is less invasive, the blood-brain barrier, which is thought to remain intact following mTBI (335,336), attenuates the ability of biomarkers to enter the blood. Some biomarkers may be bound to carrier proteins or undergo degradation in the blood, further decreasing their measurable concentration, while extracerebral sources of some biomarkers also exist. Consequently, it has been difficult to identify reliable blood markers for mTBI (337). With specific regards to the evaluation of myelin, myelin basic protein (MBP) is the best available fluid biomarker of myelin integrity. No studies are currently available regarding the effects of mTBI on MBP levels in the CSF. While

the specificity of MBP in the blood for TBI appears to be adequate (96%), this marker lacks the sensitivity (44%) required to be clinically useful (338). In contrast, myelin water imaging enables us to reliably observe pathophysiological reductions in myelin behind a potentially intact blood-brain barrier, and thus appears to be a more appropriate *in-vivo* method towards answering research questions related to changes in myelination.

Myelin water imaging is a preferable technique to diffusion tensor imaging (DTI) for the evaluation of myelin integrity following mTBI (112). DTI is sensitive to diffusion properties of the various tissues within the brain, and has often been used as an indirect marker of myelin integrity in mTBI research. In white matter, the neurofibrils, axonal membrane, and myelin sheath promote water diffusion axially (along the length of the axons) relative to perpendicular flow, a phenomenon termed anisotropic diffusion (96) and quantified as fractional anisotropy (FA). Reports on diffusivity changes following mTBI have been equivocal, and have been reviewed in detail previously (97-99). While many studies have reported a decrease in FA following mTBI (100-102), numerous other studies have demonstrated an increase in FA (103-105), while other studies have reported no changes (106,107), or FA changes in both directions (24,105). At least part of these discrepancies may be related to inconsistent injury-to-scan intervals across studies, as the time course of changes in FA post-injury is not fully understood. Nevertheless, many studies infer that changes in DTI metrics reflect compromises in white matter and, more specifically, myelin integrity (108). Such interpretations must be made with caution, however, as myelin changes are not necessary or sufficient to yield changes in metrics of anisotropic diffusion (96). In fact, a fundamental limitation of interpreting changes in DTI metrics is the inability of this technique to distinguish between multiple factors that influence

them, including fiber diameter and density, fiber orientation, membrane permeability, edema, and demyelination (110). In contrast, T_2 relaxation-based myelin water imaging *directly* evaluates myelin content in the brain, and has been validated histopathologically (111-113).

There are several limitations to the current preliminary study. Firstly, the sample size of concussed athletes is relatively small, which precludes an in-depth analysis of any differences between male and female athletes, or the relationship between myelin water fraction and neuropsychological scores. The prospective nature of this preliminary study limited the sample size but allowed us to directly compare intra-individual changes before and after injury. Nevertheless, the authors feel that this approach is preferable to a cross-sectional case-control design with a higher sample size. By definition, the specificity of the myelin water imaging technique to myelin comes at the expense of a decreased signal-to-noise ratio, as myelin water accounts for only ~15% of total brain water content (Sled et al. 2004 in (112)). Further, different individuals have different degrees of myelination within their white matter. As such, it is imperative to obtain pre-injury data when using this imaging approach to mitigate the effects of inter-individual variation. Unfortunately, not all subjects completed all MRI scans. However, the statistical power is similar at all time points with eight out of 11 concussed subjects scanned at 72 hours, 10 out of 11 scanned at two weeks, and nine out of 11 scanned at two months. An upgrade to the MRI scanner gradient system occurred during the course of the study, which included a replacement of the gradient coil. The exact date of this upgrade was known and was included as a covariate in the statistical analysis, but did not alter the results. Furthermore, monthly scans of a geometry phantom were acquired during this study, which were evaluated for field distortions due to gradient non-linearities; no such distortions were found in the phantom

data. Our statistical approach (TBSS) is exploratory in nature, unable to elucidate subject-specific MWF changes as it limits analysis to the central cores of large WM tracts common across all subjects. Therefore, TBSS also does not examine the grey matter-white matter junction, where traumatic axonal injury also occurs. Nevertheless, the fact that significant MWF changes are seen in a preliminary study with a small sample size emphasizes the need for more in-depth prospective investigations into the effects of mTBI on myelin dynamics, including analytical approaches to assess within-subject changes (24,266).

2.6 Conclusions

In order to mitigate both the incidence and severity of concussive-type mild traumatic brain injuries, a better understanding of their neurobiological underpinnings is needed. This will facilitate the development of objective tools to improve the detection and management of these injuries. Although not currently appropriate for clinical application, myelin water imaging appears to be a promising approach to understanding the role of myelin following sport-related concussion. In this exploratory study, we detected significant reductions in myelin content in the brain of concussed athletes 2 weeks following injury, which recovered by 2 months. While validation is required in a larger number of subjects, the results of this preliminary study may indicate a process of transient myelin disruption following mTBI, and encourage the development of additional investigation into the effects of repetitive concussive and subclinical head impacts on the time course of myelination changes in the brain. Furthermore, exploration of the relationships between age, sex, and myelination on susceptibility to injury, as well as between myelin status and neurocognitive performance, is warranted.

Chapter 3: A transcranial Doppler ultrasound-based evaluation of the acute and cumulative effects of sport-related concussion on indices of dynamic cerebral autoregulation

3.1 Summary

Sport-related concussion is a global public health concern, with the Centers for Disease Control and Prevention considering the injury at epidemic levels. The objective of the current prospective cohort study collected between September 2013 and December 2016 was to determine the acute and cumulative effects of sport-related concussion on indices of dynamic cerebral autoregulation. 179 elite, junior-level (age 19.6 ± 1.5 years) contact sport (ice hockey, American football) athletes were recruited for preseason testing, 42 with zero prior concussions and 31 with three or more previous concussions. 18 athletes sustained a concussion during the competitive season, and completed follow-up testing at 72-hours, 2-weeks, and 1-month post-injury. Beat-by-beat mean arterial pressure (MAP) and middle cerebral artery blood velocity (MCAv) were recorded using finger photoplethysmography and transcranial Doppler ultrasound, respectively. Five minutes of repetitive squat-stand manoeuvres induced MAP oscillations at 0.05 Hz and 0.10 Hz. The MAP-MCAv relationship was quantified using transfer function analysis to estimate *Coherence* (correlation), *Gain* (amplitude ratio), and *Phase* (response latency). Repeated-measures ANOVA indicated 0.10 Hz *Phase* was reduced (mean difference ± SE) following acute concussion, compared to preseason, by 23% (-0.136 ± 0.033 rads) at 72-hours and by 18% (-0.105 ± 0.029 rads) at 2-weeks post-injury, and was related to performance on the Standardized Assessment of Concussion; recovery occurred by 1-month. Symptoms

resolved by 2-weeks, and athletes were cleared for return-to-play in a median of 14 days, implying physiologic dysfunction persisted beyond clinical recovery in many cases. When comparing autoregulatory behaviour between athletes with three or more prior concussions and those with zero, no differences were observed despite persistent concussion-like symptoms. Acute sport-related concussion induces transient impairments in the capacity of the cerebrovascular pressure-buffering system that may persist beyond clinical recovery, and may be due to a period of autonomic dysregulation. Athletes with a history of three or more concussions did not exhibit impairments relative to those with zero prior concussions, suggesting recovery of function over time. Findings from this preliminary study support the potential need to consider physiological recovery in deciding when patients should return-to-play following a concussion.

3.2 Introduction

Sport-related concussions (SRC) are a public health concern; recent reports estimate 1.1 to 1.9 million occur each year in US youth alone (4). Whereas the majority of patients recover clinically within two weeks (9), concussions are characterized by a period of increased cerebral vulnerability to additional trauma (9,42,339). Additional research has shown the effects of SRC may be cumulative, with reports demonstrating collegiate athletes with a history of three or more concussions exhibit a three-fold increased risk of a subsequent concussion (11).

The increased cerebral vulnerability observed post-SRC might reflect a distinction between *physiological* and *clinical* recovery, the latter being heavily based on symptom resolution to guide return-to-learn/return-to-play decisions. Alterations in physiological parameters persist far

beyond the typical 7-10 days to clinical recovery: among other physiological markers, cortical connectivity (340), myelin content (Chapter 2:)(317), cardiac autonomic function (303,305), and cerebral blood flow (CBF) (2,43,136,137) have demonstrated recovery profiles on the order of 30^{+} days. In both paediatric and adult patients, local and global reductions in CBF have been reported following SRC that may persist beyond 30 days (2,43,136). Meier et al. (43) report regional reductions in CBF at 1-month in slower-to-recover athletes that were related to initial symptom severity (43). Contrastingly, Barlow and colleagues reported reduced CBF in asymptomatic subjects at 1-month post-injury, whereas symptomatic participants displayed increased CBF compared to controls (137). Despite demonstrated CBF alterations following concussion in the literature, surprisingly little work has focused on the effects of SRC on CBF control mechanisms.

Concussion-induced CBF disturbances may arise from disruptions in cerebral autoregulation (CA) – the ability of the cerebral blood vessels to buffer blood pressure (BP) changes (189). Normally, CA behaves as a high-pass filter, whereby higher frequency BP oscillations (>0.20 Hz) are transferred to the cerebrovasculature, while lower frequency oscillations are dampened (189). Myogenic, neurogenic, and metabolic mechanisms are involved in CA, functioning to protect against BP surges during periods of relative hypertension and against ischemia during hypotensive episodes (190,341). Disrupted CA may be a mechanism underlying persistent post-concussive symptoms (206). Impairments in CA are a significant predictor of poor outcome acutely following moderate and severe traumatic brain injury (TBI) (342,343). Impaired CA has also been observed in a small number of hospitalized mild TBI patients, the majority of whom also exhibited abnormal intracranial findings on CT (208,209). However, little is known to what

extent the dynamic, frequency-dependent relationship between BP and CBF is affected by SRC (131,175).

Accordingly, our objectives in the current study were to obtain pre- and post-injury data to provide the first evaluation of acute and cumulative effects of SRC on indices of dynamic CA. Towards this end, we sought to examine the effects of: i) acutely diagnosed SRC, and; ii) multiple (3+) previous SRC in otherwise healthy athletes.

3.3 Methods

3.3.1 Study design

179 male (mean age 19.6 ± 1.5 years) elite junior hockey (n=90) and American football (n=89) players were recruited. All participants underwent preseason baseline testing. Upon enrolment, 42 players had experienced zero concussions (Hx^{-}) while 31 had sustained three or more concussions (Hx^{3+}). Athletes diagnosed with a concussion (n=18) by an independent physician based on criteria in the 4th Consensus Statement (9) repeated testing at 72-hours, 2-weeks, and 1-month post-injury. Prior to physiological testing, participants completed the Sport Concussion Assessment Tool (SCAT3) (9), a concussion screening tool comprised of symptom characterization, the Standardized Assessment of Concussion (SAC) that probes orientation, immediate and delayed recall, and concentration, as well as performance on the modified Balance Error Scoring System (BESS) (Table 3-1). No participants were excluded based on *a priori* exclusion criteria of significant history of cardiorespiratory, cerebrovascular, neurological, or severe neurodevelopmental disorder. All subjects underwent familiarization of testing procedures, and abstained from exercise, caffeine, and alcohol for 12+ hours prior to testing. All

participants provided written informed consent, approved by the University of British Columbia Clinical Research Ethics Board.

Table 3-1. Participant characteristics. Demographics, SCAT3 performance, and resting physiological parameters during each test session for acutely concussed athletes. Data are presented as mean (SD) unless otherwise noted.

Metric	Concussion History		Concussed (n=18)			
	0 (n=42)	3+ (n=31)	Preseason	72 Hour	2 Week	1 Month
Age (years)	19.0 (1.4)	19.6 (1.9)	18.6 (1.5)			
BMI (kg/m^2)	27.6 (5.1)	27.5 (4.3)	25.5 (3.1)			
RTP (days)	N/A (preseason only)		median = 14, range 7-35 days			
# Symptoms	2.2 (2.7)	4.7 (4.3)	2.0 (3.6)	11.2 (5.5)	2.8 (3.0)	1.1 (2.1)
Symptom Severity	3.4 (4.7)	9.3 (12.0)	3.6 (3.6)	25.1 (19.0)	3.6 (3.5)	1.3 (2.3)
SAC Score (/30)	27.1 (1.7)	26.7 (1.8)	26.8 (1.7)	26.5 (2.0)	26.9 (2.9)	28.1 (1.3)
BESS Score (/10)	3.5 (3.2)	3.5 (3.3)	2.6 (2.6)	4.2 (3.1)	3.0 (2.3)	2.0 (2.0)
MCAv (cm/s)	55.1 (9.0)	55.6 (9.3)	53.8 (7.0)	53.5 (8.7)	53.3 (9.5)	53.2 (7.8)
MAP (mmHg)	95.0 (13.6)	94.1 (12.4)	92.3 (20.1)	90.4 (18.2)	92.0 (16.5)	91.2 (15.9)
$P_{ET}CO_2$ (mmHg)	37.7 (2.8)	38.1 (2.5)	37.7 (3.2)	37.6 (3.7)	37.9 (4.1)	37.6 (4.5)
HR (bpm)	75.1 (9.8)	73.6 (8.7)	79.4 (8.3)	79.7 (12.2)	82.1 (15.9)	79.5 (10.2)

SCAT3 = Sport Concussion Assessment Tool version 3; BMI = body mass index; RTP = return-to-play duration; SAC = Standardized Assessment of Concussion; BESS = Balance Error Scoring System; MAP = mean arterial pressure; MCAv = middle cerebral artery blood velocity; $P_{ET}CO_2$ = end-tidal partial pressure of carbon dioxide; HR = heart rate

3.3.2 Instrumentation

Participants were equipped with a three-lead electrocardiogram (ECG). Transcranial Doppler (TCD) ultrasound (ST3, Spencer Technologies, Seattle, WA) recorded blood velocity in the middle cerebral artery (MCA*v*). After the vessel was identified and signal optimized according to depth, waveform, and velocity, the ultrasound probe was locked in place with a fitted head frame (Spencer Technologies, Seattle, WA). Our research group has demonstrated this method of indexing CBF to be highly reliable and reproducible in a similarly aged population, with within-

subject coefficient of variations reported to be 2-3% (210). Beat-to-beat BP was recorded with finger photoplethysmography (Finometer PRO, Finapres Medical Systems, Amsterdam, Netherlands). End-tidal partial pressure of carbon dioxide (P_{ETCO_2}) was monitored using an online gas analyzer (ML206, AD Instruments, Colorado Springs, CO). All data were sampled at 1000 Hz (PowerLab 8/30 ML880, AD Instruments) and stored for offline analysis using commercially available software (LabChart version 7.1, AD Instruments).

3.3.3 Experimental protocols

All visits to the laboratory occurred at the same time of day (344) and involved a hemodynamic challenge protocol (repetitive squat-stand manoeuvres) (210). Resting data were recorded during five-minutes of quiet stance. During squat-stands, subjects started from standing, squatted and held a ~90˚ knee angle, and returned to standing at a pace dictated by a metronome. Squat-stands were performed for five minutes at two different frequencies – 0.05 Hz and 0.10 Hz – thought to reflect myogenic and autonomic contributions towards CA, respectively (191-193).

3.3.4 Data processing and transfer function analysis

Beat-to-beat mean arterial pressure (MAP) and MCA*v* were determined from each R-R interval. All data were analyzed with custom-designed software in LabView 14 (National Instruments, Austin, TX), as outlined previously (210). MAP and MCA*v* data were spline interpolated and resampled at 4 Hz. In accordance with best-practice guidelines for transfer function analysis (212), each 5-min recording was divided into five successive windows with 50% overlap. Data within each window were linearly detrended and passed through a Hanning window prior to fast

Fourier transform. Transfer function analysis involved determining the cross-spectrum between MAP and MCA*v*, divided by the MAP autospectrum, in order to derive transfer function *Coherence*, *Phase*, and normalized *Gain*. Similar to a correlation coefficient, *Coherence* describes the proportion of variance in the output signal (MCA*v*) explained by the input signal (MAP); a high coherence improves within-subject reliability of *Phase* and *Gain* (210). *Phase* represents the timing offset between input and output oscillations, whereas *Gain* provides a ratio of output amplitude to input amplitude. In the setting of intact CA, higher *Phase* indicates more rapid adjustment of cerebrovascular resistance to changing MAP. Low gain indicates low magnitudes of MAP oscillation transferred to the cerebrovasculature (i.e. greater buffering) (210). Metrics were sampled at 0.05 and 0.10 Hz; phase wraparound was not present for any point-estimates.

3.3.5 Statistical analyses

All statistical analyses were performed using SPSS Statistics for Macintosh (Version 22.0, IBM Corp., Armonk, NY). Shapiro-Wilks tests were used to assess for normality, while Mauchly's test was used to evaluate sphericity. Where sphericity was violated, Greenhouse-Geiser epsilon was used to adjust degrees-of-freedom for the primary F-test. Significance was determined *a priori* to achieve an experiment-wide $\alpha = 0.05$.

3.3.5.1 Effect of acute concussion

A 2 (frequency: 0.05 and 0.10 Hz) by 4 (time: preseason, 72-hours, 2-weeks and 1-month) two-way repeated-measures ANOVA was used to evaluate the effect of acute SRC on dependent variables. When omnibus tests indicated significant main effects, pre-planned t-tests

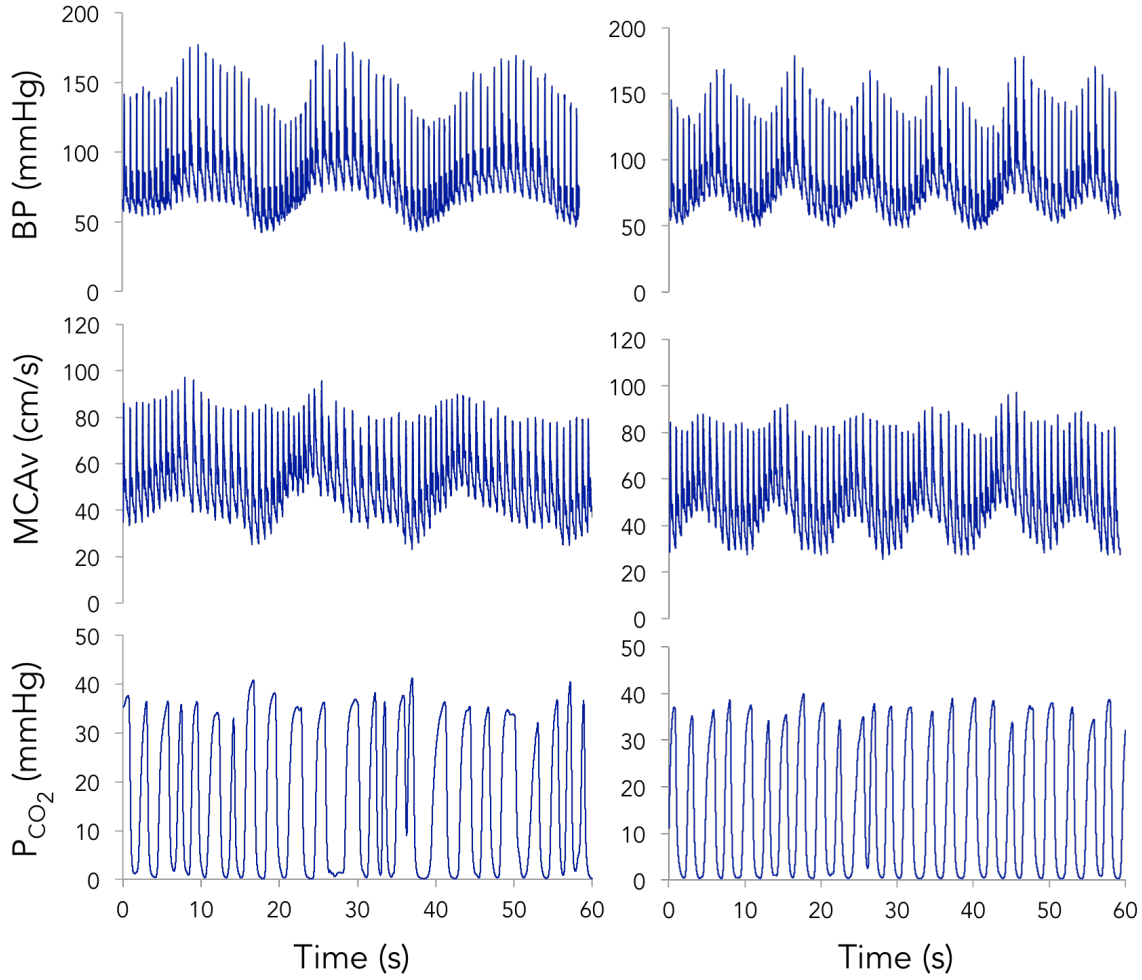

Figure 3-1. Representative traces. Representative time-series profiles showing oscillations in blood pressure (BP, top) elicited during 60 seconds of squat-stand manoeuvres performed at 0.05 Hz (left) and 0.10 Hz (right); oscillations are also observed in middle cerebral artery blood velocity (MCAv, middle); expired carbon dioxide (P_{CO2}, bottom) is less affected.

were used to evaluate each post-injury time point relative to preseason. For variables exhibiting a significant effect of time, secondary exploratory analyses were conducted to estimate the relationship between change in CA metrics and change in SCAT3 performance. Specifically, Pearson or Spearman correlation coefficients were calculated between acute change scores in the dependent variable and changes in symptom number, symptom severity, performance on the

SAC and BESS, and time to independent medical clearance for full return-to-play (RTP).

3.3.5.2 Cumulative effects of multiple previous concussions

The cumulative effect of concussions on transfer function metrics was evaluated using a 2 (frequency) x 2 (Hx^- versus Hx^{3+}) two-way mixed ANOVA.

3.4 Results

Demographic characteristics, SCAT3 performance, RTP durations and resting physiological data across are outlined in Table 3-1. Concussed athletes were cleared by their team physician to return to full-contact game participation a median 14 days post-injury. At preseason, Hx^{3+} participants reported a greater number ($p = 0.033$) and severity of symptoms ($p = 0.013$) than Hx^- participants. Physiological variables did not differ between groups ($p > 0.05$). Representative traces of MAP, MCA*v*, and $P_{ET}CO_2$ are shown in Figure 3-1. Squat-stand manoeuvres increased power in MAP and MCA*v* signals at the desired point frequencies (Figure 3-2).

3.4.1 Effect of acute concussion

Two-way RM-ANOVA indicated significant main effects of frequency for *Coherence* and *Gain* (Figure 3-3). *Coherence* ($F_{1,17}=35.6$, $p<0.001$) and *Gain* ($F_{1,17}=172.1$, $p<0.001$) were significantly higher at 0.10 Hz than at 0.05 Hz, suggesting preserved high-pass filter behaviour of the cerebrovasculature following injury. Interaction terms between frequency and time were significant for *Phase* ($F_{3,51}=4.715$, $p=0.007$), but not for *Coherence* ($F_{2.02,34.31}=0.416$, $p=0.665$) or *Gain* ($F_{3,51}=1.654$, $p=0.189$). Simple effects revealed an effect of time for 0.10 Hz *Phase* ($F_{3,51}=8.061$, $p<0.001$). Relative to preseason, planned contrasts revealed significant *Phase*

reductions (mean difference ± SEM) of 27% at 72 hours (-0.158 ± 0.028 rads, p<0.001) and 20% at 2-weeks (-0.117 ± 0.023 rads, p<0.001), suggesting a slower CA response post-SRC. By 1-month, *Phase* had recovered to near-preseason values (-0.024 ± 0.029 rads, p=0.42).

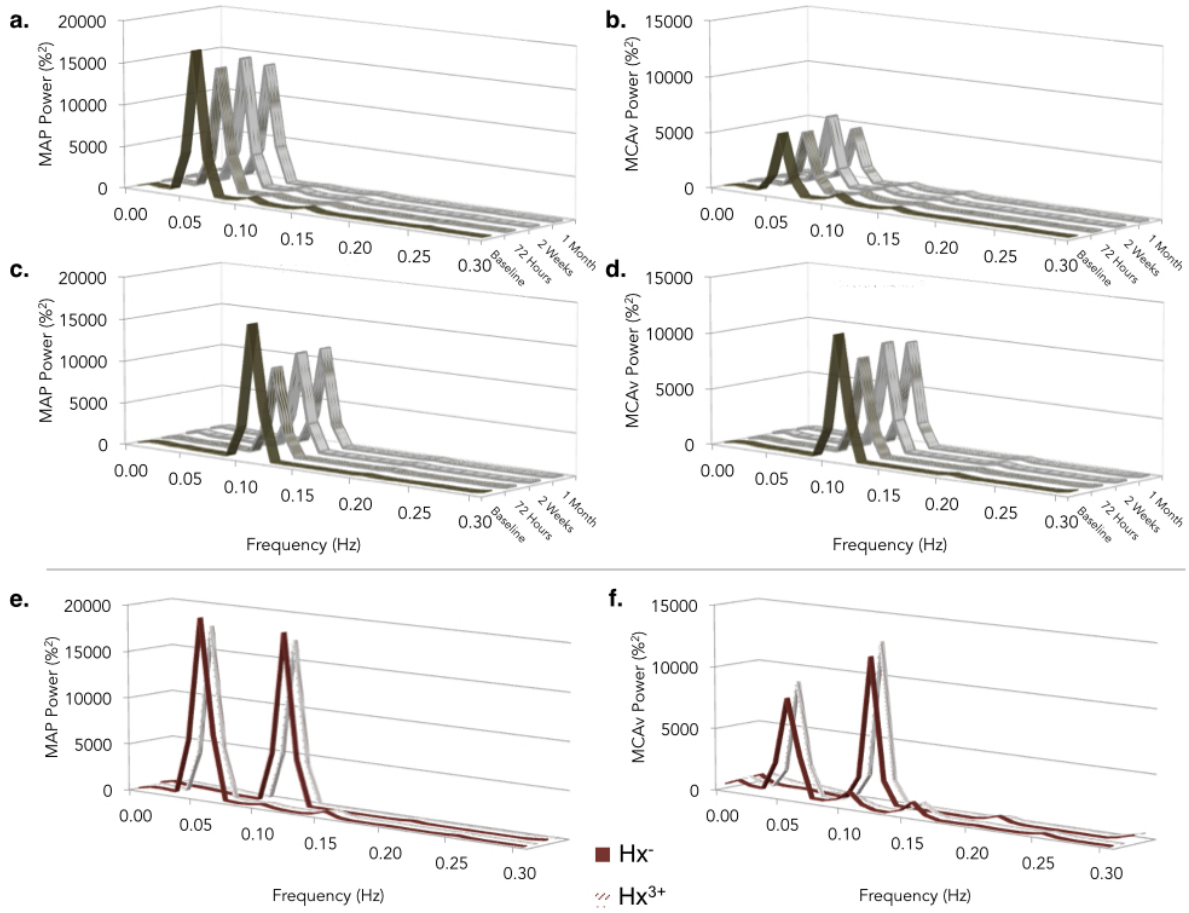

Figure 3-2. Normalized power spectra. Normalized values of power spectrum densities for mean arterial pressure (MAP; a,c) and middle cerebral artery blood velocity (MCA*v*; b,d) for preseason and post-concussion squat-stands at 0.05 Hz (a,b) and 0.10 Hz (c,d); preseason squat-stands in subjects with zero (Hx^-) versus three or more (Hx^{3+}) previous concussions (e,f). The frequencies at which power spectra reached peak amplitude (either 0.05 Hz or 0.10 Hz) were used in sampling point estimates for *Coherence*, *Phase*, and *Gain*.

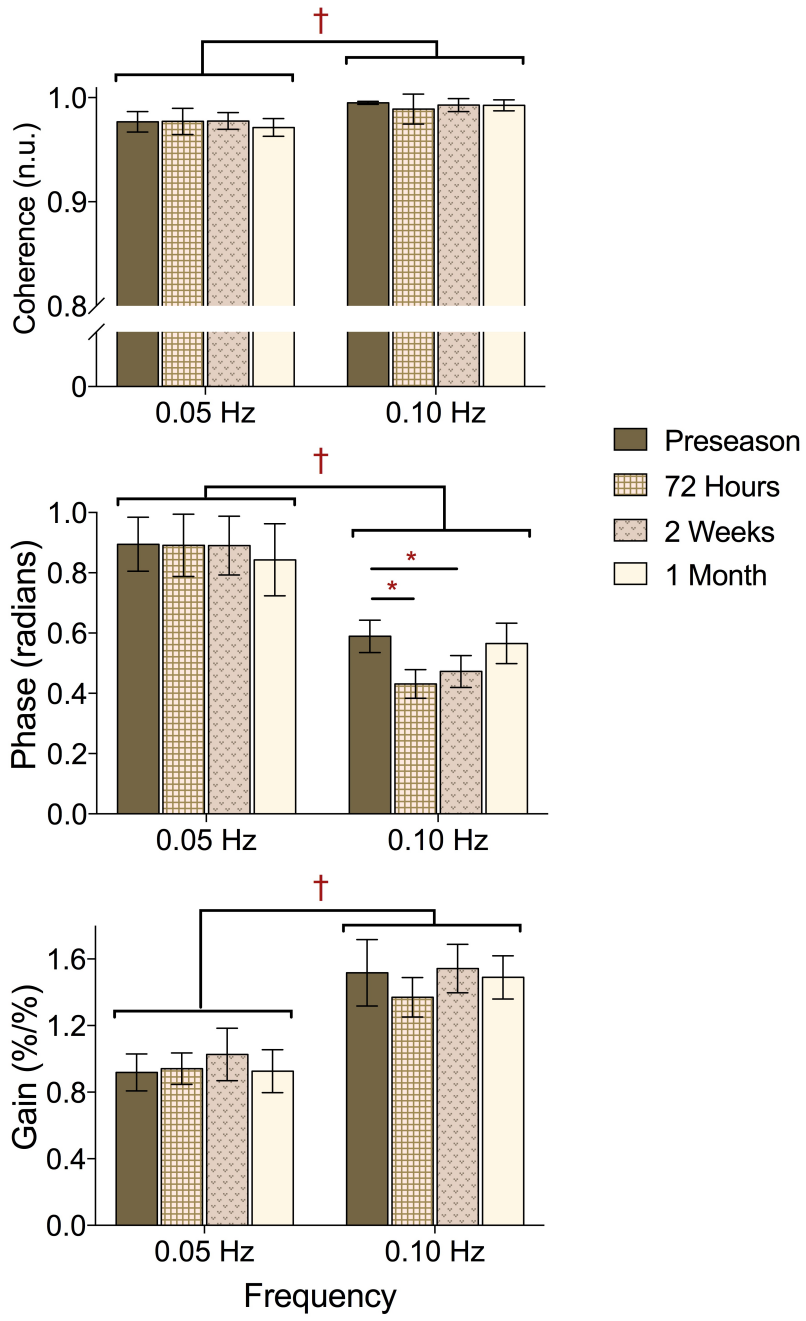

Figure 3-3: Summary of transfer function analysis outcomes describing CA function in acutely concussed athletes. *Coherence* (top), *Phase* offset (middle), and normalized *Gain* (bottom) during squat-stand manoeuvres assessed at preseason and each post-injury time point in athletes who suffered a concussion during the season. † represents significant main effect of frequency (p all <0.01), * denotes significant simple effect of time (p all < 0.001). Data presented as mean ± 95% CI.

A significant correlation was observed between $\Delta Phase_{0.10Hz}$ from preseason to 72-hours, and ΔSAC composite score (r=0.549, p=0.018). However, correlations were not significant between $\Delta Phase_{0.10Hz}$ and change in symptom number (r=-0.197, p=0.434), symptom severity (ρ=-0.168, p=0.504), headache (ρ=-0.113, p=0.656), "pressure in the head" (ρ=-0.011, p=0.967), or performance on the BESS (r=-0.133, p=0.600).

3.4.2 Cumulative effects of multiple previous concussions

Two-way mixed ANOVA did not reveal main effects of concussion history for *Coherence* ($F_{1,71}$=1.808, p=0.184), *Phase* ($F_{1,71}$=0.005, p=0.944), or *Gain* ($F_{1,71}$=0.832, p=0.365) (Figure 3-4). Significant main effects of frequency were observed for all variables (p<0.001), while interaction terms were non-significant (p=0.311, 0.398, 0.973 for *Coherence*, *Phase*, and *Gain*, respectively). As expected, *Coherence* (+1.74%, 95%CI = 0.008 – 0.026) and *Gain* (+50.4%, 95%CI = 0.398 – 0.533 %/%) were higher, while *Phase* was lower (-37.9%, 95%CI = -0.372 – -0.256 rads) at 0.10 Hz relative to 0.05 Hz, indicating intact high-pass filter behaviour.

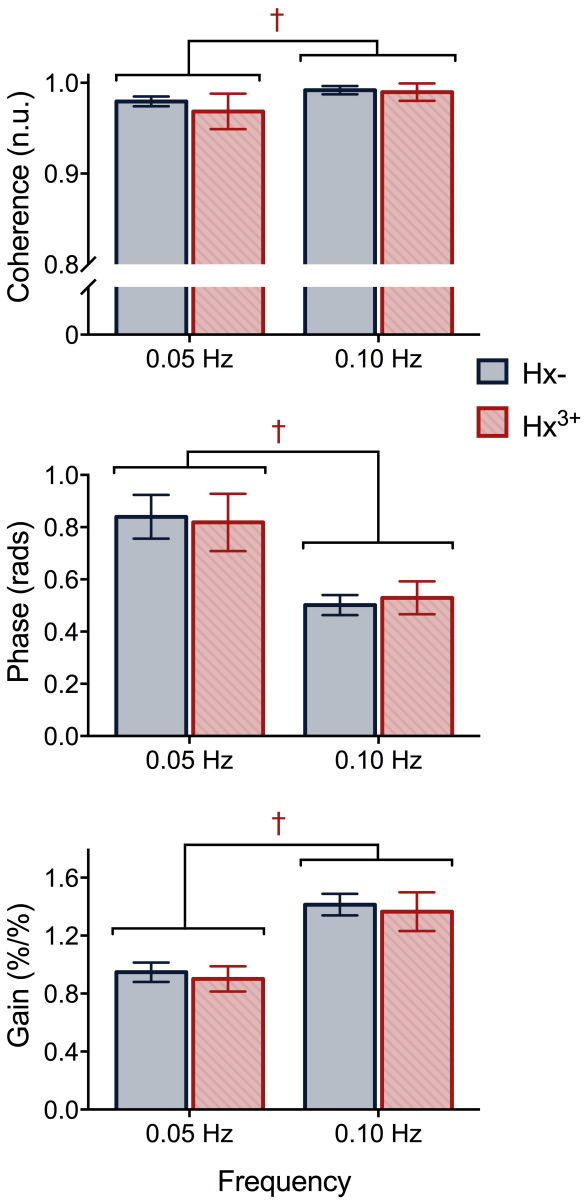

Figure 3-4. Summary of preseason autoregulatory outcomes from transfer function analysis for athletes with zero (Hx^-) and three or more (Hx^{3+}) previous concussions. *Coherence* (top), *Phase* offset (middle), and normalized *Gain* (bottom) during squat-stand manoeuvres assessed at preseason in athletes with zero and three or more previous concussions. † represents significant main effect of frequency (*p* all <0.001). Data presented as mean ± 95% CI.

3.5 Discussion

In the current report, we provide the first prospective study to evaluate the capacity of the cerebral blood vessels to modulate CBF in response to changes in BP after SRC. Transient impairments in the CA response were observed at 0.10 Hz, a frequency associated with sympathetic contributions to CA (191,345). At a within-subject level, more substantial CA impairments were related to poorer performance on the SAC, a brief cognitive screen for SRC. These impairments in pressure-buffering persisted beyond medical clearance for return-to-play in many cases, highlighting an important discrepancy between clinical (median 14 days) and physiological recovery (~30 days). However, when comparing preseason CA function in contact sport athletes with three or more previous concussions to those with zero, no differences were observed despite persistent concussion-like symptoms. These data support a process of transient SRC-induced autonomic dysfunction that does not appear to exert cumulative, lasting effects on the cerebrovasculature.

3.5.1 Cerebral blood flow alterations post-concussion

Transient disruptions in CBF following concussion have been reported previously (2,43,136,137). In the earliest post-mTBI phase (<48 hours), a period of acute hyperemia occurs with CBF peaking at 24-hours (346), although this may be age-dependent (139,140). Subsequently, global and regional reductions in CBF are reported both sub-acutely (2,43,136) and chronically (142,143), in both paediatric (2) and young adult (43,136) athlete populations. In both age groups, blood flow often recovers to near-healthy levels by ~30 days (2), and may be related to symptom resolution (43,137). Nevertheless, arterial spin labeling has shown those with a history of concussion consistently display reduced fronto-temporal CBF, independent of the

number of previous concussions (136,144). In the current study, we did not observe significant reductions in resting *MCAv* as a function of concussion (Table 3-1). This may be due to differences in sensitivity and spatial resolution between MRI and TCD to subtle changes in flow, with MRI providing higher spatial resolution. Despite the existence of CBF alterations following SRC, a paucity of evidence exists documenting effects on mechanisms controlling CBF. A comprehensive evaluation of cerebrovascular functioning should consider reactivity to CO_2, the neurovascular coupling response, and an assessment of the cerebral pressure-flow relationship (145).

3.5.2 CA impairments in traumatic brain injury

Autoregulatory mechanisms appear sensitive to acquired brain injury, as CA disruptions are well documented following moderate and severe TBI, with one small study suggesting similar deficits in a subset (8/29) of hospitalized mild TBI patients (208). Across multiple studies, 49-87% of severe TBI patients exhibit absent or impaired CA (197,199), with recovery of CA after severe TBI delayed in some cases beyond 2 weeks (199). CA evaluation has predictive value in acquired brain injury populations: in severe TBI, acutely compromised CA is a significant predictor or poor outcome (200,201); following subarachnoid haemorrhage, CA dysfunction predisposes patients to delayed cerebral ischemia and vasospasm (203-205); impaired CA is an established independent risk factor for stroke (159). The findings of the current study indicate that even comparatively mild brain injuries, such as SRC, disrupt the capacity of the brain to regulate CBF in the face of changing BP.

3.5.3 Possible mechanisms for CA impairments following concussion

The current observation of reduced 0.10 Hz *Phase* for at least two weeks implies altered autonomic regulation of the cerebrovasculature causing a delayed change in vascular resistance in response to a BP challenge. This complements previous reports of SRC-induced cardiovascular autonomic dysfunction, providing the first evidence that autonomic dysregulation affects cerebral hemodynamics. Concussion alters cardiac autonomic function, particularly during exercise; altered heart rate variability patterns – associated with resting CBF (347) – suggest increased sympathetic and / or lowered parasympathetic tone for at least 10 days post-injury (298-301). The cerebrovascular tree is richly innervated by sympathetic fibres (272), and mounting evidence points to the importance of sympathetic innervation in the dynamic regulation of BP variability (190). Pharmacological studies in healthy young adults have shown sympathetic activation reduces *Phase* at frequencies including 0.10 Hz (191,345). In accordance with the suggestion that progressive impairment of CA likely affects response latency (*Phase*) before affecting efficiency (*Gain*) (195), we observed significant changes in *Phase* only. These results indicate SRC induces autonomic dysregulation affecting cerebral hemodynamics for at least 2-weeks, but suggest no cumulative effects of multiple injuries.

The mechanism by which SRC alters autonomic function, CA, and CBF remains unclear. It is not definitively known whether vascular dysfunction precedes neuronal dysfunction, or results from primary neuronal dysfunction (348), preclinical rodent TBI models have shown reductions in the number and diameter of capillaries at the injury site (349), while impaired CA has been associated with cerebral white matter damage (350). Specifics underlying sympathetic modulation of cerebrovascular resistance remain poorly defined, but brain injury models show

lateralized control of cardiac autonomic outflow, wherein left insular efferents exert parasympathetic effects and right insular efferents generate sympathetic responses (351-353). Disruption to the corpus callosum – the white matter structure consistently reported to show damage following concussion (99,317) – could compromise balance between sympathetic and parasympathetic outflow (301). Furthermore, damage to prefrontal areas within the central autonomic network could exert a disinhibition of the central nucleus of the amygdala, causing a net increase in sympathetic activity through subsequent disinhibition of sympathoexcitatory neurons in the ventrolateral medulla (354). Increased sympathetic drive has been shown to effect a transient "stiffening" of intracerebral vessels following concussion (300), which would effectively cause a delay in vessel responsiveness and explain the observed reduction in *Phase*, although this remains speculative.

3.5.4 Limitations

There are several limitations to the current study. First, clinical guidelines for SRC management during the study period dictate physical and cognitive rest post-injury. The potential detraining influence of strict rest on CA cannot be discounted; however, CA has previously been shown to be preserved or even improved following prolonged bed rest (355). Recruiting a control group of healthy uninjured elite athletes to undergo a post-concussion rest and return-to-play protocol would allow insight, but is pragmatically unrealistic. Second, concussion remains a clinical diagnosis. As we recruited from multiple teams, three different physicians provided official concussion diagnoses, potentially magnifying the omnipresent heterogeneity within our sample. Third, we tested only male athletes, representing a significant shortcoming in the generalizability of our findings, as females are reported to sustain higher rates of SRC and longer recovery times

than males (356). Fourth, evaluation of acute effects of SRC benefitted from a repeated-measures design. Insight into the cumulative effects of multiple concussions may be better gleaned using a similar prospective approach; our between-subjects design may have precluded differences between Hx^{-} and Hx^{3+} groups due to substantial variability in the normal range of *Phase* and *Gain*. Despite the widespread use of TFA within the CA research community (212), this approach models the MAP-CBF relationship as a linear system with one input and one output. As such, the inherent non-linearity of the autoregulatory system is not captured by this analysis technique, representing an important limitation of this approach. In effort to maximize the reliabilty of our MAP-CBF characterization, we had participants complete repetitive squat-stand manoeuvres to "linearize" the system and amplify signal-to-noise ratio. Nevertheless, though the analysis parameters used followed recommendations from the international CA research network (CARNet), it is possible that using only 5 data windows within our TFA may have inflated estimates of coherence; however, coherence would still be sufficiently high to enable reliable interpretation of *Phase* and *Gain* (210). Lastly, TCD provides an index of CBF by measuring the velocity of red blood cells – that this relationship holds true requires the diameter of insonated vessel to remain constant, which cannot be verified. However, there are ongoing debates surrounding the influences of MCA diameter changes on evaluation of cerebral hemodynamics (357,358). Effects of P_{CO_2} on MCA diameter are assumed to have been minimal in the current study, as end-tidals were tightly maintained (Figure 3-1) (359). Nevertheless, that significant CA impairments were observed in a small sample emphasizes the need for in-depth prospective investigations into the effects of SRC on CBF control mechanisms.

3.6 Conclusion

In order to mitigate the incidence and severity of concussive-type mild TBI, a better understanding of their neurobiological underpinnings is needed. Although not currently appropriate for clinical management, this TCD-based assessment shows significant reductions in the pressure-buffering capacity of cerebral blood vessels in concussed athletes for at least 2-weeks that were related to performance on a cognitive screening test. While validation is required in a larger sample, these results indicate SRC induces a transient disruption in autonomic cerebrovascular function that may outlast symptom resolution and clinical recovery. Development of further prospective investigations into the effects of SRC on mechanisms controlling CBF – including relationships with age, sex, impact biomechanics, and susceptibility to injury – are warranted.

Chapter 4: A prospective transcranial Doppler ultrasound-based evaluation of the effects of repetitive subconcussive head trauma on indices of dynamic cerebral autoregulation

4.1 Summary

Recent literature has raised concern over detrimental effects of repetitive subconcussive head trauma experienced during participation in contact sport. The objective of the current prospective cohort study collected between September 2013 and December 2016 was to determine the effect of repetitive sub-concussive head trauma on indices of dynamic cerebral autoregulation. 179 elite, junior-level (age 19.6 ± 1.5 years) contact sport (ice hockey, American football) athletes recruited for pre-season testing. 52 non-concussed athletes returned for post-season testing. 15 non-contact sport athletes (age 20.4 ± 2.2) also completed pre- and postseason testing. Beat-by-beat mean arterial pressure (MAP) and middle cerebral artery blood velocity (MCAv) were recorded using finger photoplethysmography and transcranial Doppler ultrasound, respectively. MAP oscillations were induced during five minutes of repetitive squat-stand manoeuvres at 0.05 Hz and 0.10 Hz. The MAP-MCAv relationship was quantified using transfer function analysis to estimate *Coherence* (correlation), *Gain* (response amplitude), and *Phase* (response latency). In contact sport athletes, 0.10 Hz *Phase* was reduced and *Gain* was increased at post-season compared to pre-season, indicating impairment in both the latency and magnitude of the autoregulatory response to changing blood pressure. These changes were associated with increased reporting of headache and pressure in the head. Changes in *Phase* were greater in athletes experiencing a higher number and severity of head impacts. *Gain* increases were

correlated to elevations in sympathetic tone, estimated from resting heart rate variability. Contrastingly, non-contact sport controls displayed no changes in autoregulatory behaviour from pre- to post-season. Repetitive subconcussive head trauma, as experienced during one season of participation in contact sport, induces exposure-dependent impairments in the capacity of the cerebrovascular pressure-buffering system, and may be due to autonomic dysregulation. It is unknown how long these deficits persist or if they accumulate year-over-year.

4.2 Introduction

Sport-related concussion is a global public health issue, with growing concern over the effects of repetitive subconcussive head trauma (55). Subconcussion can be defined as head trauma that does not elicit signs or symptoms typical of clinical concussion (40,56). Numerous studies have demonstrated repetitive subconcussive trauma can cause deleterious alterations in brain structure and function that may be associated with the degree of subconcussive exposure (20,25,26,65-68,71,72), potentially altering the brain's susceptibility and contributing to injury risk.

Alterations in cerebral blood flow (CBF) play an important role in the pathophysiology underlying concussion, but the effects of subconcussive trauma on CBF are poorly understood. Although the brain comprises only 2% of total body weight, it accounts for 15-20% of cardiac output (145), underscoring the importance of maintaining adequate perfusion. Comprehensive evaluation of cerebrovascular function need consider the multiple controllers of CBF, including reactivity to carbon dioxide (CO_2), the neurovascular coupling response, and the blood pressure (BP) buffering system (145). In the face of changing BP, resistance within the cerebrovascular tree is altered to maintain CBF. The ability of the cerebral blood vessels to buffer changes in BP

– commonly referred to as cerebral autoregulation (CA) (189,360) – serves as an important marker of cerebrovascular function, and involves myogenic, neurogenic, and metabolic mechanisms (190,341). Disrupted CA has been demonstrated acutely post-concussion (Chapter 3:) and has been hypothesized as a mechanism underlying persistent post-concussion symptoms (206).

Disruptions in CBF have been demonstrated following sport-related head trauma. In both adult and paediatric populations, concussion has been shown to induce CBF changes that may be related to symptom recovery (2,43,137,141). Multiple studies have demonstrated alterations in reactivity of the cerebrovasculature to CO_2 following concussion (45,176,178,361). Recently, impairments in CO_2 reactivity have also been shown in asymptomatic high school football and soccer players that were not observed in non-collision athletes (18,19), and were attributed to repetitive subconcussive head trauma. While we have previously demonstrated transient CA impairments in acutely concussed athletes (Chapter 3:), it is currently unknown if subconcussive hits also affect CA.

Accordingly, our objective was to prospectively evaluate the effect of repetitive subconcussive head trauma on indices of dynamic CA, with the hypothesis that deficits would be observed at post-season relative to pre-season in a group of young adult elite contact sport athletes.

4.3 Methods

4.3.1 Study design

179 male (mean age 19.6 ± 1.5 years) junior hockey (n=90) and football (n=89) were recruited to

the study, in addition to 15 non-contact sport controls (mean age 20.4 ± 2.2 years; 12 cross-country running, 1 ultimate, 2 basketball). All participants underwent baseline laboratory testing prior to the beginning of the athletic season (preseason). Testing was repeated within two weeks of the end-of-season (post-season) for a subset of contact sport participants who did not sustain a concussion during the season (n=52), as well as the non-contact sport controls. Prior to physiological testing, all participants completed the Sport Concussion Assessment Tool, version 3 (SCAT3)(9). The SCAT3 is a concussion screening tool comprised of a graded symptom checklist (7-point Likert scale to characterize burden of 22 symptoms), the Standardized Assessment of Concussion (SAC) that probes orientation, immediate and delayed recall, and concentration, as well as the Balance Error Scoring System (BESS). No participants were excluded based on predefined criteria including a significant history of cardiorespiratory, cerebrovascular, neurological, or severe neurodevelopmental disorder. All subjects underwent familiarization of testing procedures, and abstained from exercise, caffeine, and alcoholic beverages for 12^{+} hours prior to testing. Written informed consent was obtained prior to participation, as approved by the University of British Columbia Clinical Research Ethics Board.

4.3.2 Instrumentation

Participants were equipped with a three-lead electrocardiogram (ECG) for measurement of R-R intervals and heart rate. Cerebral blood flow velocity was recorded in the middle cerebral artery (MCAv) using transcranial Doppler ultrasound (ST3, Spencer Technologies, Seattle, WA). Vessels were identified and signals optimized according to depth, waveform, and velocity, and ultrasound probes were locked in place with a fitted headframe (Spencer Technologies). Beat-to-beat BP was recorded with finger photoplethysmography (Finometer PRO, Finapres Medical

Systems, Amsterdam, The Netherlands), while partial pressure of expired CO2 (P_{ETCO_2}) was monitored using an online gas analyzer (ML206, AD Instruments, Colorado Springs, CO). All data were sampled at 1000 Hz (PowerLab 8/30 ML880, AD Instruments) and stored for offline analysis using commercially available software (LabChart version 7.1, AD Instruments).

Table 4-1. Participant characteristics. Demographics, SCAT3 performance, and resting physiological parameters for contact sport and control (non-contact sport) athletes at preseason and post-season; data are presented as mean (SD).

Metric	Contact Sport (n=52)		Control (n=15)	
	Preseason	Post-season	Preseason	Post-season
Age (years)	19.6 (1.5)		20.4 (2.2)	
BMI (kg/m^2)	28.2 (4.9)		22.6 (3.0)	
Test Interval (days)	109.2 (25.8)		100.1 (23.8)	
# of Symptoms[a]	3.7 (3.6)	5.4 (5.0)	5.5 (5.0)	5.7 (3.3)
Symptom Severity[a]	6.7 (7.8)	9.6 (10.1)	8.1 (8.3)	8.2 (7.2)
SAC Score[a]	26.6 (1.9)	26.6 (1.8)	27.7 (1.5)	27.9 (1.4)
BESS Score[a]	3.7 (3.3)	2.9 (3.2)	2.7 (2.4)	2.6 (3.6)
MCAv (cm/s)	54.5 (9.3)	53.8 (7.5)	55.6 (14.4)	57.3 (14.3)
MAP (mmHg)	92.2 (12.2)	92.6 (12.7)	93.9 (13.8)	91.3 (9.7)
$P_{ET}CO_2$ (mmHg)	38.0 (3.0)	37.1 (2.6)	37.9 (1.8)	37.9 (1.3)
HR (bpm)	74.5 (10.0)	78.3 (11.9)	71.0 (9.9)	72.8 (13.8)

SCAT3 = Sport Concussion Assessment Tool version 3; SAC = Standardized Assessment of Concussion; BESS = Balance Error Scoring System; MAP = mean arterial pressure; MCAv = middle cerebral artery velocity; $P_{ET}CO_2$ = end-tidal partial pressure of carbon dioxide; HR = heart rate

4.3.3 Experimental protocols

All testing occurred at the same time of day (344) and involved a hemodynamic challenge protocol (repetitive squat-stand manoeuvres) (210). First, spontaneous fluctuations in physiological metrics were recorded while standing quietly for five minutes. During squat-stand

manoeuvres, subjects began in a standing position, squatted to a knee angle of ~90˚, and returned to standing at a pace dictated by a metronome. Squat-stands were performed for five minutes at each of two frequencies, including 0.05 Hz and 0.10 Hz – thought to reflect myogenic and autonomic contributions towards CA, respectively (191-193) (Figure 4-1).

4.3.4 Data processing

Beat-to-beat mean arterial pressure (MAP) and MCAv were determined from each R-R interval. All data were processed and analyzed with custom-designed software in LabView 14 (National Instruments, Austin, TX), as outlined previously (210). Beat-to-beat MAP and MCAv data were spline interpolated and resampled at 4 Hz. In accordance with recently published best-practice guidelines for transfer function analysis (212), each five-minute recording was divided into five successive windows with 50% overlap. Data within each window were linearly detrended and passed through a Hanning window prior to fast Fourier transform. The transfer function $H(f)$ between the MAP and MCAv signals was calculated as:

$$H(f) = S_{MAP\text{-}MCAv}(f)/S_{MAP\text{-}MAP}(f)$$

where $S_{MAP\text{-}MAP}(f)$ is the autospectrum of the MAP signal and $S_{MAP\text{-}MCAv}(f)$ is the cross-spectrum between the two signals. The squared *Coherence* function was estimated as:

$$MSC(f) = | S_{MAP\text{-}MCAv}(f) |^2 / \cdot \{SMAP\text{-}MAP(f)\ \ SMCAv\text{-}MCAv(f)\}$$

The real $H_R(f)$ and imaginary $H_I(f)$ components of the complex transfer function were used to calculate *Gain* $| H(f) |$ and *Phase* $| \phi(f) |$:

$$| H(f) | = \{HR(f)2 + HI(f)2\}$$

$$\phi(f) = \tan^{-1}\{HI(f) / HR(f)\}$$

Coherence, *Phase*, and normalized *Gain* were sampled at the point estimate of the driven frequency (0.05 or 0.10 Hz), falling within the very low frequency (VLF; 0.02- 0.07 Hz) and low frequency (LF; 0.07- 0.20 Hz) ranges where CA is believed to be operant (189). Phase wraparound was not present for any point-estimates.

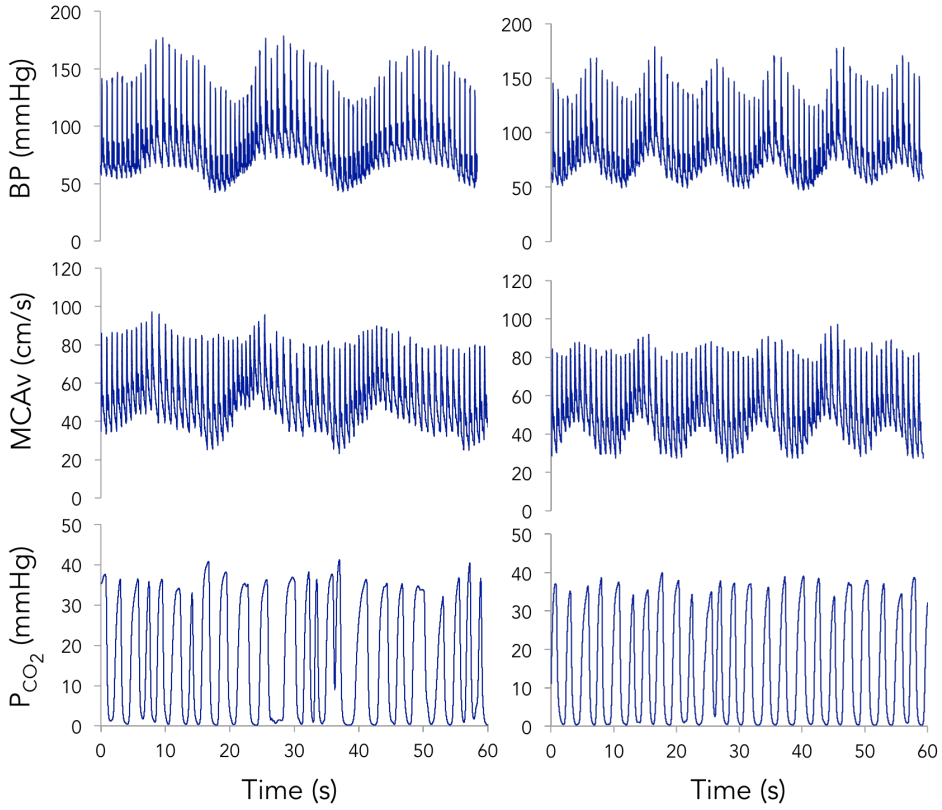

Figure 4-1. Representative traces. Representative time-series showing oscillations in blood pressure (BP, top) elicited during 60 seconds of squat-stand manoeuvres performed at 0.05 Hz (left) and 0.10 Hz (right); oscillations are also observed in middle cerebral artery blood velocity (MCAv, middle); expired carbon dioxide (P_{CO2}, bottom) is less affected.

4.3.5 Impact monitoring

During the season, impact sensors (xPatch, X2 Biosystems, Seattle, WA) were attached to the right mastoid in a subset of players (n=29) to estimate linear and rotational accelerations experienced by the head during each game played (Figure 4-2). Acceleration profiles were recorded for 100 ms at 1000 Hz when translational acceleration exceeded a 10 *g* threshold. Following each game, data were downloaded using the Head Impact Monitoring System software (X2 Biosystems). Features within the software provide estimates of peak linear (PLA) and peak rotational (PRA) acceleration for each detected impact event. Only acceleration events exceeding a 20 *g* threshold were used in an effort to focus analysis to head accelerations most likely to result from direct impacts to the head or body, as opposed to hard stops or cuts (19,362,363). Estimates of cumulative exposure to linear (*cPLA*) and rotational (*cPRA*) acceleration by summing across all impacts for the season. Non-contact sport athletes were not monitored for head acceleration events during their competitive season.

Figure 4-2. Photo of a participant wearing an impact sensor on the right mastoid process (Permission to reproduce photo obtained; credit: GreystokePhoto.com).

4.3.6 Statistical analyses

Effects of exposure to repetitive subconcussive head trauma during the course of a competitive season were estimated using a 2 (group: contact sport versus control) x 2 (time: preseason versus post-season) x 2 (frequency: 0.05 versus 0.10 Hz) three-way mixed ANOVA. Secondary exploratory analyses were conducted using independent samples t-tests to explore differences in change scores from pre- to post-season for *Phase* (Δ*Phase*) and *Gain* (Δ*Gain*) between high and low quartiles for estimated impact exposure variables (*Hits/season, cPLA*, *cPRA*) and. Spearman's correlation coefficients were calculated between Δ*Phase*/Δ*Gain* and change in symptom, SAC, and BESS scores from pre- to post-season.

All statistical analyses were performed using SPSS version 22.0 for Macintosh (IBM, Wherever, USA). Significance was determined *a priori* to achieve an experiment-wide $\alpha = 0.05$.

4.4 Results

Demographic characteristics, SAC and BESS performance, and resting physiological data are outlined in Table 4-1. Representative traces of MCAv, MAP, and $P_{ET}CO_2$ at both squat-stand frequencies are provided in Figure 4-1. These manoeuvres substantially increased power in MCAv and MAP signals at the target frequencies (Figure 4-3).

Biomechanical descriptions of impact exposure in the subset of participants wearing sensors are presented in Table 4-2. Relative to football players, ice hockey players experienced fewer hits per game, but greater cumulative number of hits due to the differences in the length of season (62 games in hockey versus 10 games in football). Individual impacts were similar in PLA

magnitude across sports, but football players experienced greater *PRA/hit* than hockey players.

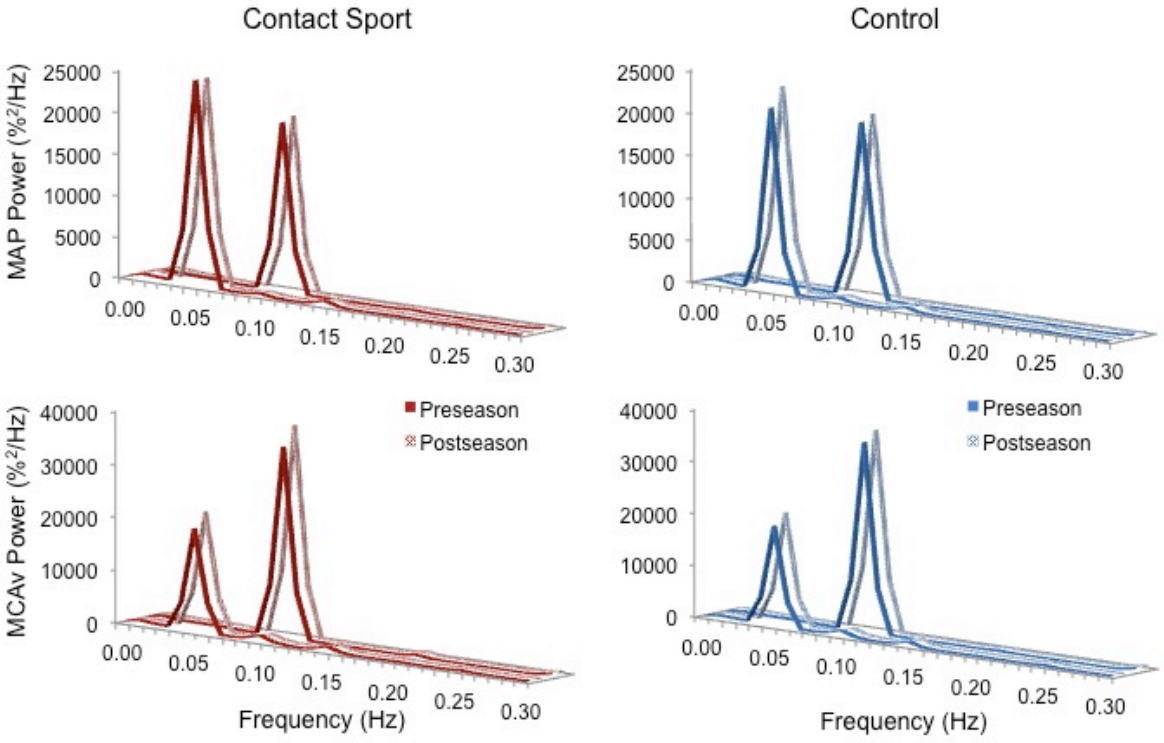

Figure 4-3. Normalized power spectra. Normalized power spectrum densities for mean arterial pressure (MAP, top) and middle cerebral artery blood velocity (MCAv, bottom) for preseason and post-season squat-stands in contact sport athletes (red, left) and in non-contact sport control athletes (blue, right). The frequency at which PSD reached peak amplitude (either 0.05 Hz or 0.10 Hz) was used for sampling point estimates for *Coherence*, *Phase*, and *Gain*.

4.4.1 Effect of exposure to repetitive subclinical head impacts

The three-way interaction term (frequency*time*group) was non-significant for *Coherence* ($F_{1,65}$=2.415, p=0.125), *Phase* ($F_{1,65}$=1.494, p=0.226), and *Gain* ($F_{1,65}$=0.007, p=0.931). Significant two-way interactions existed for *Gain* between frequency and time ($F_{1,65}$=7.577, p=0.008, partial eta^2=0.104), and between group and time ($F_{1,65}$=5.898, p=0.018, partial

eta^2=0.083), and for *Phase* between frequency and time ($F_{1,65}$=3.982, p=0.049, partial eta^2=0.059). Subsequent analysis of simple effects revealed a 12.4% relative increase in 0.10 Hz *Gain* (95%CI= +0.096 – 0.238 %MCAv/%MAP, p<0.001) and a 9.0% relative decrease in 0.10 Hz *Phase* (95%CI= -0.005 – -0.096 rads, p=0.027) from pre- to post-season in contact sport athletes (Figure 4-4), suggestive of autonomic dysfunction. No changes were observed at 0.10 Hz in control athletes (Figure 4-4) for *Gain* (95%CI= -0.079 – 0.115 %/%, p=0.696) or *Phase* (95%CI= -0.103 – 0.029 rads, p=0.247). No significant changes were observed for 0.05 Hz *Gain* or *Phase* (p > 0.05).

Table 4-2. Summary of head impact exposure in contact sport athletes. Median (IQ range) for head impact exposure variables across subset of hockey (n=10) and football (n=19) players wearing impact sensors during the season; p-values reflect results of Mann-Whitney U-tests comparing across sport.

Metric	Hockey	Football	*p*
Hits / game (#)	8.2 (6.1 - 11.2)	16.6 (8.7 - 21.2)	0.003
Hits / season (#)	353.5 (295.0 - 587.3)	166 (63.5 - 212)	0.002
PLA / hit (*g*)	36.3 (35.4 - 37.4)	36.6 (34.5 - 40.2)	0.448
cPLA (*g*)	11920.5 (10788.3 - 21570.6)	5794.2 (2507.5 - 7117.7)	0.002
PRA / hit (rad/s^2)	5036.1 (4772.3 - 5510.8)	6601.8 (6104.0 - 7441.0)	<0.001
cPRA (rad/s^2)	2016603.5 (11592.6 - 24322.9)	1057691.6 (486976.2 - 1376735.9)	0.003

PLA = peak linear acceleration; PRA = peak rotational acceleration; cPLA/cPRA = cumulative PLA/PRA

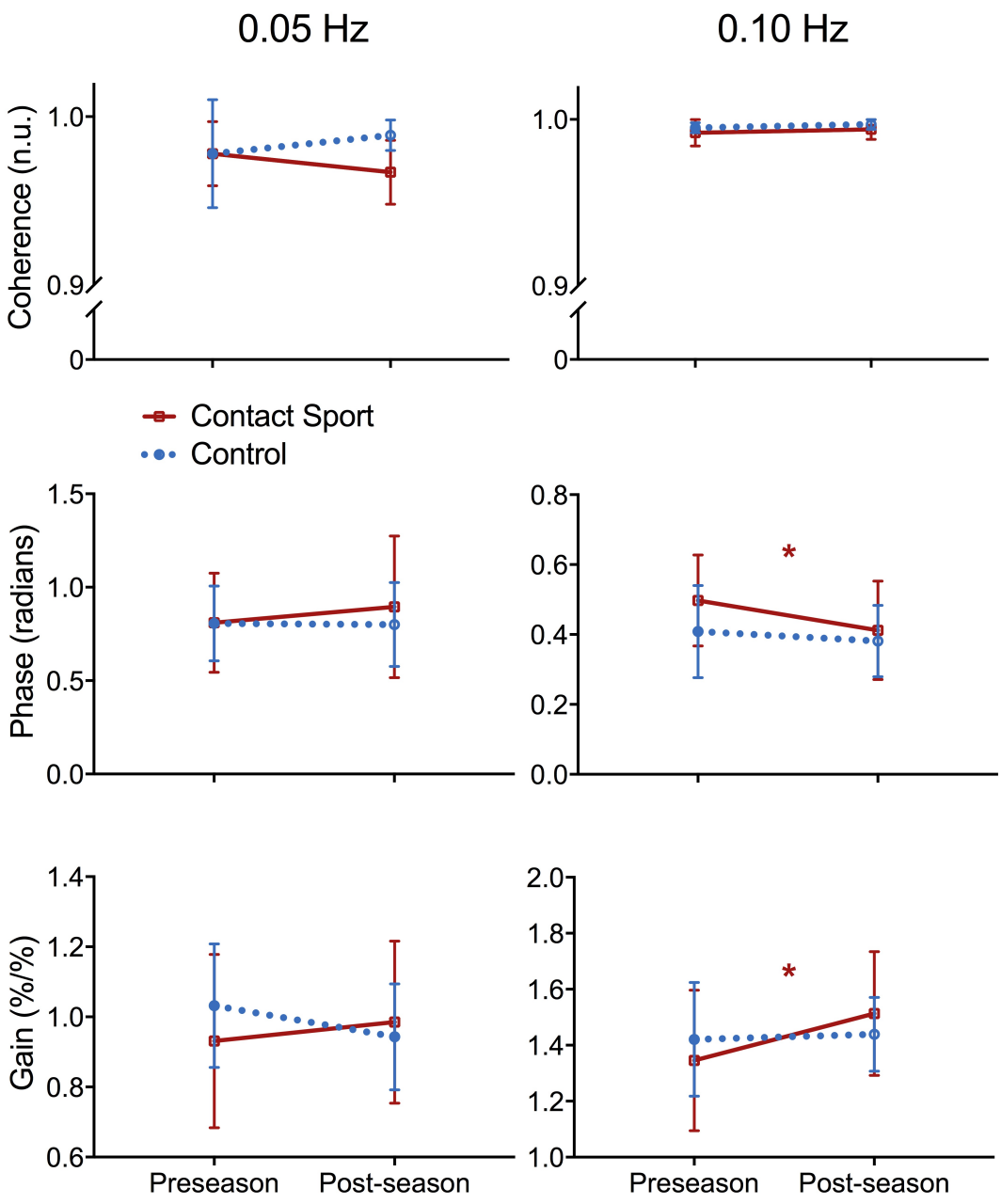

Figure 4-4. Effects of subconcussive trauma on autoregulatory outcomes derived from transfer function analysis. Transfer function analysis outcomes presented as mean ± SD for *Coherence* (top), *Phase* offset (middle), and normalized *Gain* (bottom) during squat-stand manoeuvres performed at 0.05 Hz (left) and 0.10 Hz (right), assessed at preseason and postseason in contact sport (solid red lines) and non-contact sport control (dashed blue lines) athletes. * indicates significant simple effects of time in the contact sport group; no significant changes were observed in control athletes.

Comparing high-to-low quartiles for total number of hits experienced per season in the subgroup of players who wore impact sensors, significant differences in Δ*Phase* were observed – players experiencing fewer hits exhibited a mean ± SD increase in *Phase* of 0.092 ± 0.108 rads from pre- to post-season, whereas those experiencing a higher number of hits exhibited an average decrease in *Phase* (i.e. impairment) of 0.070 ± 0.067 rads (t_{12}=2.366, p=0.036). Similarly, Δ*Phase* differed between high and low quartiles based on estimated *cPLA*, wherein players exposed to lower cumulative linear acceleration exhibited a *Phase* increase of 0.092 ± 0.108 rads, while those in the highest *cPLA* quartile exhibited a *Phase* decrease of 0.043 ± 0.107 rads (t_{12}=3.381,p=0.005). Significant Spearman's correlations were observed between Δ*Phase* and symptom scores for "pressure in the head" (rho = -0.305, p = 0.047), and between Δ*Gain* and headache scores (rho = -0.398, p = 0.008).

To further characterize the role of autonomic dysfunction following subconcussive trauma, we estimated sympathovagal balance by calculating the ratio between low frequency (0.04-0.15 Hz) and high frequency (0.15-0.40 Hz) power (*LF:HF*) in the ECG signal (Kubios v2.2, Biosignal Analysis and Medical Imaging Group, Finland). A significant positive correlation was observed between Δ*LF:HF* and Δ*Gain* (r=0.334, p=0.031), indicating increases in sympathetic output (i.e. elevated LF:HF) were associated with increased CA *Gain*.

4.5 Discussion

In the current report, we present the first prospective study of dynamic cerebral autoregulation in the context of repetitive subconcussive head trauma. Transcranial Doppler ultrasound data

revealed: 1) an impairment in both the *Gain* and *Phase* of the cerebral pressure-flow relationship in contact sport athletes who had not sustained a concussion, suggesting a delayed change in vascular resistance in response to a change in BP that lead to a greater magnitude change in CBF for a given BP shift; 2) athletes exposed to a higher number of hits and higher cumulative linear acceleration exhibited greater deficits in CA function than those exposed to lower levels of head trauma; 3) CA alterations from pre- to post-season were related to sympathovagal balance, as estimated from heart rate variability; 4) in contrast, non-contact sport athletes exhibited no change in CA function over the course of one season. Although the permanence of the observed CA impairments cannot been established, the current data suggest the potential for subconcussive trauma to impair cerebrovascular function in response to a common BP challenge.

4.5.1 Physiological disruptions following repetitive subconcussive trauma

A number of studies have reported minimal or no detrimental effects of subconcussive trauma (60,364-367), particularly on clinical measures of behaviour / neuropsychological function (reviewed in (368)). Despite exposure to more than 1000 impacts on average, one season of participation in collegiate football did not meaningfully impair scores on a computerized neuropsychological test battery (369), SAC (60,369), the Sensory Organization Test (60), BESS (60), or symptom severity (60). However, a higher number of years of collegiate playing experience was associated with worse performance on the Sensory Organization Test. It is possible that sequelae of subconcussive trauma may require more time to develop than a single season, or that these measures of neurological function may not be sensitive enough to detect subtle dysfunction. Findings to date suggest that any effect of subconcussive blows on neuropsychological performance is likely to be small and perhaps only evident in a subset of

individuals on select measures (368).

In contrast to clinical neuropsychological evaluations, more advanced techniques have revealed multiple detrimental effects of subconcussive hits. A body of evidence exists to suggest repetitive head trauma can, in the absence of symptoms, disrupt various aspects of brain structure and function, including white matter microstructure (24-26,65,66,370-372), cerebral metabolism (67,68,70,364) cortical activation patterns (20,69), vestibular function (373), functional connectivity (21,71), and blood-brain barrier integrity (66,77). For example, collegiate soccer players exhibited gray matter atrophy in anterior temporal regions compared to non-soccer playing controls (23); at baseline, resting-state fMRI revealed hyperconnectivity in the default mode network of high-school football players compared to controls that increased over one season (71); both American football and soccer players have shown disrupted white matter microstructure on diffusion tensor imaging (24,25). The current results – obtained using Transcranial Doppler – support the potential for subconcussive trauma to impair physiological function following only one season of play, including the integrity of cerebral blood flow control. Overall, however, the long-term functional and clinical implications of the outlined changes remain unclear.

4.5.2 Disruptions in control of cerebral blood flow following subconcussive trauma

Although acute and chronic disruptions in CBF have been well-documented following concussions (2,43,141), our understanding of the consequences of subconcussive head trauma on cerebral perfusion is comparatively scant. Only three other studies to-date have examined the effect of subconcussive trauma on mechanisms controlling CBF. Svaldi and colleagues

demonstrated impairments in reactivity of the cerebrovasculature to CO_2 in collegiate football players following the onset of the season (18). Similar deficits in CO_2 reactivity were reported in soccer players beginning in the second half of the season that persisted for at least 3-4 months after the end of the season (19). While the subconcussive nature of head trauma in professional boxing is debatable, Bailey and colleagues reported impairments in CO_2 reactivity that were related to the volume and intensity of sparring but not the frequency of knock-outs suffered or number of rounds fought (17). Considered alongside the current data these findings are concerning, as the underlying mechanisms governing CO_2 reactivity and CA are though to represent distinct processes (158,374), suggesting repetitive subconcussive trauma may induce deficits to multiple aspects of cerebrovascular control.

4.5.3 Autonomic role for subconcussion-induced impairments in CA

An impaired CA response to changes in MAP occurring at 0.10 Hz implies dysregulation in the autonomic control of the cerebrovasculature (191,192). Increasingly, the rich sympathetic innervation of the cerebrovascular tree is recognized to play an important role in the dynamic regulation of BP variability (190). Numerous lines of evidence have outlined the detrimental effects of concussion on autonomic function (298-301). To further characterize autonomic dysfunction in our contact sport athletes, ECG signals were used to calculate the low to high frequency power ratio – reflective of sympathovagal balance (290). The observed increase in CA *Gain* correlated significantly with increased sympathetic-to-vagal output, providing an additional line of support for autonomic contributions towards the observed cerebrovascular dysfunction. In a related study, our group demonstrated acute concussion imparts a ~20% reduction in the latency (*Phase*) of the CA response that persists for at least two weeks post-injury (Chapter 3:).

Disruptions were observed in both the latency and magnitude of the CA response following subconcussive trauma, indicating that even mild repetitive head trauma may incrementally disrupt the capacity of the cerebral blood vessels to regulate CBF in the face of changing BP. Additional research is warranted to further clarify the underlying mechanisms of cerebrovascular dysfunction, which may be influenced by age, sex, neck strength, previous impact exposure, and baseline autonomic function.

4.5.4 Evidence linking head impact exposure with physiological disruption

Prior work has demonstrated relationships between sport-related head impact exposure and outcomes on functional MRI (20-22,76,375), white matter diffusion (26,376,377), balance (60), ocular near point of convergence (56), and cerebral metabolism (70). For example, in non-concussed high school football players, Talavage and colleagues demonstrated pre-to-post season changes in cortical activation on fMRI during a working memory task that correlated with the total number of head impacts experienced during the season (22). In collegiate football and ice hockey players, McAllister et al. noted changes in fractional anisotropy (a metric describing water diffusion patters reflective of white matter microstructure) in multiple brain regions that was associated with head impact exposure, including total number of hits across the season and exposure to linear acceleration (26). Recent work in collegiate football players has demonstrated the time between subconcussive hits may influence the effects on white matter diffusivity metrics (377). While it has been established that no true biomechanical threshold exists towards the incidence of concussion (48,52), data suggest subconcussive trauma may influence injury risk. Reports indicate football players sustain a higher number and severity of head impacts on days of diagnosed concussion than on days without, suggesting incremental changes occur

following subconcussive impacts that may lower the injury threshold (378). The current findings associating change in CA function with impact exposure should be considered preliminary, and further study is warranted to investigate potential long-term consequences of repetitive hits on cerebrovascular and neurologic function. However, these data reinforce the hypothesis that the effects of repetitive blows to the head are cumulative, with repeated exposure contributing to pathologically altered neurophysiology.

4.5.5 Limitations

There are several limitations to the current preliminary study. Transcranial Doppler ultrasound measures the velocity of red blood cells, rather than CBF explicitly. For velocity to approximate flow in this scenario, the diameter of the insonated vessel must remain constant, which cannot be verified. However, debate continues over the importance of diameter changes in evaluating cerebral hemodynamics, particularly when P_{CO2} is held stable (357-359), as in this study (Figure 4-1). While previous research has demonstrated head impact exposure is typically greater during games than practices (49,379), impact data were collected during games only and therefore underestimate absolute exposure. Furthermore, the skin-worn impact sensors used in this study have recently been shown to exhibit substantial in-vivo overestimation error when compared to mouthguard-based sensors due to non-rigid skull coupling (380). Consistent overestimation of the severity of individual impacts likely did not affect the comparisons made within this study, however. Nevertheless, that significant CA impairments were observed in a small sample of contact sport athletes, but not in non-contact control athletes, emphasizes the need for in-depth prospective investigations into the effects of subconcussive trauma on CBF control mechanisms.

4.6 Conclusion

There is growing concern that even low-magnitude, subconcussive head impacts can cause lasting neurological injury. While behavioural changes in response to subconcussive trauma have been difficult to identify, this exploratory study suggests the cerebral autoregulatory system is vulnerable to repetitive head trauma. Our data provide evidence of cumulative impairment in the CA response of contact sport athletes associated with exposure to repetitive head trauma. Non-contact athletes exhibited no such changes in CA integrity. Future prospective cohort studies in a larger number of subjects are warranted to investigate the clinical relevance of CA changes induced by subconcussive impacts towards injury susceptibility and long-term outcomes.

Chapter 5: A prospective transcranial Doppler ultrasound-based evaluation of the acute and cumulative effects of sport-related concussion on dynamics of the neurovascular coupling response

5.1 Summary

Sport-related concussion has been shown to alter cerebral blood flow (CBF) both acutely and chronically, with suggestions that effects may be cumulative across multiple injuries. Such dysfunction may be mediated by trauma-induced deficits to CBF control mechanisms, though our understanding of such effects is limited, including the dynamics of the neurovascular coupling (NVC) response (i.e. CBF responses to neurologic demand). 179 junior-level contact sport athletes completed preseason testing – 42 had never experienced a concussion (Hx^{-}) and 31 had endured three or more (Hx^{3+}). 18 athletes suffered concussions during the study period and were re-tested 3-days, 2-weeks, and 1-month post-injury. NVC dynamics were estimated by measuring blood flow velocity in the posterior (PCAv) and middle (MCAv) cerebral arteries during cycles of 20-s eyes-closed and 40-s eyes-open to a visual stimulus (reading). Acutely following concussion, peak rate of PCAv increase during the activation phase was delayed by over 50%, and the magnitude of the PCAv response elevated by over 30% compared to preseason, although these alterations were largely resolved by 2-weeks. Independent medical clearance for full return-to-play was inversely related to the magnitude of increase in the NVC response. No changes in MCAv, blood pressure, or end-tidal carbon dioxide responses to visual stimulation were observed following concussion. No differences in NVC dynamics were observed between Hx^{-} and Hx^{3+} groups at preseason. Via multiple potential mechanisms, acute

sport-related concussion induces transient impairments in the dynamics of the NVC response that may be related to clinical recovery. Such effects do not appear to accumulate across multiple injuries.

5.2 Introduction

Sport-related concussion is a global public health concern, with recent reports estimating incidences of 1.1 to 1.9 million injuries each year in US youth alone (4). Defined as a "complex pathophysiological process affecting the brain, induced by biomechanical forces" (9), concussions are characterized by a period of increased cerebral vulnerability during which normal metabolism within the brain is disrupted, leading to heightened sensitivity to additional trauma during the recovery period (9,42,79). Cerebral metabolism is critically dependent on the regulation of cerebral blood flow (CBF). In both paediatric and adult patients, it has been demonstrated that the brain exhibits local and global reductions in CBF following sport-related concussion (2,43,141). Animal models have shown concussive-type injuries to reduce the number and diameter of capillaries, both adjacent and distal to the injury site (349). As such, alterations in cerebral blood flow (CBF) are thought to play an important role the pathophysiology underlying concussion (131), though effects on mechanisms controlling CBF are poorly understood.

Comprehensive assessment of cerebrovascular function should include evaluation of the cerebrovascular reactivity to carbon dioxide (CO_2), the cerebral pressure-flow relationship, as well as the neurovascular coupling response (145). Neurovascular coupling (NVC), a concept first proposed in the late 1800's, describes alterations in CBF in response to changes in neuronal

activity. Although direct microvascular measurements of CBF are challenging to obtain in humans *in vivo*, NVC can be characterized indirectly using transcranial Doppler ultrasound (TCD) to measure CBF velocity (CBF*v*) in the posterior cerebral artery (PCA) – the primary source of blood supply to the visual cortex – during visual stimulation protocols such as reading (156,213,241,242,381). Neurovascular coupling is also the basis for the blood oxygen level-dependent (BOLD) signal obtained using functional MRI (fMRI), which is a function of the ratio of oxy-to-deoxyhemoglobin, CBF, and cerebral blood volume.

In the context of sport-related concussion, transient impairments in cerebrovascular function have been observed following injury, including reduced capacity of the cerebrovasculature to buffer changes in blood pressure (Chapter 3:) and altered reactivity to CO2 (132). Concussion-induced CBF disturbances may also be affected by alterations in the ability of cerebral blood vessels to respond to changes in cortical activation (131). Multiple fMRI studies have shown concussed brains recruit additional cortical resources to successfully perform working memory and attentional tasks (255-257); however, these studies have typically derived a single parameter to estimate the magnitude of the BOLD response, which assumes the shape of the hemodynamic response is unaltered by injury. Only one study has considered whether NVC dynamics are affected by mTBI, reporting increased activation in the visual cortex of mTBI patients during a visual-auditory sensorimotor task, particularly during the earliest (2-4 seconds) response phase (265). Furthermore, fMRI studies have largely used case-control designs without pre-injury data, precluding insights into the magnitude of within-subject effects. The extent to which the dynamics of the neurovascular coupling response are affected by concussive head trauma may be better estimated by obtaining pre-injury data in a prospective cohort design with techniques

providing better temporal resolution, such as TCD.

Accordingly, our objectives in the current study were to prospectively evaluate the effects of sport-related concussion on the dynamics of the neurovascular coupling response using TCD ultrasound. Towards this end, we sought to examine: i) the effect of clinically diagnosed concussion, and; ii) whether effects are cumulative across multiple (3+) previous concussions in otherwise healthy and fully-recovered athletes on the neurovascular coupling response.

5.3 Methods

5.3.1 Study design

179 male (mean age 19.6 ± 1.5 years) elite junior hockey (n=90) and American football (n=89) players were recruited. All participants underwent preseason baseline testing. Upon enrolment, 42 players had experienced zero prior concussions (Hx^{-}) while 31 had sustained three or more (Hx^{3+}: mean (SD) = 4.5 (2.4), range = 3-12). Athletes diagnosed with a concussion by an independent physician during the competitive season (n=18), based on criteria in the 4th Consensus Statement (9), repeated testing at 72-hours, 2-weeks, and 1-month post-injury. Prior to physiological testing, participants completed the Sport Concussion Assessment Tool (SCAT3) (9), a concussion screening tool comprised of a graded symptom checklist, the Standardized Assessment of Concussion (SAC) – probing orientation, immediate and delayed recall, and concentration – as well as performance on the modified Balance Error Scoring System (BESS) (Table 5-1). No participants were excluded based on *a-priori* criteria including significant history of cardiorespiratory, cerebrovascular, neurological, or severe neurodevelopmental disorder. All participants provided written informed consent, abstained from exercise, caffeine,

and alcohol for 12+ hours, and were familiarized with testing procedures prior commencing this study, approved by the University of British Columbia Clinical Research Ethics Board.

Table 5-1. Participant characteristics. Demographics, SCAT3 performance, return-to-play duration, and resting physiological parameters during each test session for acutely concussed athletes.

Metric	Concussion History		Concussed (n=18)			
	0 (n=42)	3+ (n=31)	Preseason	72 Hour	2 Week	1 Month
Age (years)	19.0 (1.4)	19.6 (1.9)	18.6 (1.5)			
BMI (kg/m^2)	27.6 (5.1)	27.5 (4.3)	25.5 (3.1)			
RTP (days)	N/A (preseason only)		median = 14, range 7-35 days			
# Symptoms	2.2 (2.7)	4.7 (4.3)	2.0 (3.6)	11.2 (5.5)	2.8 (3.0)	1.1 (2.1)
Symptom Severity	3.4 (4.7)	9.3 (12.0)	3.6 (3.6)	25.1 (19.0)	3.6 (3.5)	1.3 (2.3)
SAC Score	27.1 (1.7)	26.7 (1.8)	26.8 (1.7)	26.5 (2.0)	26.9 (2.9)	28.1 (1.3)
BESS Score	3.5 (3.2)	3.5 (3.3)	2.6 (2.6)	4.2 (3.1)	3.0 (2.3)	2.0 (2.0)
PCAv (cm/s)	35.8 (4.9)	35.7 (5.8)	35.4 (3.5)	35.3 (8.2)	34.8 (5.3)	34.9 (6.2)
MCAv (cm/s)	55.1 (9.0)	55.6 (9.3)	53.8 (7.0)	53.5 (8.7)	53.3 (9.5)	53.2 (7.8)
MAP (mmHg)	95.0 (13.6)	94.1 (12.4)	92.3 (20.1)	90.4 (18.2)	92.0 (16.5)	91.2 (15.9)
$P_{ET}CO_2$ (mmHg)	37.7 (2.8)	38.1 (2.5)	37.7 (3.2)	37.6 (3.7)	37.9 (4.1)	37.6 (4.5)
HR (bpm)	75.1 (9.8)	73.6 (8.7)	79.4 (8.3)	79.7 (12.2)	82.1 (15.9)	79.5 (10.2)

BMI = body mass index; RTP = return-to-play; SAC = Standardized Assessment of Concussion; BESS = Balance Error Scoring System; PCAv = posterior cerebral artery blood velocity; MCAv = middle cerebral artery blood velocity; MAP = mean arterial pressure; $P_{ET}CO_2$ = end-tidal partial pressure of CO2; HR = heart rate

5.3.2 Instrumentation

Participants were equipped with a three-lead electrocardiogram (ECG). Cerebral blood flow was indexed using TCD (ST3, Spencer Technologies, Seattle, WA) to record CBF velocities in the right middle (MCA*v*) and left posterior (PCA*v*) cerebral arteries. After vessels were identified and signals optimized according to depth, waveform, velocity, and response to carotid compression / visual stimulation tests, ultrasound probes were locked in place with a fitted head

frame. Beat-to-beat blood pressure was recorded using finger photoplethysmography with a brachial cuff to adjust for height differences between the finger and the brachial artery (Finometer PRO, Finapres Medical Systems, Amsterdam, The Netherlands), while partial pressure of expired carbon dioxide ($P_{ET}CO_2$) was monitored using an online gas analyzer (ML206, AD Instruments, Colorado Springs, CO). All data were sampled at 1000 Hz (PowerLab 8/30 ML880, AD Instruments) and stored for offline analysis using commercially available software (LabChart version 7.1, AD Instruments).

5.3.3 Experimental protocols

All visits to the laboratory occurred at the same time of day during which the NVC response was evaluated in the PCA using a visual stimulation paradigm, as outlined previously (213). Resting physiological data were recorded while sitting quietly for one-minute. Participants were seated 50-60 cm from the visual screen (27" Apple iMac) with a 50 cm x 35 cm visual field. Subjects completed five cycles of 20 seconds eyes-closed, 40 seconds eyes-open to a visual stimulus (reading an article of interest to the participant), consistent with other NVC research (241,242,244,247,381); all visual stimuli were presented in the centre of the visual field, with screen brightness set to maximum and unchanged across participants or test sessions.

5.3.4 Data processing

All data were processed using a custom-written script in the Matlab environment (vR2013a, Mathworks, Natick, MA). Beat-to-beat values of systolic and diastolic BP, MCA*v*, and PCA*v* were determined from each R-R interval and used to calculate mean arterial pressure (MAP) and

mean velocity traces. Breath-to-breath peak expired CO_2 values were extracted to measure $P_{ET}CO_2$. All signals were visually inspected for artifacts and corrected by cubic spline interpolation. Data were then filtered with a dual-pass, 4th order digital Butterworth filter with a 2 Hz cutoff frequency. Time-series profiles for each trial were time-aligned to stimulus onset (eyes open) and all trials averaged within-participant to generate a representative NVC response for that session (Figure 1). To account for the unknown insonation angle of the TCD probes, relative changes in CBF velocity were calculated relative to the average velocity during the 3-5 second window before stimulus onset. Responses to visual stimulation were quantified by calculating the maximum slope of the activation phase ($slope_{max}$), time to $slope_{max}$ ($t_{slopemax}$), peak relative change in CBF*v* (v_{max}), time-to-peak-change (t_{vmax}), and area-under-the-curve during the first 25 seconds after stimulus onset (AUC_{25}) (Figure 5-1).

5.3.5 Statistical analyses

All statistical analyses were performed using SPSS version 22.0 for Macintosh (IBM, Wherever, USA). Shapiro-Wilks tests were used to assess for normality, while Mauchly's test was used to evaluate sphericity. In cases where the assumption of sphericity was violated, Greenhouse-Geiser epsilon was used to adjust degrees-of-freedom for the primary F-test. Significance was determined *a priori* to achieve an experiment-wide $\alpha = 0.05$.

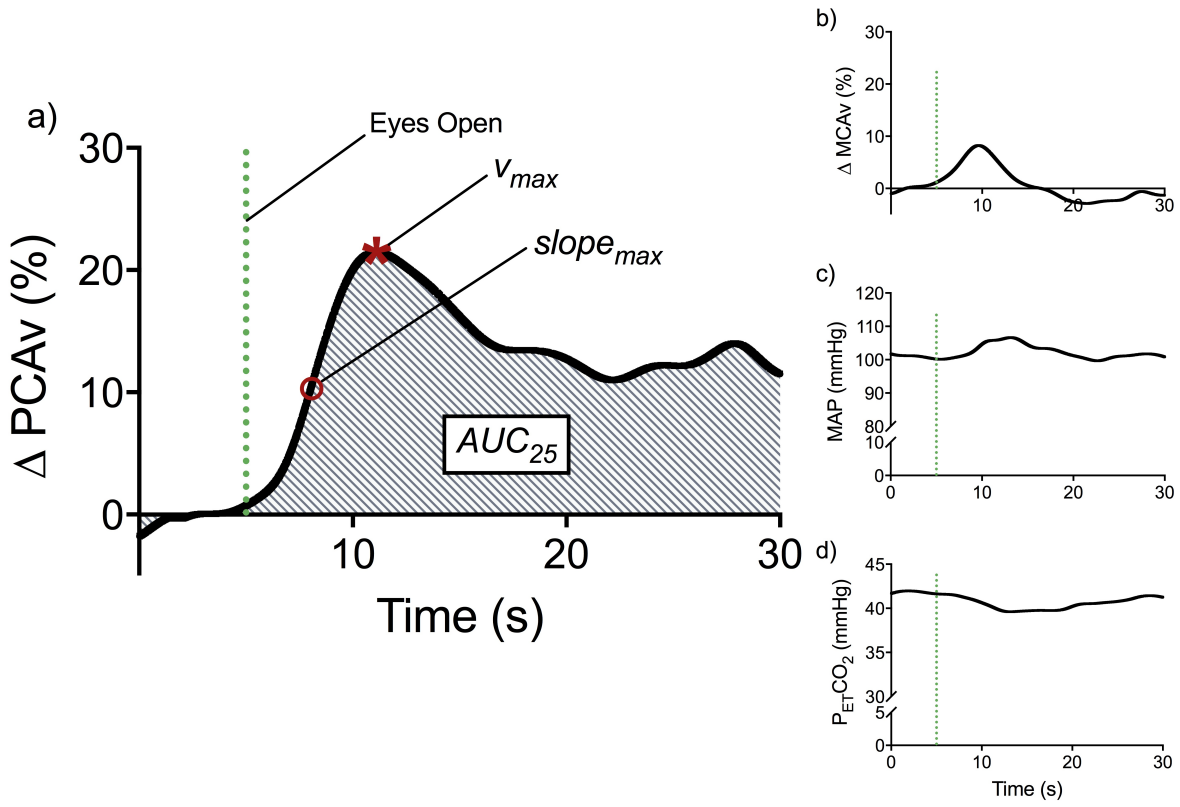

Figure 5-1. Representative traces of changes in physiological variables during one trial of the neurovascular coupling task. a) change posterior cerebral artery velocity (PCAv) – outcome metrics include timing and magnitude of peak slope during activation phase ($t_{slopemax}$, $slope_{max}$), timing and magnitude of peak relative change in PCAv (t_{vmax}, v_{max}), and area-under-the-curve over the first 25 seconds of eyes open (AUC_{25}); b) change in middle cerebral artery velocity (MCAv); c) mean arterial pressure (MAP); and d) end-tidal partial pressure of CO_2 ($P_{ET}CO_2$); vertical green dashed lines indicates stimulus onset (eyes-open).

5.3.5.1 Effect of acute concussion

A one-way (time) repeated-measures ANOVA was used to evaluate the effect of acute sport-related concussion on NVC metrics. Where omnibus tests indicated significant main effects, pre-planned t-tests were used to evaluate specific pairwise contrasts (i.e. each post-injury time point relative to preseason).

5.3.5.2 Cumulative effects of multiple previous concussions

The effect of multiple prior concussions on NVC metrics was evaluated using independent samples t-tests (Hx^{-} versus Hx^{3+}).

5.4 Results

Demographic characteristics, SCAT3 performance, return-to-play durations and resting physiological data across are outlined in Table 5-1. Concussed athletes were cleared by their team physician to return to full-contact game participation a median 14 days post-injury. At preseason, Hx^{3+} participants reported a greater number ($p = 0.033$) and severity of symptoms ($p = 0.013$) than Hx^{-} participants. Physiological variables did not differ between groups ($p > 0.05$). Representative traces for PCAv, MCAv, MAP, and $P_{ET}CO_2$ are shown in Figure 5-1.

5.4.1 Effect of acute concussion

Time-series profiles for each primary physiological measure, averaged across subjects, are presented in Figure 5-2.

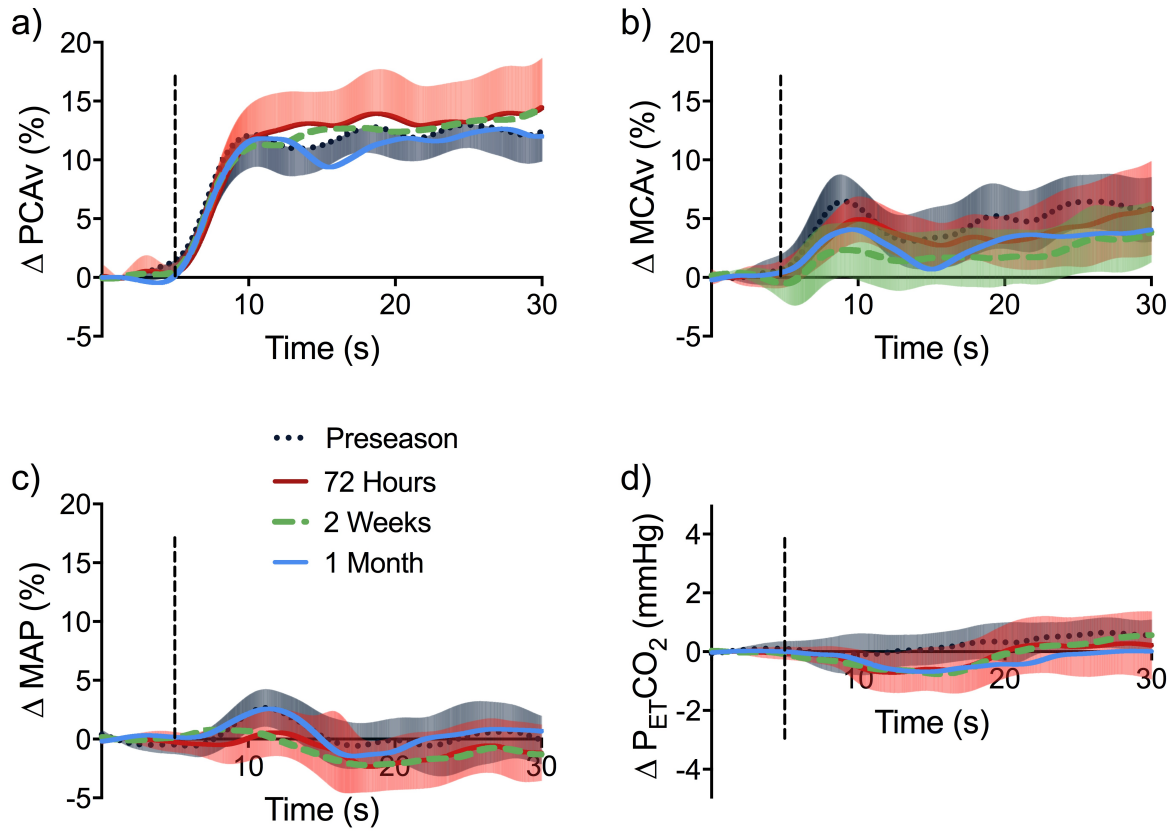

Figure 5-2. Time-series profiles of physiological variables – averaged across subjects – at each pre- and post-concussion time point following visual stimulation for a) change posterior cerebral artery velocity (ΔPCAv); b) change in middle cerebral artery velocity (ΔMCAv); c) mean arterial pressure (ΔMAP); and d) change in end-tidal partial pressure of CO_2 ($P_{ET}CO_2$); vertical black dashed lines at t=5s indicates stimulus onset (eyes-open); error bars represent 95% confidence intervals – note: not all error bars are shown for purposes of visual clarity.

Evaluating the NVC response in the PCA, repeated measures-ANOVA indicated a significant main effect of time for $t_{slopemax}$ ($F_{2.48,42.14}$=3.157, p=0.043, partial eta^2=0.157), v_{max} ($F_{3,51}$=4.172, p=0.01, partial eta^2=0.197) and AUC_{25} ($F_{3,51}$=2.924, p=0.043, partial eta^2=0.147). As outlined in Figure 5-3, post-hoc analyses revealed an absolute increase in PCA-$t_{slopemax}$ of 1.2 seconds (56.6% slower) at 72-hours post-injury (95%CI=0.01-2.42 seconds, adjusted p=0.049) and a

trend towards a 1.1 second increase at 2-weeks (95%CI=-0.16-2.36 seconds, p=0.08), but returned to near-preseason levels by 1-month.

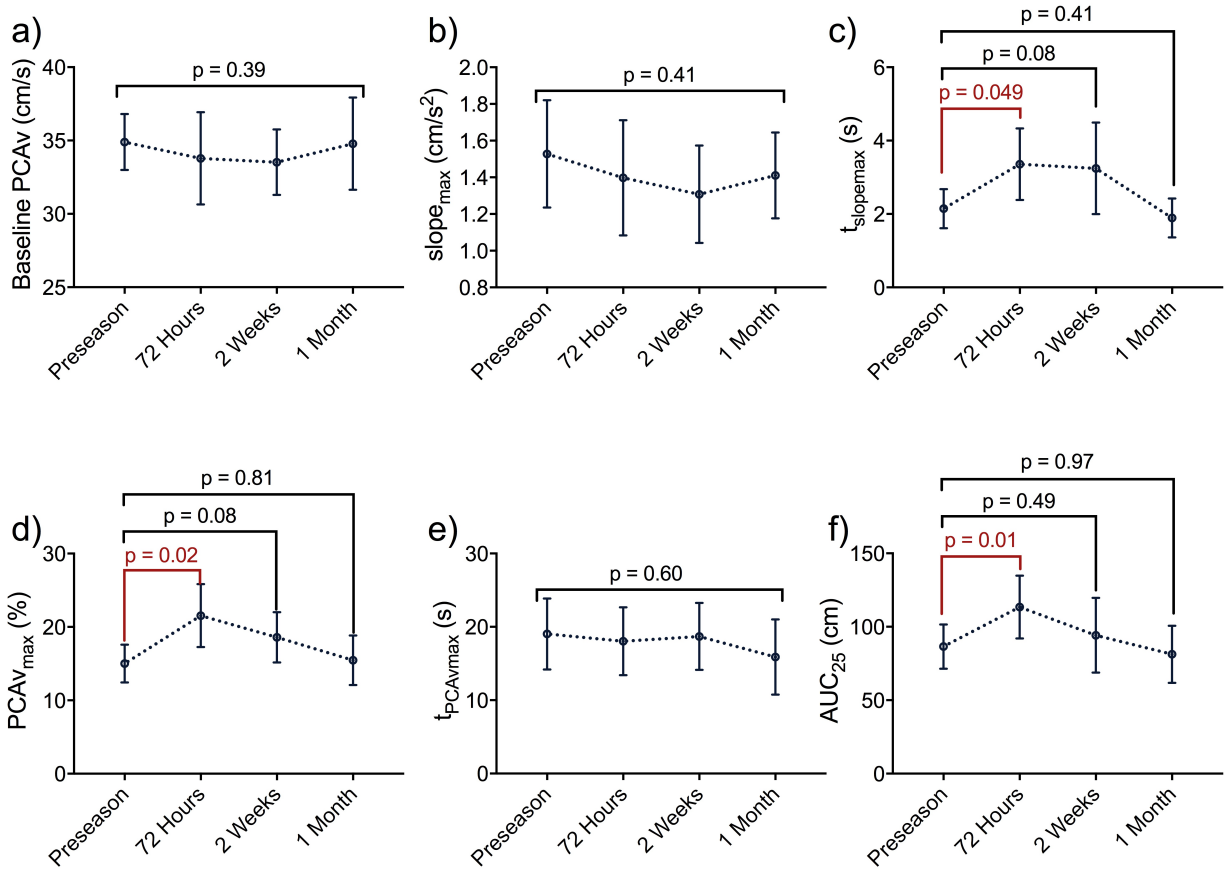

Figure 5-3. Summary of neurovascular coupling metrics in the posterior cerebral artery (PCA) to visual stimulation at preseason and each post-concussion time point; a) eyes-closed PCA blood velocity (Baseline PCAv); b) maximum PCAv slope ($slope_{max}$), and c) time to maximum ($t_{slopemax}$) PCAv slope during activation phase; d) peak relative change (v_{max}), and e) time to peak relative change (t_{vmax}) in PCAv; f) area-under-the-curve during the first 25 seconds after stimulus onset (AUC_{25}); unless pairwise comparisons are indicated, p-values represent main effect of time; error bars represent 95% confidence intervals.

Absolute PCA-v_{max} increased by 6.5% – a 45.6% relative increase – at 72-hours (95%CI=2.2–

10.8%, adjusted p=0.015) and 3.6% at 2-weeks post-injury (95%CI=0.4–6.7%, adjusted p=0.084); PCA-v_{max} did not differ from preseason by 1-month post-injury. Similarly, PCA-AUC_{25} was elevated by 31.1% at 72-hours (95%CI=10.9–43.0 cm, adjusted p=0.009), but did not differ from preseason at 2-weeks or 1-month post-injury.

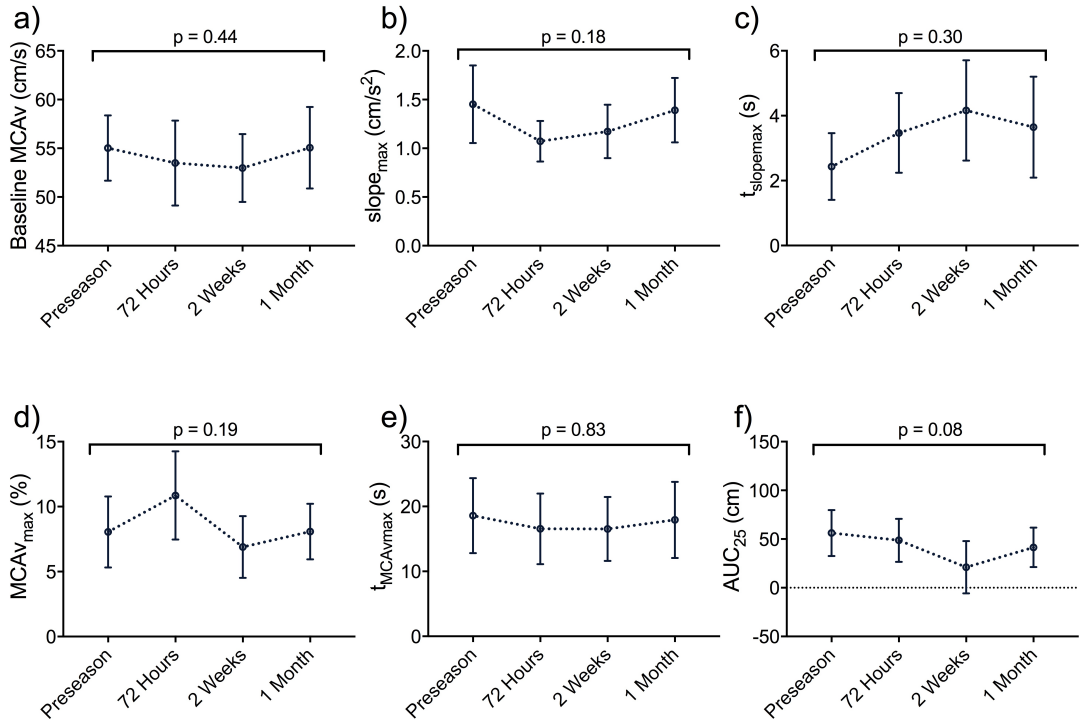

Figure 5-4. Summary of neurovascular coupling response metrics to visual stimulation in the middle cerebral artery (MCA) at preseason and each post-concussion time point (error bars represent 95% confidence intervals): a) eyes-closed MCA blood velocity (Baseline MCAv); b) maximum MCAv slope ($slope_{max}$), and c) time to maximum ($t_{slopemax}$) MCAv slope during activation phase; d) peak relative change (MCAv_{max}), and e) time to peak relative change ($t_{MCAvmax}$) in MCAv; f) area-under-the-curve during the first 25 seconds after stimulus onset (AUC_{25}); p-values represent main effect of time from repeated measures ANOVA.

An association was observed between ΔPCA-v_{max} and time to clearance for return-to-play (RTP) (Spearman's rho = -0.491, p=0.05), indicating those who exhibited the greatest increases in PCA-v_{max} immediately following injury relative to preseason responses recovered more quickly on clinical grounds. Concussion did not significantly affect MCAv, MAP, or CO_2 responses to visual stimulation (Figure 5-4, Table 5-2).

Table 5-2. Summary of responses to visual stimulation for secondary physiological variables.

Metric	Concussion History			Concussed (n=18)				
	Hx^- (n=42)	Hx^{3+} (n=31)	*p*	Preseason	72 Hour	2 Week	1 Month	*p*
MAP_{base} (mmHg)	90.1 (8.6)	91.3 (13.1)	*0.669*	93.9 (14.0)	92.5 (13.0)	91.4 (17.5)	91.1 (11.5)	*0.603*
ΔMAP_{max} (%)	4.1 (3.1)	6.1 (3.8)	*0.013**	4.3 (2.9)	3.3 (3.0)	3.7 (2.5)	4.5 (2.2)	*0.459*
$P_{ET}CO_{2base}$ (mmHg)	35.6 (8.7)	37.0 (6.9)	*0.211*	38.7 (0.7)	37.3 (1.1)	36.1 (1.4)	36.9 (0.9)	*0.102*
$\Delta P_{ET}CO_{2max}$ (mmHg)	1.7 (2.7)	1.3 (1.3)	*0.447*	1.0 (1.0)	1.2 (1.5)	1.3 (1.1)	1.1 (1.1)	*0.611*
HR_{base} (bpm)	64.6 (9.9)	65.1 (9.6)	*0.853*	62.9 (1.9)	63.8 (1.9)	65.5 (2.4)	65.2 (2.0)	*0.563*
ΔHR_{max} (bpm)	5.0 (3.8)	5.4 (3.4)	*0.687*	4.8 (3.1)	5.0 (2.8)	6.3 (4.0)	5.9 (3.2)	*0.258*

MAP_{base} = baseline MAP (eyes-closed); ΔMAP_{max} = peak relative change in MAP following stimulus onset; $P_{ET}CO_{2base}$ = baseline end-tidal PCO_2 (eyes-closed); $\Delta P_{ET}CO_{2max}$ = peak absolute change in $P_{ET}CO_2$ following stimulus onset; HR_{base} = baseline heart rate (eyes-closed); ΔHR_{max} = peak absolute change in heart rate following stimulus onset; p-values for Concussion History reflect independent samples t-tests; p-values for Concussed reflect omnibus F-test from one-way repeated measures ANOVA; * indicates significant finding.

5.4.2 Cumulative effects of multiple previous concussions

No significant differences were observed between Hx^- and Hx^{3+} groups with respect to any NVC metric in either the PCA or MCA (Figure 5-5, Figure 5-6, Figure 5-7, Table 5-2).

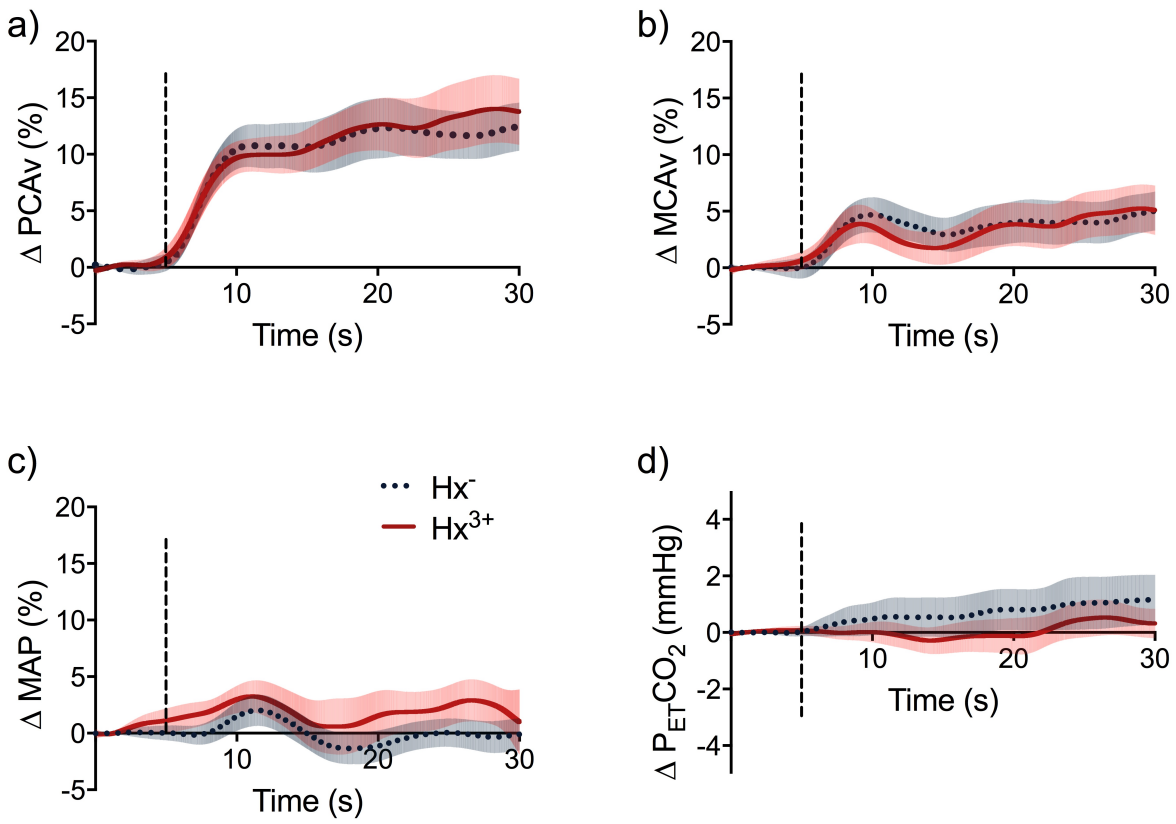

Figure 5-5. Preseason time-series profiles – averaged across participants with zero (Hx-) versus three or more (Hx3+) prior concussions – following visual stimulation for a) change posterior cerebral artery velocity (ΔPCAv); b) change in middle cerebral artery velocity (ΔMCAv); c) mean arterial pressure (ΔMAP); and d) change in end-tidal partial pressure of CO_2 ($P_{ET}CO_2$); vertical black dashed lines indicates stimulus onset (eyes-open); error bars represent 95% confidence intervals**.**

Statistically, participants in the Hx^{3+} group exhibited a significantly greater peak change in MAP following stimulus onset (t_{71} = -2.55, p=0.013), though the magnitude of the difference was only 2.0%.

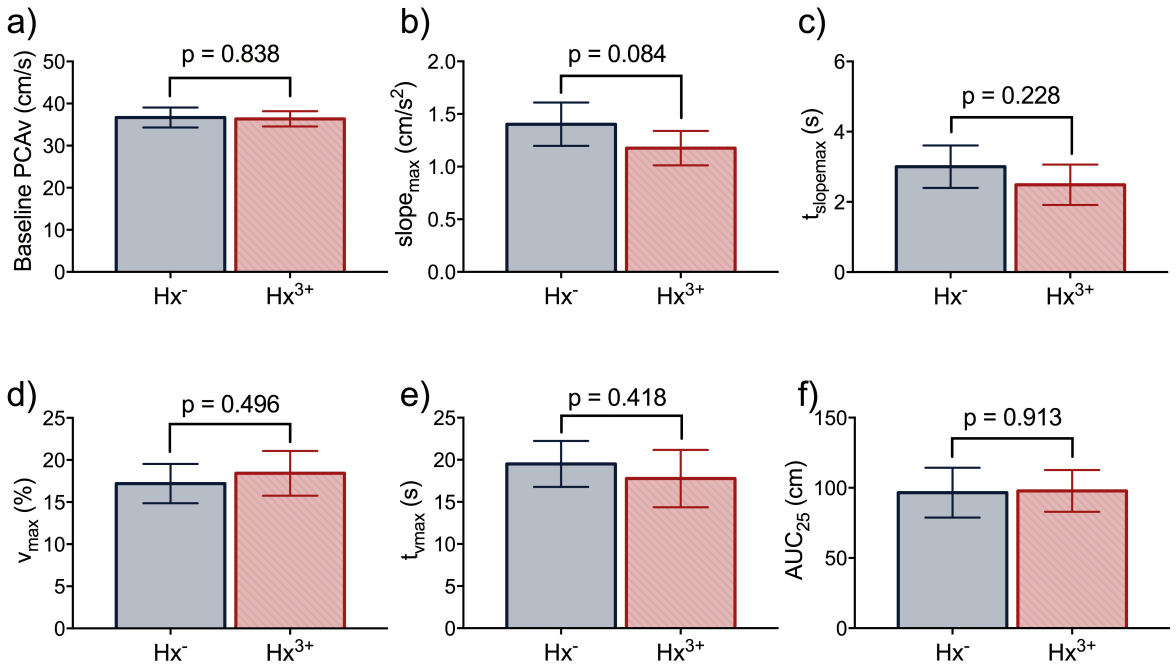

Figure 5-6. Summary of preseason neurovascular coupling responses to visual stimulation in the posterior cerebral artery (PCA) across participants with zero (Hx^{-}) versus three or more (Hx^{3+}) prior concussions; a) eyes-closed PCA blood velocity (Baseline PCAv); b) maximum PCAv slope ($slope_{max}$), and c) time to maximum ($t_{slopemax}$) PCAv slope during activation phase; d) peak relative change (v_{max}), and e) time to peak relative change (t_{vmax}) in PCAv; f) area-under-the-curve during the first 25 seconds after stimulus onset (AUC_{25}); no differences were observed between groups; error bars represent 95% confidence intervals.

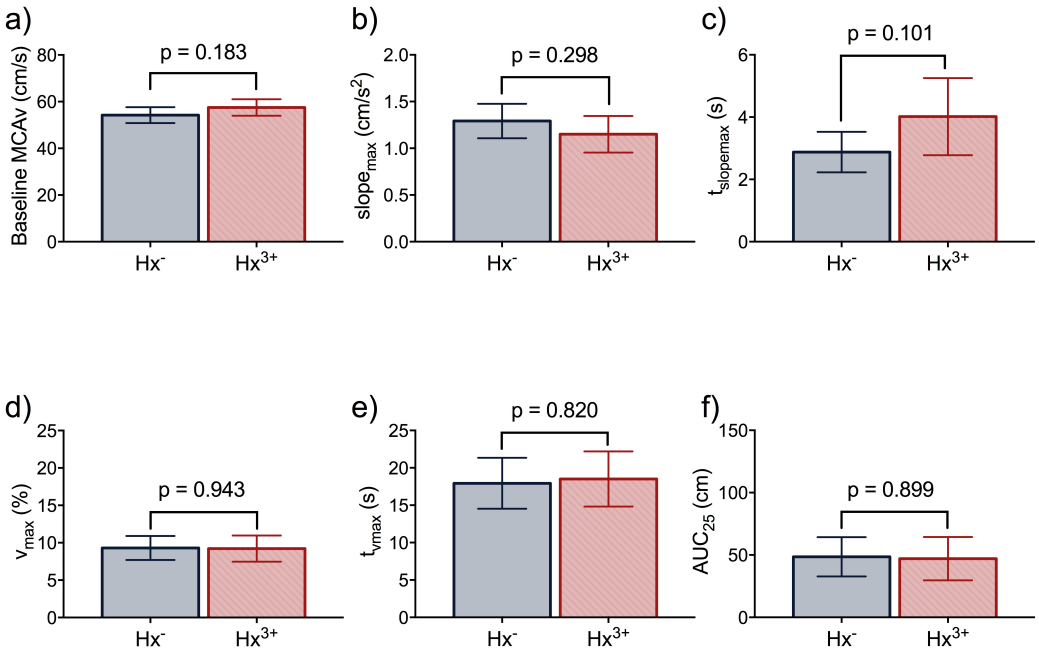

Figure 5-7. Summary of preseason neurovascular coupling responses to visual stimulation in the middle cerebral artery (MCA) across participants with zero (Hx^{-}) versus three or more (Hx^{3+}) prior concussions; a) eyes-closed MCA blood velocity (Baseline MCAv); b) maximum MCAv slope ($slope_{max}$), and c) time to maximum ($t_{slopemax}$) MCAv slope during activation phase; d) peak relative change (v_{max}), and e) time to peak relative change (t_{vmax}) in MCAv; f) area-under-the-curve during the first 25 seconds after stimulus onset (AUC_{25}); no differences were observed between groups.

5.5 Discussion

In this study, we present the first evaluation into the effects of sport-related concussion on the neurovascular coupling response to visual stimulation using transcranial Doppler ultrasound, and revealed three primary findings: i) activation of the blood flow response in the posterior cerebral artery to visual stimulation was delayed, and the magnitude of the response augmented, acutely following injury, but returned to preseason values by 1-month; ii) the magnitude of increase in blood flow response was inversely related to clinical recovery, as estimated by time to medical

clearance for return-to-play; and; iii) effects of acute concussion do not appear to be cumulative, wherein comparison of NVC responses at preseason revealed no differences between individuals who had never experienced a concussion versus those who had endured three or more prior concussions.

5.5.1 Cerebral blood flow alterations following sport-related concussion

The brain comprises only 2% of total body weight, yet receives 15-20% of cardiac output at rest, highlighting its high metabolic demand and the consequent importance of maintaining adequate CBF for proper functioning. Cerebral blood flow has repeatedly shown alterations following concussive injury (2,43,136,137,346). In the immediate post-injury phase CBF may be elevated (346), with evidence demonstrating subsequent reductions in global and regional CBF both sub-acutely (2,43,136) and chronically (142,143). Recovery of CBF to near-healthy levels occurs over ~30 days (2) and may be related to symptom resolution (43,137), although some studies have shown previous concussions may invoke chronic reductions in regional CBF (136,144). In the current study, we did not observe significant changes in resting *PCAv* or *MCAv* following injury (Table 5-1), although this may be heavily influenced by methodological differences between MRI- and TCD-derived estimates of CBF; the improved spatial resolution of MRI provides heightened sensitivity for subtle changes in flow, while TCD-indexed CBF is susceptible to changes in the diameter of the insonated vessel. However, TCD provides superior temporal resolution, allowing insights into CBF dynamics. Despite the known CBF alterations following concussion, our understanding of the effects of such injuries on mechanisms controlling CBF is comparatively lacking, including the dynamics of the neurovascular coupling response.

5.5.2 Mechanisms underlying the neurovascular coupling response

Regional increases in neural activity – and the associated elevation in metabolism – require appropriate and coordinated responses by the neurovascular unit to modulate local cerebrovascular resistance and direct nutrient-rich blood to active cortical regions. Failure to appropriately match CBF with local metabolic demands may lead to neurological dysfunction. Multiple pathways have been proposed to contribute to the NVC response, including local accumulation of vasoactive metabolic by-products (e.g. CO_2, nitric oxide (NO), adenosine, arachidonic acid metabolites), direct neural control of the cerebrovasculature, astrocyte-mediated vasosignaling, and pericyte-mediated changes in capillary tone (reviewed in: (145,220)). The entire cerebrovascular tree is innervated with sympathetic and parasympathetic fibres and growing evidence supports a role for autonomic activity in modulating CBF via its effects on cerebrovascular resistance (145,225,226). Indeed, the fast initial rise in PCAv in the current visual stimulation paradigm is thought to result from sympathetic activation within the visual cortex (145,213,220). Astrocytes are thought to contribute to a slow-onset (3-4 seconds) neurovascular coupling response, but may play a lesser role in the immediate hyperemic effect (< 1 second) following neural activation (232). Excitatory and inhibitory neurons synapse on both astrocytes and GABAergic interneurons that are closely associated with astrocytic end-feet enveloping penetrating arterioles. The release of glutamate from active neurons triggers metabotropic receptor-mediated calcium uptake into neighbouring astrocytes (reviewed in: (232)) and causes release of vasoactive modulators (adenosine, nitric oxide, prostaglandins and other arachidonic acid derivatives) onto arteriolar smooth muscle cells and vascular endothelial cells (234-236). Inhibitory interneurons communicating with neurons, astrocytes, and

microvessels play a role in integrating local signals and releasing vasoactive substances (e.g. NO, acetylcholine, neuropeptide-Y, vasoactive intestinal peptide) to precisely regulate CBF (237,238). Ultimately, gap junctions between adjacent vascular smooth muscle cells are thought to facilitate intramural propagation of vascular signals to yield vasoactivity in remote pial arterioles up-stream of the signal origin (221-223). Although our understanding of the precise mechanisms controlling the NVC response in humans remains incomplete, it is clear that multiple redundant pathways exist to ensure CBF is appropriately altered in response to changes in local neural activity (220).

5.5.3 Functional magnetic resonance imaging in concussion

The majority of task-based functional MRI studies into the effects of concussion and mTBI on the hemodynamic response have used tasks related to frontal lobe/executive function, and have typically shown increases in cortical activation relative to healthy controls in multiple brain regions (reviewed in (67)). Cortical hyperactivation is demonstrated often despite no between-group differences in task performance, and may be related to symptom severity (255-257). The timeline to recovery of functional alterations following concussion is unclear; a recent prospective study demonstrated gradual reductions in the degree of global hyperactivation over time, showing elevated BOLD responses in multiple frontoparietal regions at three days post-injury, in the dorsolateral prefrontal cortex (DLPFC) and inferior parietal lobe persisting for two weeks, and in the DLPFC only at two months (257). In a study of concussed high-school athletes in the first week post-injury, hyperactivation in Brodman's Area 6 during a working memory task was associated with prolonged recovery (262); this contrasts the current findings in a slightly older population, wherein those who exhibited an augmented NVC response acutely

post-injury experienced shorter recovery times. However, the former study also reported higher activation associated with lower symptom scores (262). Further investigation is warranted to determine if this discrepancy is a function of age group or other clinical factors. In the only study to evaluate the effects of mTBI on the dynamics of the hemodynamic response, Mayer and colleagues reported greater activation in the visual cortex in mTBI patients presenting to the emergency department relative to controls, particularly in the 2-4 second window after stimulus onset, suggesting a shorter time-to-peak response (265). Our within-subject results similarly showed increased peak responses (v_{max}, Figure 5-3), but delayed response initiation ($t_{slopemax}$) and no difference in time to peak response (t_{vmax}, Figure 5-3). Nevertheless, elevated task-based activation in the brains of concussed patients has generally been interpreted as recruitment of additional neural resources to compensate for cognitive and/or attentional deficits to support task performance (257,264).

Other fMRI studies have demonstrated concussion-induced *hypo*activation in similar areas to those mentioned above, particularly in individuals with persistent symptoms, though task performance was not always as equal between groups (258-261,382). Participants reporting more post-concussion syndrome symptoms (persisting > 1 year post-injury) exhibited reduced activation in task-irrelevant areas but greater activation in attention-related areas as cognitive load was increased during a top-down attentional task (263). Previous studies have observed, similar to our results, no cumulative effects of multiple previous concussions on the hemodynamic response to cortical activation in otherwise healthy young adult athletes (269,270). However, multiple previous concussions were shown to negatively affect recruitment patterns in the relational memory network of former professional football players (271), raising the

possibility that multiple concussions in addition to repetitive subconcussive trauma may be detrimental over the course of a long career. Although inconsistency across fMRI studies may be partly attributed to methodological differences and injury heterogeneity across samples (266), a recent meta-analysis of fMRI findings in mTBI observed hyperactivation during continuous tasks – similar to the current reading paradigm – and hypoactivation during tasks requiring discrete periods of working memory (267). Nevertheless, additional investigations are warranted to determine whether conflicting results are a function of task complexity or other clinical factors.

5.5.4 Possible mechanisms for observed effects of acute concussion

Concussive injuries may alter the NVC response by multiple mechanisms. Concussions induce autonomic dysregulation in the form of elevated sympathetic and / or depressed parasympathetic tone (298,299,301,305) that affects the stiffness of peripheral arteries (300). Such autonomic dysfunction has previously been shown to delay the response of cerebral blood vessels to changes in blood pressure, though the magnitude of the response was unaffected (Chapter 3:). Neuronal damage caused by concussion, including disruptions in myelination (Chapter 2:)(317), is thought to reduce the speed and efficiency of signal processing within the brain. Taken together, these findings complement the current observation of a delay in achieving peak activation slope of the NVC response (Figure 5-3). Animal mTBI models have shown leukocyte invasion initially occurs across pial microvessels; elevated chemokine expression from astrocytes provides a chemotactic gradient for subsequent perivascular migration of leukocytes into brain parenchyma, suggesting a contributing role for astrocytes in the post-concussion neuroinflammatory response (383). Increased astrocyte signaling could also contribute to the

observed increase in the magnitude of the NVC response, given its role outlined above, though this remains speculative. Furthermore, concussion induces a decoupling between CBF and glutamate metabolism, followed by a more global reduction in cerebral metabolism (42,79,104). Consequently, the demand placed on neural networks involved in performing a given task may be elevated, requiring compensatory activation of additional neural resources (255). Indeed, the effects of acute concussion appear similar to observations in non-concussed healthy athletes of increases in PCA-v_{max} and PCA-AUC_{25} as the complexity of the visual stimulus increased (213). As mentioned previously, multiple redundant mechanisms are thought affect the NVC response in normal humans, and there are multiple ways in which concussive trauma may interfere with normal functioning. Taken alongside results from fMRI, the current data support a concussion-induced alteration in the CBF response to task-based cortical activation.

5.5.5 Limitations

There are several limitations to the current study. First, our sample consisted exclusively of male athletes, representing a significant shortcoming in the generalizability of our findings as females sustain higher rates of SRC and endure longer recovery trajectories (356). Second, while TCD provides excellent resolution in the time domain, the spatial sensitivity of this technique is limited to measurement of blood velocity in major cerebral arteries; given the known heterogeneity in biomechanical and spatial localization of concussive injuries, this approach is unable to discern subtle regional damage and any impairments in NVC function at a microvascular level. Furthermore, our sample was drawn from multiple athletic teams with different medical staff; the clinical nature of concussion diagnosis raises the possibility of magnifying the heterogeneity of injuries across participants. Third, TCD does not measure blood

flow, but rather CBF velocity; for this index to accurately represent flow requires the diameter of insonated vessels to remain unchanged, which cannot be verified. However, any effects of CO_2 on vessel diameters are presumed to have been minimal as end-tidals were largely unchanged (Figure 5-2, Figure 5-5). Lastly, clinical guidelines outlining appropriate management of concussed patients dictate a period of physical and cognitive rest post-injury. As such, the potential effect of inactivity on our results cannot be discredited. Nevertheless, significant alterations in the dynamics of the NVC response were revealed in a small sample of concussed young adult athletes, encouraging further prospective investigations into the effects of sport-related head trauma on CBF control mechanisms.

5.6 Conclusion

A better understanding of the pathophysiological underpinnings of concussion is needed to limit the burden of these injuries at an individual and population level. Although not appropriate for clinical decision-making, this TCD-based evaluation revealed acute alterations in the neurovascular coupling response in the posterior cerebral artery of concussed athletes that were related to clinical recovery. However, it appears that such effects are transient in nature and do not accumulate with multiple injuries. These findings add to our growing appreciation for the detrimental effects of sport-related concussion on multiple aspects of cerebrovascular function. Development of further prospective investigations in larger samples towards delineating the effects of concussion on mechanisms controlling CBF – including relationships with age, sex, biomechanical exposure, and susceptibility to injury – are warranted.

Chapter 6: A prospective transcranial Doppler ultrasound-based evaluation of the effects of repetitive subconcussive head trauma on neurovascular coupling dynamics

6.1 Summary

Recent research has implicated repetitive subconcussive head trauma towards short- and long-term deficits in brain structure and function, including changes in the integrity of mechanisms controlling cerebral blood flow (CBF). However, the degree to which dynamics of the neurovascular coupling (NVC) response (i.e. CBF response to neural activity) are altered following a season of contact sport participation is unknown. 179 junior-level contact sport athletes (ice hockey and football: age 19.6 ± 1.5 years) completed preseason testing – 52 returned for follow-up testing after completion of the athletic season. 15 age- and education-matched non-contact sport athletes (primarily cross-country running) also completed pre- and post-season testing. NVC dynamics were estimated during cycles of 20-s eyes-closed and 40-s eyes-open to a visual stimulus (reading) by measuring blood flow velocity in the posterior (PCA) and middle (MCA) cerebral arteries using transcranial Doppler ultrasound. No significant differences in NVC dynamics in the PCA or MCA were observed between pre- and post-season in either athlete group. Furthermore, no significant relationships were found between change scores in NVC metrics from pre- to post-season and estimates of cumulative head impact exposure as derived from skin-worn impact sensors. Within the context of growing concern over detrimental effects of repetitive subconcussive trauma, the current results suggest that the dynamics of NVC responses are not affected by one season of participation in junior-level ice

hockey or football.

6.2 Introduction

There is growing concern over the effects of repetitive subconcussive head trauma towards long-term deficits in neurologic function (55). Subconcussion has been defined as trauma that does not result in signs or symptoms of concussion (40,56). Over the past five years, multiple studies have shown repetitive trauma to the head can induce deficits in brain structure and function associated with impact exposure (25,26,65-68,71,72), including metrics influenced by the cerebral blood flow (CBF) response to changes in neural activity (20,22).

Brain metabolism is critically dependent on the adequate supply of nutrients, accomplished via multiple mechanisms that contribute to the tight regulation of CBF (145). In addition to influences of blood pressure (cerebral autoregulation) and carbon dioxide (CO_2 reactivity), CBF is also sensitive to regional neural activity through a process known as neurovascular coupling (NVC). The NVC phenomenon may be observed using transcranial Doppler (TCD) ultrasound to index blood flow in the posterior cerebral artery – the primary supplier of blood to the occipital cortex – during visual stimulation paradigms such as reading (156,213,241,242,381). Functional MRI (fMRI) also capitalizes on this hemodynamic response: local cortical activation elicits a relative excess of delivered blood, termed functional hyperemia, thereby altering the ratio of oxy-to-deoxyhemoglobin and thus providing the basis for the blood oxygen level-dependent (BOLD) signal obtained using fMRI.

Cerebral blood flow plays an important role in the pathophysiology of concussion (131), but the

effects of subconcussion on CBF are less well understood. Alterations in CBF have been repeatedly demonstrated following diagnosed sport-related concussion (2,43,136,137), with evidence also indicating changes in the integrity of CBF control mechanisms including cerebral autoregulation (Chapter 3:), CO_2 reactivity (reviewed in (132)), and NVC dynamics (Chapter 5:)(265). Recently, impairments in CO_2 reactivity (18,19) have also been attributed to repetitive subconcussive trauma in collegiate soccer and football, while deficits in cerebral autoregulation have been associated with number of head hits and cumulative exposure to linear acceleration over the course of one season of football or ice hockey (Chapter 4:). Importantly, these alterations in cerebrovascular function were not observed in non-contact athletes. Studies employing fMRI suggest subconcussive trauma may also alter NVC; for example, differences were observed in task-based BOLD signal in the occipital cortex were related to the number of head impacts experienced (20). However, fMRI studies have typically derived one parameter to estimate the magnitude of the BOLD response, which assumes the dynamics of the NVC response are unaltered by trauma (67). To probe this assumption, evaluation of NVC dynamics using techniques providing better temporal resolution, such as TCD, are required.

As such, our objective was to prospectively evaluate the effect of repetitive subconcussive head trauma on the dynamics of the NVC response, with the hypothesis that alterations would be observed at post-season relative to pre-season in a group of young adult elite contact sport athletes.

6.3 Methods

6.3.1 Study design

179 elite male (mean age 19.6±1.5 years) junior hockey (n=90) and football (n=89) athletes were recruited to the study, in addition to 15 non-contact sport controls (mean age 20.4±2.2 years: 12 cross-country running, 1 ultimate, 2 basketball). All participants completed baseline laboratory testing prior to the beginning of the athletic season (preseason). Testing was repeated within two weeks of the end-of-season (post-season) in a subset of contact sport participants who were not concussed during the season (n=52), as well as all non-contact sport controls (Table 6-1). Prior to testing, participants completed the Sport Concussion Assessment Tool, version 3 (SCAT3) (9). The SCAT3 is comprised of a graded symptom checklist (7-point Likert scale for 22 concussion symptoms), the Standardized Assessment of Concussion (SAC) probing orientation, concentration, and immediate and delayed recall, as well as a balance assessment in the form of the modified Balance Error Scoring System (BESS). Exclusion criteria included significant history of cardiorespiratory, cerebrovascular, neurological, or severe neurodevelopmental disorder, however no participants were excluded on these grounds. All subjects were familiarized with testing procedures and abstained from caffeine, exercise, and alcoholic beverages for at least 12 hours before testing. Written informed consent was obtained before participating in the study, which was approved by the University of British Columbia Clinical Research Ethics Board.

Table 6-1. Participant characteristics. Demographics, SCAT3 performance, and resting physiological parameters for contact sport and control (non-contact sport) athletes at preseason and post-season; data are presented as mean (SD).

Metric	Contact Sport (n=52)		Control (n=15)	
	Preseason	Post-season	Preseason	Post-season
Age (years)	19.6 (1.5)		20.4 (2.2)	
BMI (kg/m^2)	28.2 (4.9)		22.6 (3.0)	
Test Interval (days)	109.2 (25.8)		100.1 (23.8)	
# of Symptoms[a]	3.7 (3.6)	5.4 (5.0)	5.5 (5.0)	5.7 (3.3)
Symptom Severity[a]	6.7 (7.8)	9.6 (10.1)	8.1 (8.3)	8.2 (7.2)
SAC Score[a]	26.6 (1.9)	26.6 (1.8)	27.7 (1.5)	27.9 (1.4)
BESS Score[a]	3.7 (3.3)	2.9 (3.2)	2.7 (2.4)	2.6 (3.6)
PCAv (cm/s)	34.6 (5.7)	36.1 (7.7)	35.9 (6.2)	37.0 (1.3)
MCAv (cm/s)	54.5 (9.3)	53.8 (7.5)	55.6 (14.4)	57.3 (14.3)
MAP (mmHg)	92.2 (12.2)	92.6 (12.7)	93.9 (13.8)	91.3 (9.7)
$P_{ET}CO_2$ (mmHg)	38.0 (3.0)	37.1 (2.6)	37.9 (1.8)	37.9 (1.3)
HR (bpm)	74.5 (10.0)	78.3 (11.9)	71.0 (9.9)	72.8 (13.8)

SCAT3 = Sport Concussion Assessment Tool version 3; SAC = Standardized Assessment of Concussion; BESS = Balance Error Scoring System; MAP = mean arterial pressure; PCAv = posterior cerebral artery blood velocity; MCAv = middle cerebral artery velocity; $P_{ET}CO_2$ = end-tidal partial pressure of carbon dioxide; HR = heart rate

6.3.2 Instrumentation

Participants wore a standard three-lead electrocardiogram (ECG). Cerebral blood flow velocity was recorded using 2 MHz TCD ultrasound probes (ST3, Spencer Technologies, Seattle, WA); the P-1 segment of the PCA was insonated on the left side of the head (PCA*v*), and the M-1 segment of middle cerebral artery (MCA*v*) was insonated on the right side. Vessels were identified and signals optimized according to depth, waveform, velocity, and response to carotid compression / visual stimulation tests (150), and ultrasound probes were locked in place with a fitted head frame (Spencer Technologies). Beat-to-beat blood pressure was recorded via finger

photoplethysmography and a brachial cuff to adjust for height differences between the finger and brachial artery (Finometer PRO, Finapres Medical Systems, Amsterdam, The Netherlands). Partial pressure of expired CO_2 was recorded via an online gas analyzer (ML206, AD Instruments, Colorado Springs, CO) and used to measure end-tidal CO_2 levels ($P_{ET}CO_2$). Data were sampled at 1000 Hz (PowerLab 8/30 ML880, AD Instruments) and stored for offline analysis using commercially available software (LabChart version 7.1, AD Instruments).

6.3.3 Experimental protocols

As outlined previously (Chapter 5:, 213) baseline physiological metrics were established during a one-minute resting trial prior to evaluating NVC dynamics using a visual stimulation paradigm (Table 1). The visual stimulation protocol entailed five cycles of 20 seconds eyes-closed followed by 40 seconds eyes-open and reading an article of interest to the participants, consistent with other neurovascular coupling research (213,241,242,244,247,381). Subjects were seated 50-60 cm from the computer monitor (27" Apple iMac) with a 50 cm x 35 cm visual field, with screen brightness set to maximum.

6.3.4 Data processing

All data were processed using a custom program (Matlab vR2013a, Mathworks, Natick, MA). Beat-to-beat values of systolic and diastolic blood pressure, MCA*v*, and PCA*v* were determined from each R-R interval and used to calculate mean arterial pressure (MAP) and mean velocity traces. Breath-to-breath peak expired CO_2 values were extracted to measure $P_{ET}CO_2$. All signals were visually inspected for artifacts and corrected by cubic spline interpolation before filtering

with a dual-pass, 4th order digital Butterworth filter with a 2 Hz cutoff frequency. Trials were time-aligned to stimulus onset (eyes open) and averaged within-subject to generate a representative NVC trace for each session. Given the unknown insonation angle of the TCD probes, relative changes in CBF velocity were calculated as a percent change from the average velocity during the 3-5-second window prior to stimulus onset (244). Dynamics of the NVC response were quantified as peak rate of increase in CBF velocity during the activation phase ($slope_{max}$), time to $slope_{max}$ ($t_{slopemax}$), peak relative change in CBF velocity (v_{max}), time to v_{max} (t_{vmax}), and area-under-the-curve to 25 seconds (AUC_{25}) (Figure 6-1).

6.3.5 Impact monitoring

During the season, a subset of contact sport participants who completed both pre- and post-season testing (n=29) wore impact sensors (xPatch, X2 Biosystems, Seattle, WA) on the right mastoid process to estimate linear and rotational acceleration profiles for each head impact experienced during games. Acceleration profiles were sampled for 100 ms (10 ms pre-impact, 90 ms post-impact) at 1000 Hz when translational acceleration exceeded a 10 *g* threshold. Data were uploaded using the Head Impact Monitoring System (X2 Biosystems) after each game. Peak linear (PLA) and peak rotational (PRA) acceleration were estimated for each detected impact. In an attempt to restrict analysis to head accelerations most likely to result from direct impacts rather than hard stops or cuts, only acceleration events exceeding a 20 *g* threshold were used (19,362,363). Cumulative exposure to linear (*cPLA*) and rotational (*cPRA*) acceleration were estimated by summing across all impacts for the season. Non-contact sport athletes did not wear impact sensors.

6.3.6 Statistical analyses

Statistical analyses were performed in SPSS for Macintosh (v22.0, IBM Corp., Armonk, NY). Normality was evaluated using Shapiro-Wilks tests. Significance was determined *a priori* to achieve an experiment-wide $\alpha = 0.05$. Effects of exposure to repetitive subconcussive trauma during the course of one competitive season were estimated via a 2 (contact versus non-contact sport) x 2 (pre-season versus post-season) two-way mixed ANOVA using type III sums of squares to account for unbalanced sample sizes across groups. Secondary exploratory analyses on the relationship between change scores in $slope_{max}$, $t_{slopemax}$, v_{max}, t_{vmax}, and AUC_{25} from pre- to post-season and *cPLA* and *cPRA* were conducted using Spearman's correlation coefficients. Spearman's correlation coefficients were also calculated between NVC change metrics and changes in symptom, SAC, and BESS scores from pre- to post-season.

6.4 Results

Demographic characteristics, SCAT3 scores, and resting physiological metrics across testing sessions and groups are outlined in Table 6-1. Representative traces of PCAv, MCAv, MAP, and $P_{ET}CO_2$ responses to visual stimulation are provided in Figure 6-1.

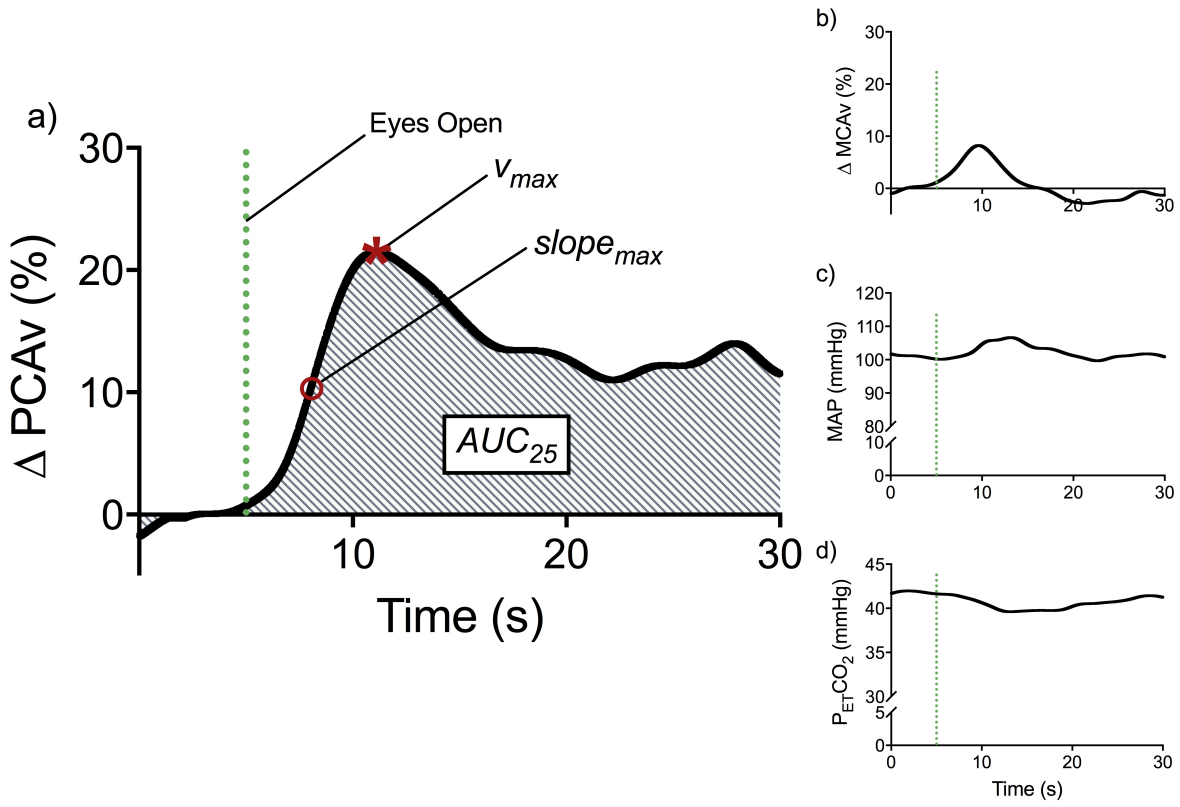

Figure 6-1. Representative traces of physiological responses to visual stimulation during one trial of the neurovascular coupling task a) change posterior cerebral artery velocity (PCAv) – outcome metrics include timing and magnitude of peak slope during activation phase ($slope_{max}$), timing and magnitude of peak relative change in PCAv (v_{max}), and area-under-the-curve over the first 25 seconds of eyes open (AUC_{25}); b) change in middle cerebral artery velocity (MCAv); c) mean arterial pressure (MAP); and d) end-tidal partial pressure of CO_2 ($P_{ET}CO_2$); vertical green dashed lines indicates stimulus onset (eyes-open).

Biomechanical descriptors of impact exposure are presented in Table 6-2. Relative to football players, ice hockey players experienced fewer hits per game, but greater cumulative number of hits due to season length differences (62 games in hockey versus 10 games in football).

Table 6-2. Summary of biomechanical variables describing head impact exposure in contact sport athletes.

Median (IQ range) for head impact exposure variables across subset of hockey (n=10) and football (n=19) players wearing impact sensors during the season; p-values reflect results of Mann-Whitney U-tests comparing across sport.

Metric	Hockey	Football	*p*
Hits / game (#)	8.2 (6.1 - 11.2)	16.6 (8.7 - 21.2)	0.003
Hits / season (#)	353.5 (295.0 - 587.3)	166 (63.5 - 212)	0.002
PLA / hit (*g*)	36.3 (35.4 - 37.4)	36.6 (34.5 - 40.2)	0.448
cPLA (*g*)	11920.5 (10788.3 - 21570.6)	5794.2 (2507.5 - 7117.7)	0.002
PRA / hit (rad/s^2)	5036.1 (4772.3 - 5510.8)	6601.8 (6104.0 - 7441.0)	<0.001
cPRA (rad/s^2)	2016603.5 (11592.6 - 24322.9)	1057691.6 (486976.2 - 1376735.9)	0.003

PLA = peak linear acceleration; PRA = peak rotational acceleration; cPLA/cPRA = cumulative PLA/PRA

Averaged time-series profiles for physiological responses to visual stimulation are presented in Figure 6-2.

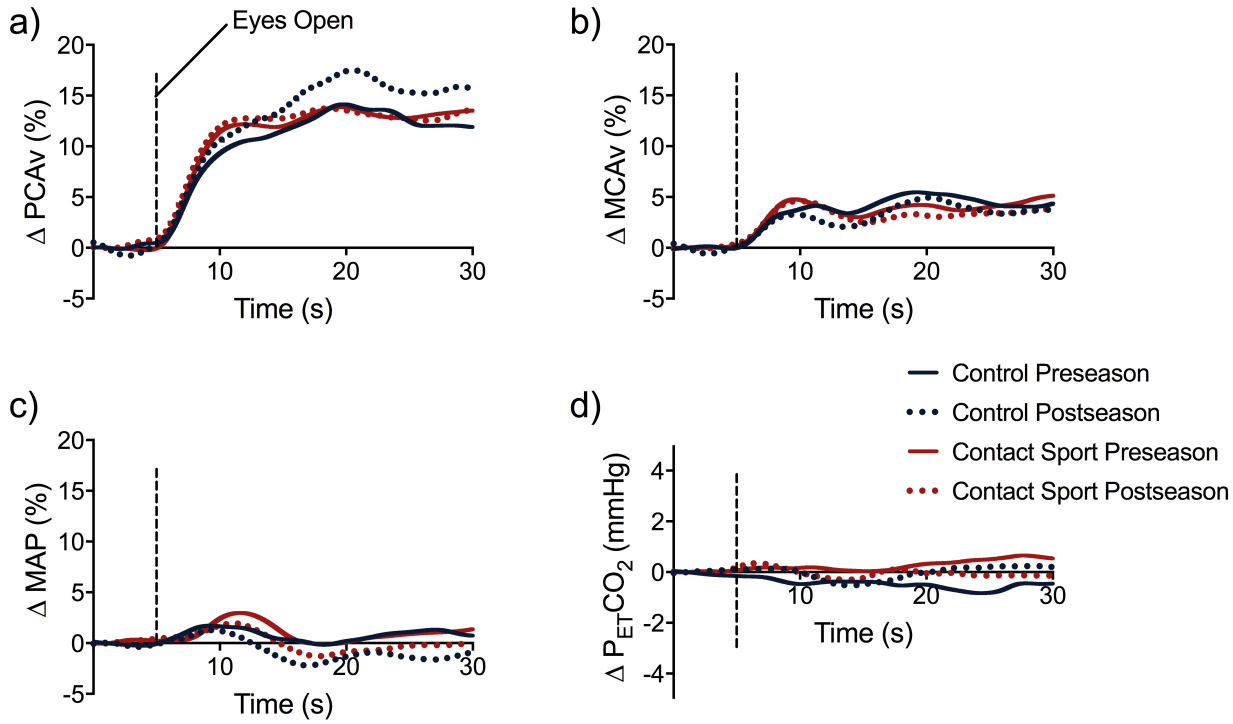

Figure 6-2. Average time-series profiles to visual stimulation across physiological variables a) posterior cerebral artery blood velocity (ΔPCAv); b) middle cerebral artery blood velocity (ΔMCAv); c) mean arterial pressure (ΔMAP); and d) end-tidal partial pressure of CO_2 ($\Delta P_{ET}CO_2$); vertical black dashed lines indicates stimulus onset (eyes-open); note: error bars are note shown for purposes of visual clarity.

Two-way mixed ANOVA revealed no significant group-time interactions for any NVC metric in the PCA (Figure 6-3) or MCA (Figure 6-4), nor were any significant main effects found for group or time (p all >0.05).

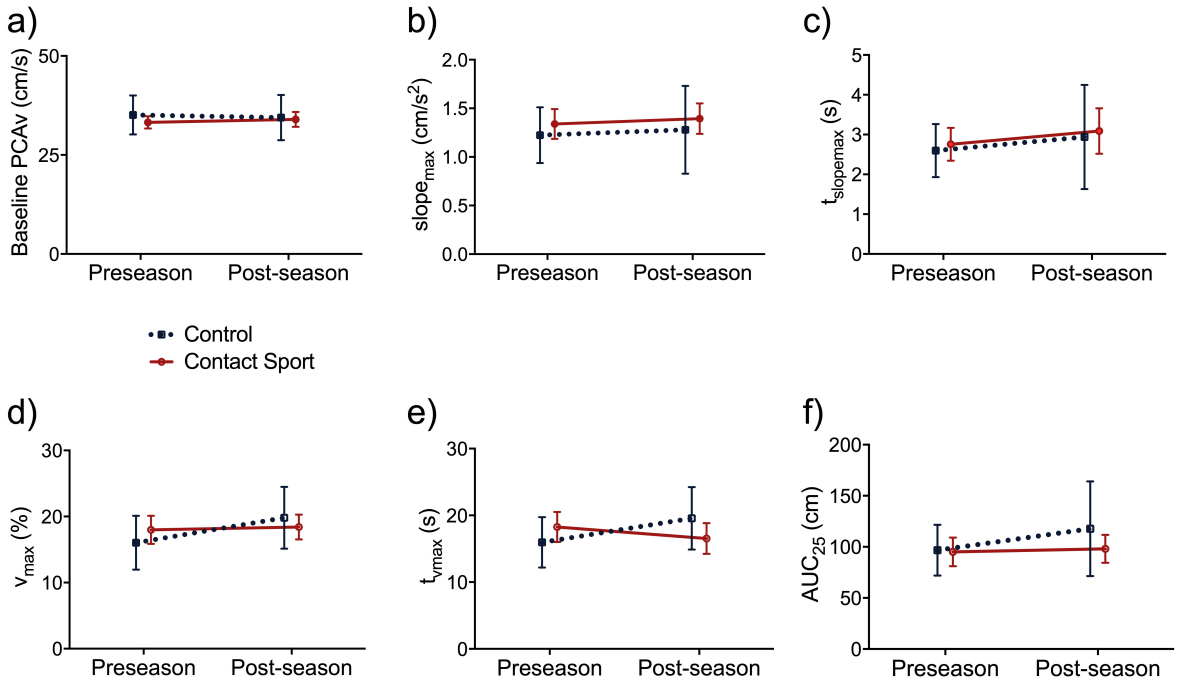

Figure 6-3. Summary blood velocity responses to visual stimulation in the posterior cerebral artery (PCA) a) baseline resting PCA blood velocity (PCAv); b) maximum ($slope_{max}$), and c) time to maximum ($t_{slopemax}$) PCAv slope during activation phase; d) peak relative change (v_{max}), and e) time to peak relative change (t_{vmax}) in PCAv; f) area-under-the-curve during the first 25 seconds after stimulus onset (AUC_{25}); 2-way mixed ANOVA revealed no significant main or interaction effects for any variable; error bars represent 95% confidence intervals.

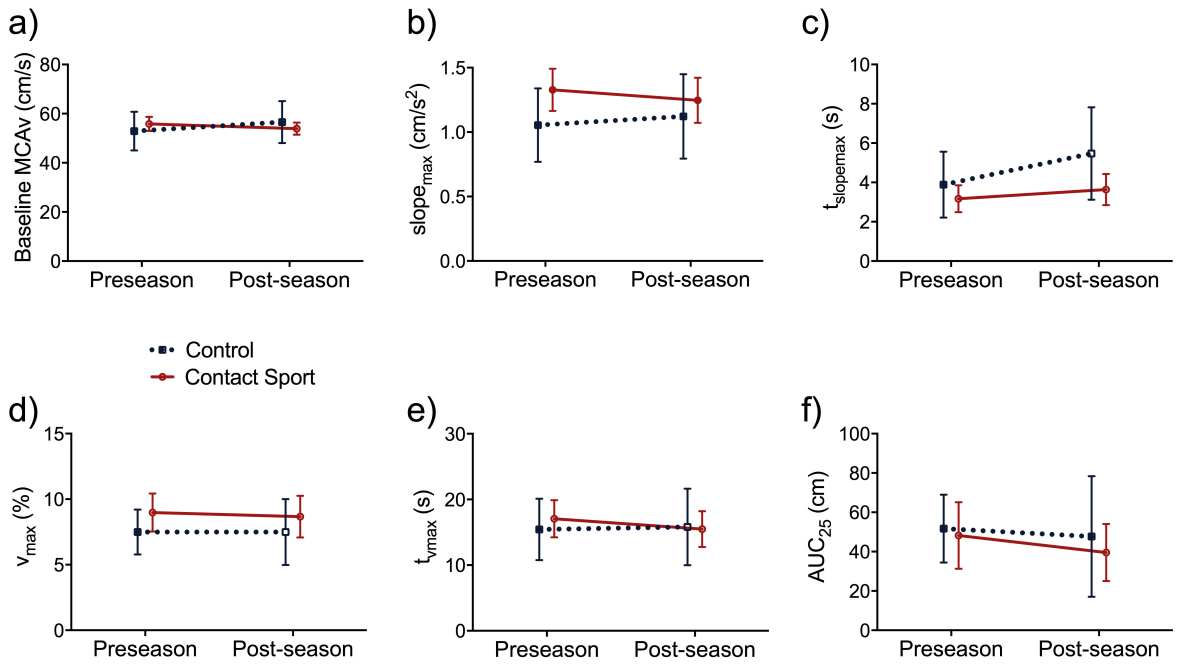

Figure 6-4. Summary of blood velocity responses to visual stimulation in the middle cerebral artery (MCA) a) baseline resting MCA blood velocity (MCAv); b) maximum ($slope_{max}$), and c) time to maximum ($t_{slopemax}$) MCAv slope during activation phase; d) peak relative change (v_{max}), and e) time to peak relative change (t_{vmax}) in MCAv; f) area-under-the-curve during the first 25 seconds after stimulus onset (AUC_{25}); 2-way mixed ANOVA revealed no significant main effects of group or time, nor significant interactions for any variable ($p > 0.05$) ; error bars represent 95% confidence intervals.

As shown in Table 6-3, control subjects exhibited a lower heart rate than contact sport subjects during eyes-closed periods, but heart rate responses to visual stimulation did not differ. No significant correlations were observed between changes in NVC dynamics from pre- to post-season and any impact exposure metric, nor were there any significant relationships with change scores on components of the SCAT3 (p all >0.05).

Table 6-3. Summary of responses to visual stimulation for secondary physiological variables.

Metric	Contact Sport (n=52)		Control (n=15)		$p_{interaction}$	p_{time}	p_{group}
	Preseason	Post-season	Preseason	Post-season			
MAP_{base} (mmHg)	90.3 (9.8)	89.7 (10.5)	86.3 (19.0)	88.4 (10.4)	0.571	0.747	0.323
ΔMAP_{max} (%)	5.1 (3.6)	4.2 (3.7)	4.3 (4.6)	2.9 (2.1)	0.694	0.144	0.213
$P_{ET}CO_{2base}$ (mmHg)	37.7 (4.2)	38.4 (4.8)	40.1 (2.5)	39.0 (2.4)	0.213	0.757	0.160
$\Delta P_{ET}CO_{2max}$ (mmHg)	1.5 (2.3)	1.1 (2.4)	0.8 (0.5)	1.3 (0.9)	0.361	0.895	0.131
HR_{base} (bpm)	64.9 (10.0)	66.2 (10.2)	58.7 (7.5)	59.3 (10.2)	0.791	0.509	*0.020**
ΔHR_{max} (bpm)	5.2 (3.8)	4.7 (3.6)	4.8 (3.5)	4.6 (3.4)	0.785	0.604	0.792

MAP_{base} = baseline MAP (eyes-closed); ΔMAP_{max} = peak relative change in MAP following stimulus onset; $P_{ET}CO_{2base}$ = baseline end-tidal PCO_2 (eyes-closed); $\Delta P_{ET}CO_{2max}$ = peak absolute change in $P_{ET}CO_2$ following stimulus onset; HR_{base} = baseline heart rate (eyes-closed); ΔHR_{max} = peak absolute change in heart rate following stimulus onset; p-values reflect 2-way mixed ANOVA; * indicates significant finding.

6.5 Discussion

In the current study, the influence of repetitive sub-concussive head trauma – as experienced during one season of competitive athletic play – on indices of neurovascular coupling dynamics was examined using a visual stimulation paradigm in contact sport and non-contact sport athletes. It was hypothesized that the timing and amplitude of NVC responses in the posterior cerebral artery would be negatively influenced by repetitive head trauma, as described by five variables: (i) the peak rate of increase in PCAv following stimulus onset ($slope_{max}$); (ii) time between stimulus onset and peak PCAv slope ($t_{slopemax}$); (iii) the peak elevation (v_{max}) and; (iv) time to peak elevation (t_{vmax}) in PCAv; and (v) area-under-the-curve during the first 25 seconds after visual stimulation (Figure 6-1). The results did not support this hypothesis, with no statistically significant effects of group or time detected by two-way mixed ANOVA. Taken together, the results suggest that exposure to repetitive subconcussive trauma over the course of one season induces minimal effects on TCD-derived characteristics of the NVC response following visual stimulation.

6.5.1 Mechanisms underlying the neurovascular coupling response

Cortical activation increases local cerebral metabolism, which requires a coordinated response of the neurovascular unit to modulate resistance within cerebral blood vessels to distribute blood to necessary areas. The dynamics of the NVC response likely involve a multitude of mechanisms, including accumulation of vasoactive metabolic by-products (e.g. CO_2, nitric oxide (NO), adenosine, arachidonic acid metabolites), direct neural control of the cerebrovasculature, astrocyte-mediated vasosignaling, and pericyte-mediated changes in capillary tone (reviewed in: (145,220)). The entire cerebrovascular tree is directly innervated with autonomic fibres, implicating a role for autonomic activity in modulating cerebrovascular resistance and thus CBF (145,225,226). Astrocytes with end-feet that envelope penetrating arterioles are thought to play an important role in the slow component to the NVC response (3-4 seconds), but may serve a limited role in the immediate hyperemic effect (< 1 second) following neural activation (232). Glutamate release from active neurons causes nearby astrocytes to uptake calcium ions and alter arteriolar smooth muscle tone following the release of vasoactive substances (adenosine, nitric oxide, prostaglandins and other arachidonic acid derivatives) (reviewed in: (232)). Inhibitory interneurons are pivotal in the precise regulation of CBF via their interactions with neurons, astrocytes, and microvessels to integrate signals and release vasoactive substances (e.g. NO, acetylcholine, neuropeptide-Y, vasoactive intestinal peptide) (237,238). Gap junctions between vascular smooth muscle cells enable signal propagation and vasoactivity in upsteam pial arterioles (221-223). While the extent to which each of these pathways regulate NVC dynamics in humans remains incompletely understood, multiple overlapping mechanisms exist to modulate cerebrovascular resistance and appropriately distribute CBF following changes in neural activity,

providing multiple avenues by which head trauma may influence NVC responses (220).

6.5.2 Evidence of functional changes following subconcussive trauma

That subconcussive trauma can detrimentally alter brain structure and function has been established. Transient damage to the blood-brain barrier in the form of elevated serum levels of the astrocytic protein S100B has been observed as a function of the number of subconcussive hits in football players (66), while dynamic contrast enhanced MRI showed blood-brain barrier pathology in football players interpreted to result from subconcussive trauma (77). Multiple studies have established an association between subconcussive trauma and changes in white matter diffusion (24-26,65,370,372). Deficits in aspects of cerebrovascular function other than neurovascular coupling have been attributed to repetitive subconcussive trauma (Chapter 4:)(17-19). In the same sample of participants as the current study, impairments in the cerebrovascular response to changes in blood pressure were related to total number of head impacts and cumulative exposure to linear acceleration (Chapter 4:). Furthermore, these changes were related to alterations in sympathovagal balance from pre- to post-season (Chapter 4:). Alterations in CO_2 reactivity in football and soccer players have been associated with cumulative impact exposure and persisted for up to 4-5 months post-season (18,19). Impairments in CO_2 reactivity were related to the volume and intensity of sparring, but not the frequency of knock-outs suffered or number of rounds fought in a sample of professional boxers (17). Cerebral metabolism is affected by repetitive head hits, wherein the total number hits greater than 60 *g* in the week preceding evaluation was associated with alterations in a variety of metabolites; larger blows to the head yielded disrupted energy metabolism and damage to glial cells (70). Relationships with cerebral metabolites suggested high magnitude trauma induces an initial state of

hypermetabolism followed by hypometabolic responses that are influenced by the timing, number, magnitude, and location of hits to the head (70). Similar changes in cerebral metabolites were observed in retired professional soccer players without a history of concussion, suggesting the potential for chronic metabolic disturbances resulting from subconcussive trauma (68).

Multiple recent task-based fMRI studies in high-school football athletes have demonstrated changes in hemodynamic response patterns to cortical activation as a function of head impact exposure (20,22,69,76,268). During in-season assessments, those who performed abnormally on cognitive tests displayed reduced fMRI activity within temporal and occipital cortices, and experienced higher numbers of hits to the top-front of the head relative to subjects performing normally (22). Relative to preseason, scans during periods of the athletic season with high levels of contact revealed greater within-subject variation in visual working memory BOLD signal that tended to normalize after contact was ceased (69). fMRI changes persisting after the conclusion of the season were significantly more common in athletes receiving 50+ hits per week during the season (268); 80% of subjects who sustained > 900 cumulative hits were flagged for abnormal functional findings 2-5 months post-season, whereas those who received < 600 impacts were flagged 52% of the time. Thus, while the timing and magnitude of the NVC response multiple studies have reported changes in cortical activation as a result of subconcussive trauma.

6.5.3 Biomechanical characteristics of subconcussive trauma

The lack of significant findings in the current study may be partly explained by differences in impact exposure from other studies. The average total number of hits per subject in this study (ranging from 5 to 677) was lower than has been reported in most studies (379,384-387), with

only five subjects (four hockey players, one football) sustaining at least 500 hits. In collegiate football players, cumulative impact numbers have averaged well over 500 per player (>14.4 g) (379,385,387). The average linear magnitude of impacts in the present study (Table 2) was higher than reported in other studies (379,384-387), but in line with those that have used a similar threshold (>20 g) for recording valid impacts (363). Nevertheless, subconcussive impacts may incrementally alter cerebral susceptibility to injury, with reports that football players were exposed to a higher magnitude and volume of head impacts on days of diagnosed concussion than on days without concussion (378). Other research has suggested individual playing style – and the influence on the spatial distribution of hits to the head – may influence the changes in hemodynamic responses to cortical activity (76); however, we did not assess relationships between impact location and NVC dynamics in the current study due to the known error of our impact sensors in this estimate (380). Another possible explanation for the observed lack of differences in NVC dynamics could relate to the sensitivity of the chosen outcome variables to the effects of head trauma. However, previous work has demonstrated NVC dynamics in the PCA are altered following acute concussion, including a within-subject delay in achieving peak rate of increase in PCAv (by ~50%) and elevations in the magnitude of the response (by over 30%) compared to preseason values (Chapter 5:). Thus, the chosen outcome parameters are sensitive to head trauma; however, exposure to repetitive trauma as experienced during the current study period did not significantly alter NVC dynamics.

6.5.4 Limitations

The conclusions drawn from this study are subject to multiple limitations. This was a modest sample of contact sport athletes, but was drawn from a local setting of elite male athletes aged

17-24, limiting generalizability to other patient populations including females. Transcranial Doppler ultrasound provides excellent temporal resolution, allowing beat-by-beat analysis of CBF dynamics; however, the spatial resolution of this technique is limited to large cortical territories supplied by the major cerebral arteries that can be reliably insonated. As such, any potential changes in NVC dynamics restricted to focal occipital areas as a result of repetitive head trauma may have been masked. Nevertheless, NVC dynamics in the major upstream feeding vessels were not affected. Furthermore, TCD provides an index of CBF via measurement CBF velocity; this association holds true only so long as the insonated vessel does not appreciably change diameter, which cannot be confirmed, although any influences of CO_2 and blood pressure on vessel diameter are presumed to have been minimal given the relative consistency of these measures during the task (Figures 1 & 2). It is pertinent to note that impact sensors were worn during games only, thereby underestimating true impact burden. The number and magnitude of hits have repeatedly been shown to be higher in games than practices (50,387), and between-subject trends in exposure based on individual playing style and position are likely still captured in the current data set. The error in estimates of impact exposure is compounded by recent reports demonstrating substantial in-vivo overestimation bias using skin-worn impact sensors when compared to mouthguard-based sensors due to non-rigid skull coupling (380). Consistent overestimation of the severity of individual impacts is unlikely to affect the conclusions drawn from the current data.

6.6 Conclusions

There is growing concern over the potential short- and long-term effects of contact sports and repetitive subconcussive head impacts. While deficits in other aspects of cerebrovascular

function have been reported as a consequence of subconcussive trauma, this exploratory study suggests the dynamics of the neurovascular coupling response are unaltered following a single season of contact sport participation. Non-contact sport athletes also exhibited no changes in NVC dynamics. Future prospective cohort studies in a larger number of subjects with greater impact exposure are warranted to confirm the current findings.

Chapter 7: Conclusion.

7.1 Overview

The global motivation underlying my doctoral research was to elucidate physiological changes that occur following sport-related head trauma towards facilitating the development of novel and objective methods to improve the identification, management, and treatment protocols for sport-related head injury. Sport-induced concussion occurs at an alarmingly high rate, affecting millions each year, with estimates of the prevalence in adolescent athletes reaching 10-25% (6,7). Despite vastly increased attention to the issue over recent years, sport-related head injury remains one of the most poorly understood injuries in the fields of sports medicine and neuroscience. Clinical management of concussion is based heavily on resolution of patient-reported symptoms (9), highlighting the pressing need for more objective identification and management techniques. Intentional underreporting of symptoms – in the hopes of avoiding being withdrawn from play, or to expedite return-to-play – is a well-established problem that increases risk for further concussions as well as longer-term consequences associated with repetitive head trauma (8,12-14).

Emerging evidence also suggests a link between repetitive subconcussive head impacts, multiple clinically manifest concussions, and the development of long-term neurodegeneration termed chronic traumatic encephalopathy (15). There is a growing evidence base supporting a link between repetitive subconcussive trauma specifically, and impairments in learning (16), alterations in functional brain connectivity (20-22), changes in gray matter density and volume (23), and altered diffusion in white matter tracts (24-26). Long-lasting effects of sport-related head trauma may include problems with affect regulation, attention, memory, and depression,

though the physiological changes underlying such effects remain poorly understood. It is imperative that we achieve a more thorough understanding of the precise mechanisms by which sport-related head trauma affects the brain.

Though concussions have been traditionally thought to represent a functional disturbance in the brain rather than a structural injury, recent research has challenged this assumption, including data presented in Chapter 2: of this thesis. Myelin is increasingly regarded as an important player in TBI pathophysiology, though its role is poorly understood (83). Clinical deficits following concussion have been associated with regional microstructural white matter changes observed using diffusion tensor imaging (103,104,388-390). However, the inability of this technique to provide information on the underlying reason for changes in water diffusion (e.g. demyelination versus increased membrane permeability versus overlapping axons) (110) has contributed to inconsistent findings across studies (97,99). While various animal models have implicated myelin fragmentation and degradation following traumatic injury (82,95), the initial study in this thesis provides the first direct, *in-vivo* assessment of myelin integrity following sport-related concussion (and mTBI more broadly) in humans. The axonal pathology observed in concussion is thought to evolve over days to weeks following traumatic insult, and this notion is supported by our findings of reductions in myelin water content at 3-days and 2-weeks post-injury. Given the foundational role of myelin towards enhancing the speed and efficiency of signal conduction, these results also support the proposition that demyelination may be responsible for slower interhemispheric transfer times associated with reduced fractional anisotropy (FA) in the corpus callosum of pediatric mTBI patients (108) and might partly explain the cognitive deficits observed post-concussion (e.g. reduced processing speed). Myelin disruptions were observed in

several areas, including the corpus callosum and posterior limb of the internal capsule – tracts known to be involved in the communication of autonomic information. Interestingly, recent animal work has suggested that myelin precursor cells contribute to the integrity of the blood-brain barrier (86); as such, it is possible to the disruptions in myelin content observed in Chapter 2: could also contribute to increased blood-brain barrier permeability that has been observed with head trauma (66,77), though this remains speculative. Myelin content returned to near-preseason levels by 2-months post-injury, suggesting remyelination may occur; however, the potential for disruptions in myelin to outlast clinical recovery raises concern that – under current management protocols – athletes may be returned to play during a period when the brain is not yet recovered structurally or physiologically. Further research is warranted to directly investigate the influence of myelin disruptions on cerebral metabolism, autonomic function, and cerebral susceptibility to injury.

Recent work has also delineated an important role for compromised cerebrovascular function in the disturbances associated with concussion (131). Cerebral metabolism is critically dependent on the regulation of CBF. Concussed patients have consistently exhibited global and regional alterations in CBF (2,43,136,137), with some studies suggesting sport-related head trauma can yield impairments in the mechanisms controlling CBF (17,19,177,179). Although they represent key components in the assessment of cerebrovascular function (150), no studies prior to those presented herein have used techniques with sufficient temporal resolution to explicitly evaluate the dynamics of CA or NVC responses in concussed athletes or in athletes exposed to a high frequency of subconcussive trauma. The aim of Chapters 3-6 was to prospectively determine how these CBF control mechanisms are affected by sport-related concussion and repetitive

subconcussive trauma. To address this question, these two aspects of cerebrovascular function were evaluated using TCD ultrasound in a cohort of individuals with a high likelihood of enduring repetitive head trauma and sustaining concussions – namely, contact sport athletes.

Chapter 3: presents a TCD-based assessment of autoregulatory function following concussion, and revealed delays in the cerebrovascular response to a common blood pressure challenge – change in posture – that persisted for at least 2-weeks and appear to be autonomically-mediated. These deficits were related to poorer performance on a cognitive screening test, but do not appear to be cumulative based on comparison of athletes with a history of 3^{+} concussions to concussion-naïve individuals. Physiologically, cerebral autoregulation is a multiple input system that likely exhibits features of non-stationarity and non-linearity (391). Despite the widespread use of TFA within the CA research community (212), this approach models the MAP-CBF relationship as a linear system with one input and one output. As such, the inherent complexity of autoregulation is not captured within this analysis technique, representing an important limitation of this approach. In effort to maximize the reliabilty of our MAP-CBF characterization, we had participants complete repetitive squat-stand manoeuvres to "linearize" the system and amplify signal-to-noise ratio (210). While validation is still required in a larger sample, the results suggest concussion induces transient disruptions in autonomic cerebrovascular function that may outlast symptom resolution and clinical recovery, highlighting the emerging notion that physiological recovery and clinical recovery may not equate.

Chapter 4: extends from the findings in Chapter 3, and demonstrates impairments in the latency and magnitude of autoregulatory responses following exposure to one season of repetitive

subconcussive trauma during contact sport. The degree to which autoregulatory capacity was impaired was directly related to: i) the total number and cumulative linear magnitude of head impacts experienced during the season, as well as; ii) the relative increase in sympathetic:parasympathetic output observed post-season. Non-contact sport athletes exhibited no such changes in autoregulatory or autonomic function. The clinical relevance of these autoregulatory changes – which also appear to be autonomically mediated – towards rendering the brain more susceptible to injury remains unknown. Furthermore, any cumulative, year-over-year effects are not discernable from this data. Despite multiple published hypotheses that CA disruptions play a pivotal role in the persistence of post-concussion symptoms (131,133,206), these studies provide the first direct, prospective evidence of dysfunction in cerebral autoreglatory behaviour in the context of sport-related head trauma.

In addition to alterations in CO_2 reactivity and cerebral autoregulation, CBF is highly sensitive to cortical activity. In Chapter 5:, alterations in PCA*v* response dynamics following visual stimulation were apparent at 3-days post-injury in concussed athletes. The rise in PCA*v* during the early activation phase – thought to be mediated by autonomic activity (213) – was delayed by over 50% compared to preseason. This increased response latency was accompanied by a >30% increase in the magnitude of the response that was inversely related to the time until medical clearance for return-to-play. Such effects appear to be transient, largely resolving by 2-weeks post-injury, and were not cumulative across multiple concussions in otherwise healthy athletes. These findings add to the growing understanding that acute sport-related concussion exerts detrimental effects on multiple aspects of cerebrovascular function, possibly via effects of autonomic modulation of CBF dynamics.

Despite reported deficits in other aspects of cerebrovascular function following exposure to repetitive subconcussive trauma – including CO_2 reactivity (17-19) and cerebral autoregulation (Chapter 4:) – the data presented in Chapter 6: suggest the dynamics of NVC responses in the PCA are unaltered following exposure to one season of repetitive subconcussive trauma. Non-contact sport athletes also exhibited no change in NVC dynamics. Considered in conjunction with the observed effects of subconcussive trauma on indices of cerebral autoregulation in the same participant pool (Chapter 4:), these results highlight the importance of comprehensive evaluations of cerebrovascular function with the understanding that the three major CBF control mechanisms (CO_2, blood pressure, NVC) reflect different and uncorrelated aspects of cerebrovascular control (158).

Autonomic nervous system dysfunction is increasingly recognized as a major contributing factor to the effects of sport-related head trauma, and this theme resonates throughout the current thesis. Multiple reports have outlined the effects of concussion on cardiac autonomic function, consistently demonstrating reduced heart rate variability in ways that suggest elevated sympathetic and / or reduced parasympathetic outflow – particularly in physically active states – that may persist beyond clinical recovery, (298-306). The results presented in Chapters 3-5 provide the first evidence supporting a role for autonomic dysregulation in the control of CBF in the context of sport-related head trauma. Specifics underlying the autonomic modulation of cerebrovascular resistance in healthy humans, let alone post-concussion, remain poorly defined (190). However, brain injury models suggest a lateralized control of autonomic outflow wherein left insular efferents exert parasympathetic effects and right insular efferents generate

sympathetic responses (351-353). It has been demonstrated that altered white matter microstructure in the corpus callosum and posterior limb of the internal capsule contribute to paroxysmal sympathetic hyperactivity (392). Damage to these regions – both of which exhibited disruptions in myelin integrity in Chapter 2: – could compromise balance between sympathetic and parasympathetic outflow (301). Damage to prefrontal areas within the central autonomic network could further exert a disinhibition of the central nucleus of the amygdala, causing a net increase in sympathetic activity through subsequent disinhibition of sympathoexcitatory neurons in the ventrolateral medulla (354). Increased sympathetic drive has been shown to effect a transient "stiffening" of peripheral blood vessels following concussion (300); if also true in the cerebrovasculature, this would explain the increased latencies in cerebrovascular responses outlined in Chapters 3-5. Reduced compliance in cerebral vessels secondary to trauma-induced autonomic dysregulation could subject the microvasculature to higher mechanical forces as a result of elevated pulsatile flow (393) and potentially contribute to microvascular damage and transient disruptions in blood-brain barrier permeability that have previously been reported (66,77).

Beyond providing a theoretical mechanism for trauma-induced disruptions in cardiac and cerebrovascular function, alterations in autonomic function may have direct clinical implications in the setting of sport-related head trauma. In patients with a history of mTBI, early autonomic dysfunction is thought to contribute to enduring emotional dysfunction, particularly in female patients (296,297). It has also been suggested that autonomic dysfunction may contribute to the increased mortality in patients with a history of mTBI (292-295). In more severe injuries, direct associations have been demonstrated between acute cardiac autonomic dysfunction and high

mortality rates in TBI patients (291). Further research is warranted to determine what contribution, if any, is made by alterations in autonomic behaviour towards the development of long-term neurodegenerative changes and clinical outcomes following a lifetime of contact sport participation.

7.2 Future directions

This thesis describes the effects of head trauma, both concussive and subconcussive, on myelin content in the brain and on mechanisms controlling blood flow to the brain. Though the techniques described in this thesis represent promising avenues for future research with the potential for integration into clinical decision making, the results presented herein provide multiple avenues for future research that have yet to be thoroughly addressed experimentally.

1. **When is it safe for concussed athletes to return-to-play?**

   In addition to the results presented in Chapter 2: and Chapter 3:, other avenues of research continue to document disruptions in physiological function that persist well beyond symptom resolution and clinical recovery (42,44,45). The obvious question that arises pertains to the safety of relying on symptom reports to guide clinical decision-making, and whether physiological recovery need be considered to avoid placing athletes at undue risk by prematurely approving return-to-play. Indeed, there are calls within the field for a shift away from symptom-based assessments of sport-related head trauma towards structured physical examinations rooted in evaluations of physiologic function (46). Clearly, the clinical relevance of various persistent physiological disruptions –

including those reported in this thesis – towards injury susceptibility needs to be established.

2. **What is the role of autonomic dysregulation of cerebrovascular function in patients who develop persistent post-concussion syndrome?**

   While recent research has increasingly suggested the deficits of acute concussion are mediated by alterations in cerebrovascular function, the extent to which these alterations also underlie the clinical manifestations of post-concussion syndrome is unknown. It will be of great clinical value to compare the structural and functional imaging patterns in patients with PCS to those in patients who recover spontaneously and in more typical timeframes. Pioneering work from the group at the University of Buffalo has sought to stratify PCS patients into operational sub-types on physiological grounds, using graded exercise and other clinical tests to evaluate the presence of global physiological dysfunction versus isolated dysfunction of particular neurological sub-systems (133). In their schema, "physiological post-concussion disorder" (PCD) is posited to be mediated by a persistence of the same biochemical, autonomic, and cerebrovascular alterations that characterize acute concussion. Techniques used in the current thesis will enable insight into these mechanisms and not only enhance our understanding of the mechanisms underlying the various proposed subtypes of PCD, but also complement and facilitate the individualized assessment and targeted treatment of these patients based on documented physiological deficits.

3. **What is the potential role for rehabilitating autonomic dysfunction following injury, including the role for exercise?**

The relationship between exercise intolerance and CBF dysregulation post-concussion is poorly understood, but may be mediated by altered autonomic function. As such, autonomic function may represent a potential therapeutic target for intervention and rehabilitation of patients suffering from post-concussion sequelae, similar to the emerging pattern in management of stroke patients (394). Indeed, the suggested mainstay of treatment for physiological PCD is tailored sub-maximal aerobic exercise (133). Evidence suggests exercise therapy may improve autonomic function by increasing vagal tone and decreasing sympathetic activity (reviewed in (290)), potentially through effects on angiotensin II and nitric oxide, both of which have potent effects on CBF. Exercise training has been shown to increase central arterial compliance and consequently reduce pulsatile flow in the middle cerebral artery (393). Along a similar line of reasoning, the potential for direct therapeutic modulation of autonomic activity following concussion is intriguing. Indeed, optimizing parasympathetic nervous system activation has shown neuroprotective effects in preclinical models of stroke – including increased CBF and improved neurogenesis (395) – and has been successful in the treatment of other brain disorders including depression and epilepsy (396,397). Excitotoxicity may be mitigated by vagal nerve stimulation, reducing extracellular glutamate levels within 15-20 minutes after 5 minutes of experimental transient global ischemia (395,398). Numerous autonomic structures are located in an area of the pterygopalatine fossa that is accessible via minimally invasive neuromodulation techniques. Neuromodulation of autonomic

fibres has been suggested as a promising approach in the management of headache disorders, stroke, and cerebral vasospasm (399). One case report has recently been published outlining the successful treatment of refractory chronic posttraumatic headache in a concussed high school football player using sphenopalatine ganglion block (400).

4. **What are the differences, if any, in physiological responses to sport-related head trauma between sexes and across age groups?**

   Perhaps the biggest limitation of this thesis (particularly Chapters 3-6) involves the exclusive examination of males – a problem not limited to the current studies but one that plagues the entire field of research in sport and exercise science (401). Although this outcome was not intended during planning of the current thesis, it will be especially important to evaluate the role of cerebrovascular and autonomic dysfunction in females considering the evidence suggesting females tend to fair more poorly than males with respect to post-concussive symptoms and recovery trajectories (356). Importantly, the physiological underpinnings for such sex-specific differences, and whether the underlying brain pathology is different between men and women with concussion, remain unknown (98). Differential responses to trauma may also occur across age groups, particularly in the developing brains of children who reportedly experience worse symptoms and longer recoveries than their adult counterparts (402,403). Answering these questions will be especially important as children participate in organized sport in greater numbers and at younger ages.

5. **To what extent might the observed deficits in autoregulatory function contribute to the development of long-term neurodegenerative changes such as chronic traumatic encephalopathy?**

   Interestingly, the pathological hallmarks of CTE include perivascular deposition of hyperphosphorylated tau (15); though it remains entirely speculative, trauma-induced impairments in CA would subject the microvasculature to higher mechanical forces as a result of elevated pulsatile flow, conceivably contributing to the blood-brain barrier disruption that has been observed with concussive and subconcussive trauma. Over the course of a long playing career in contact sports, repetitive exposure of the brain to traumatic insults could ostensibly contribute to the pattern of pathology observed in CTE. Large, longitudinal, multi-disciplinary studies are warranted to better elucidate a cause and effect relationship between repetitive head trauma and CTE.

7.3 Conclusion and significance

A better understanding of the pathophysiological underpinnings of concussion and subconcussive trauma is needed to objectively and reliably identify and manage dangerous levels of sport-related head trauma, thereby limiting the burden of sport-related head injuries at an individual and population level. Although not yet appropriate for clinical use, the methods described in this thesis provide tremendous opportunity to further investigate physiological dysfunction following head trauma. In particular, the utility of ultrasound as a portable tool to characterize cerebrovascular function in athletes is by no means exhausted. This thesis provides valuable insights into the capacity of sport-related head trauma to induce structural and functional disturbances in the brains of young adult contact sport athletes, demonstrating

disruptions in both myelin integrity and mechanisms controlling CBF following concussive and subconcussive trauma. That such disruptions persisted in some cases beyond symptom resolution and clinical recovery suggests the need to consider the relevance of clinical versus physiological recovery. All of the deficits observed post-concussion resolved within 1-2 months and did not appear to accumulate across multiple injuries. Large prospective cohort studies using a multidisciplinary approach in subjects representing a variety of contact and non-contact sports are warranted to further delineate the short- and long-term clinical utility of these findings.

Bibliography

1. Thompson J, Sebastianelli W, and Slobounov S. EEG and postural correlates of mild traumatic brain injury in athletes. Neurosci Lett. Ireland; 2005;377(3):158-63.

2. Maugans TA, Farley C, Altaye M, Leach J, and Cecil KM. Pediatric sports-related concussion produces cerebral blood flow alterations. Pediatrics. United States; 2012;129(1):28-37.

3. Williams VB, and Danan IJ. A historical perspective on sports concussion: where we have been and where we are going. Current Pain and Headache Reports. Springer US; 2016;20(6):43.

4. Bryan MA, Rowhani-Rahbar A, Comstock RD, Rivara F, and Seattle Sports Concussion Research Collaborative. Sports- and recreation-related concussions in US youth. Pediatrics. United States; 2016;138(1).

5. Langlois JA, Rutland-Brown W, and Wald MM. The epidemiology and impact of traumatic brain injury: a brief overview. J Head Trauma Rehabil. United States; 2006;21(5):375-8.

6. Echlin PS, Tator CH, Cusimano MD, Cantu RC, Taunton JE, Upshur RE, Hall CR, Johnson AM, Forwell LA, and Skopelja EN. A prospective study of physician-observed concussions during junior ice hockey: implications for incidence rates. Neurosurg Focus. United States; 2010;29(5):E4.

7. Johnson LS. Concussion in youth ice hockey: it's time to break the cycle. CMAJ. Canada; 2011;183(8):921-4.

8. McCrea M, Hammeke T, Olsen G, Leo P, and Guskiewicz K. Unreported concussion in high school football players: implications for prevention. Clin J Sport Med. United States; 2004;14(1):13-7.

9. McCrory P, Meeuwisse WH, Aubry M, Cantu B, Dvorák J, Echemendia RJ, Engebretsen L, Johnston K, Kutcher JS, Raftery M, Sills A, Benson BW, Davis GA, Ellenbogen RG, Guskiewicz K, Herring SA, Iverson GL, Jordan BD, Kissick J, McCrea M, McIntosh AS, Maddocks D, Makdissi M, Purcell L, Putukian M, Schneider K, Tator CH, and Turner M. Consensus statement on concussion in sport: the 4th International Conference on Concussion in Sport held in Zurich, November 2012. Br J Sports Med. England; 2013;47(5):250-8.

10. Canadian Pediatric Society. Evaluation and management of children and adolescents with sports-related concussion. 2012.

11. Guskiewicz KM, McCrea M, Marshall SW, Cantu RC, Randolph C, Barr W, Onate JA, and Kelly JP. Cumulative effects associated with recurrent concussion in collegiate football players: the NCAA Concussion Study. JAMA. United States; 2003;290(19):2549-55.

12. Iverson GL, Brooks BL, Collins MW, and Lovell MR. Tracking neuropsychological recovery following concussion in sport. Brain Inj. England; 2006;20(3):245-52.

13. Gavett BE, Stern RA, and McKee AC. Chronic traumatic encephalopathy: a potential late effect of sport-related concussive and subconcussive head trauma. Clin Sports Med. United States; 2011;30(1):179-88, xi.

14. McKee AC, Cantu RC, Nowinski CJ, Hedley-Whyte ET, Gavett BE, Budson AE, Santini VE, Lee HS, Kubilus CA, and Stern RA. Chronic traumatic encephalopathy in athletes: progressive tauopathy after repetitive head injury. J Neuropathol Exp Neurol. United States; 2009;68(7):709-35.

15. McKee AC, Stein TD, Nowinski CJ, Stern RA, Daneshvar DH, Alvarez VE, Lee HS, Hall G, Wojtowicz SM, Baugh CM, Riley DO, Kubilus CA, Cormier KA, Jacobs MA, Martin BR,

Abraham CR, Ikezu T, Reichard RR, Wolozin BL, Budson AE, Goldstein LE, Kowall NW, and Cantu RC. The spectrum of disease in chronic traumatic encephalopathy. Brain. England; 2013;136(Pt 1):43-64.

16. McAllister TW, Flashman LA, Maerlender A, Greenwald RM, Beckwith JG, Tosteson TD, Crisco JJ, Brolinson PG, Duma SM, Duhaime AC, Grove MR, and Turco JH. Cognitive effects of one season of head impacts in a cohort of collegiate contact sport athletes. Neurology. United States; 2012;78(22):1777-84.
17. Bailey DM, Jones DW, Sinnott A, Brugniaux JV, New KJ, Hodson D, Marley CJ, Smirl JD, Ogoh S, and Ainslie PN. Impaired cerebral haemodynamic function associated with chronic traumatic brain injury in professional boxers. Clin Sci (Lond). England; 2013;124(3):177-89.
18. Svaldi DO, Joshi C, Robinson ME, Shenk TE, Abbas K, Nauman EA, Leverenz LJ, and Talavage TM. Cerebrovascular reactivity alterations in asymptomatic high school football players. Dev Neuropsychol. England; 2015;40(2):80-4.
19. Svaldi DO, McCuen EC, Joshi C, Robinson ME, Nho Y, Hannemann R, Nauman EA, Leverenz LJ, and Talavage TM. Cerebrovascular reactivity changes in asymptomatic female athletes attributable to high school soccer participation. Brain Imaging Behav. 2016;.
20. Breedlove EL, Robinson M, Talavage TM, Morigaki KE, Yoruk U, O'Keefe K, King J, Leverenz LJ, Gilger JW, and Nauman EA. Biomechanical correlates of symptomatic and asymptomatic neurophysiological impairment in high school football. J Biomech. United States; 2012;45(7):1265-72.

21. Johnson B, Neuberger T, Gay M, Hallett M, and Slobounov S. Effects of subconcussive head trauma on the default mode network of the brain. J Neurotrauma. United States; 2014;31(23):1907-13.

22. Talavage TM, Nauman EA, Breedlove EL, Yoruk U, Dye AE, Morigaki KE, Feuer H, and Leverenz LJ. Functionally-detected cognitive impairment in high school football players without clinically-diagnosed concussion. J Neurotrauma. United States; 2014;31(4):327-38.

23. Adams J, Adler CM, Jarvis K, DelBello MP, and Strakowski SM. Evidence of anterior temporal atrophy in college-level soccer players. Clin J Sport Med. United States; 2007;17(4):304-6.

24. Bazarian JJ, Zhu T, Blyth B, Borrino A, and Zhong J. Subject-specific changes in brain white matter on diffusion tensor imaging after sports-related concussion. Magn Reson Imaging. Netherlands; 2012;30(2):171-80.

25. Lipton ML, Kim N, Zimmerman ME, Kim M, Stewart WF, Branch CA, and Lipton RB. Soccer heading is associated with white matter microstructural and cognitive abnormalities. Radiology. United States; 2013;268(3):850-7.

26. McAllister TW, Ford JC, Flashman LA, Maerlender A, Greenwald RM, Beckwith JG, Bolander RP, Tosteson TD, Turco JH, Raman R, and Jain S. Effect of head impacts on diffusivity measures in a cohort of collegiate contact sport athletes. Neurology. United States; 2014;82(1):63-9.

27. McCrory PR, and Berkovic SF. Concussion: the history of clinical and pathophysiological concepts and misconceptions. Neurology. United States; 2001;57(12):2283-9.

28. CNS. Committee on Head Injury Nomenclature, including some definitions of injury to the cervical spine. Clinical Neurosurgery. 1966;12:386-94.

29. Kay T, Harrington DE, Adams R, Anderson T, Berrol S, Cicerone K, Dahlberg C, Gerber D, Goka R, Harley P, Hilt J, Horn L, Lehmkuhl D, and Malec J. Definition of mild traumatic brain injury. J Head Trauma Rehabil. 1993;8(3):86-87.

30. AAP. The management of minor closed head injury in children. Committee on Quality Improvement, American Academy of Pediatrics. Commission on Clinical Policies and Research, American Academy of Family Physicians. Pediatrics. United States; 1999;104(6):1407-15.

31. National Center for Injury Prevention and Control. Report to Congress on mild traumatic brain injury in the United States: Steps to prevent a serious public health problem. Atlanta, GA: CDC; 2003.

32. Carroll LJ, Cassidy JD, Peloso PM, Borg J, von Holst H, Holm L, Paniak C, Pépin M, and WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. Prognosis for mild traumatic brain injury: results of the WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. J Rehabil Med. Sweden; 2004;(43 Suppl):84-105.

33. Tator CH. Let's standardize the definition of concussion and get reliable incidence data. Can J Neurol Sci. England; 2009;36(4):405-6.

34. Cantu RC. Posttraumatic retrograde and anterograde amnesia: pathophysiology and implications in grading and safe return to play. J Athl Train. United States; 2001;36(3):244-248.

35. Colorado Medical Society. Report of the Sports Medicine Committee: Guidelines for the management of concussion in sports (revised). Denver: Colorado Medical Society; 1991.

36. AAN. Practice parameter: the management of concussion in sports (summary statement). Report of the Quality Standards Subcommittee. Neurology. United States; 1997;48(3):581-5.

37. Aubry M, Cantu R, Dvorak J, Graf-Baumann T, Johnston K, Kelly J, Lovell M, McCrory P, Meeuwisse W, Schamasch P, and Concussion in Sport Group. Summary and agreement statement of the First International Conference on Concussion in Sport, Vienna 2001. Recommendations for the improvement of safety and health of athletes who may suffer concussive injuries. Br J Sports Med. England; 2002;36(1):6-10.

38. McCrory P, Johnston K, Meeuwisse W, Aubry M, Cantu R, Dvorak J, Graf-Baumann T, Kelly J, Lovell M, and Schamasch P. Summary and agreement statement of the 2nd International Conference on Concussion in Sport, Prague 2004. Br J Sports Med. England; 2005;39(4):196-204.

39. McCrory P, Meeuwisse W, Johnston K, Dvorak J, Aubry M, Molloy M, and Cantu R. Consensus statement on Concussion in Sport--the 3rd International Conference on Concussion in Sport held in Zurich, November 2008. J Sci Med Sport. Australia; 2009;12(3):340-51.

40. Bailes JE, Petraglia AL, Omalu BI, Nauman E, and Talavage T. Role of subconcussion in repetitive mild traumatic brain injury. J Neurosurg. United States; 2013;119(5):1235-45.

41. Davenport EM, Urban JE, Mokhtari F, Lowther EL, Van Horn JD, Vaughan CG, Gioia GA, Whitlow CT, Stitzel JD, and Maldjian JA. Subconcussive impacts and imaging findings over a season of contact sports. Concussion. Future Medicine; 2016;(00):CNC19.

42. Vagnozzi R, Signoretti S, Tavazzi B, Floris R, Ludovici A, Marziali S, Tarascio G, Amorini AM, Di Pietro V, Delfini R, and Lazzarino G. Temporal window of metabolic brain vulnerability to concussion: a pilot 1H-magnetic resonance spectroscopic study in concussed athletes--part III. Neurosurgery. United States; 2008;62(6):1286-95; discussion 1295-6.

43. Meier TB, Bellgowan PS, Singh R, Kuplicki R, Polanski DW, and Mayer AR. Recovery of cerebral blood flow following sports-related concussion. JAMA Neurol. 2015.

44. Lancaster MA, Olson DV, McCrea MA, Nelson LD, LaRoche AA, and Muftuler LT. Acute white matter changes following sport-related concussion: A serial diffusion tensor and diffusion kurtosis tensor imaging study. Hum Brain Mapp. United States; 2016;37(11):3821-3834.

45. Mutch WA, Ellis MJ, Ryner LN, Morissette MP, Pries PJ, Dufault B, Essig M, Mikulis DJ, Duffin J, and Fisher JA. Longitudinal brain magnetic resonance imaging CO2 stress testing in individual adolescent sports-related concussion patients: a pilot study. Front Neurol. Switzerland; 2016;7:107.

46. Willer BS, and Leddy JJ. Time to change from a symptom-based concussion assessment to a structured physical examination. Acad Emerg Med. United States; 2016;23(4):495-6.

47. Zhang L, Yang KH, and King AI. A proposed injury threshold for mild traumatic brain injury. J Biomech Eng. United States; 2004;126(2):226-36.

48. Guskiewicz KM, and Mihalik JP. Biomechanics of sport concussion: quest for the elusive injury threshold. Exerc Sport Sci Rev. United States; 2011;39(1):4-11.

49. Broglio SP, Schnebel B, Sosnoff JJ, Shin S, Fend X, He X, and Zimmerman J. Biomechanical properties of concussions in high school football. Med Sci Sports Exerc. United States; 2010;42(11):2064-71.

50. Broglio SP, Surma T, and Ashton-Miller JA. High school and collegiate football athlete concussions: a biomechanical review. Ann Biomed Eng. United States; 2012;40(1):37-46.

51. Broglio SP, Eckner JT, Surma T, and Kutcher JS. Post-concussion cognitive declines and symptomatology are not related to concussion biomechanics in high school football players. J Neurotrauma. United States; 2011;28(10):2061-8.

52. Guskiewicz KM, Mihalik JP, Shankar V, Marshall SW, Crowell DH, Oliaro SM, Ciocca MF, and Hooker DN. Measurement of head impacts in collegiate football players: relationship between head impact biomechanics and acute clinical outcome after concussion. Neurosugery. 2007;61(6):1244-1253.

53. Stein TD, Alvarez VE, and McKee AC. Concussion in chronic traumatic encephalopathy. Curr Pain Headache Rep. United States; 2015;19(10):47.

54. Bieniek KF, Ross OA, Cormier KA, Walton RL, Soto-Ortolaza A, Johnston AE, DeSaro P, Boylan KB, Graff-Radford NR, Wszolek ZK, Rademakers R, Boeve BF, McKee AC, and Dickson DW. Chronic traumatic encephalopathy pathology in a neurodegenerative disorders brain bank. Acta Neuropathol. Germany; 2015;130(6):877-89.

55. Huber BR, Alosco ML, Stein TD, and McKee AC. Potential long-term consequences of concussive and subconcussive injury. Phys Med Rehabil Clin N Am. United States; 2016;27(2):503-11.

56. Kawata K, Rubin LH, Lee JH, Sim T, Takahagi M, Szwanki V, Bellamy A, Darvish K, Assari S, Henderer JD, Tierney R, and Langford D. Association of football subconcussive head impacts with ocular near point of convergence. JAMA Ophthalmol. United States; 2016;134(7):763-9.

57. Stamm JM, Koerte IK, Muehlmann M, Pasternak O, Bourlas AP, Baugh CM, Giwerc MY, Zhu A, Coleman MJ, Bouix S, Fritts NG, Martin BM, Chaisson C, McClean MD, Lin AP, Cantu RC, Tripodis Y, Stern RA, and Shenton ME. Age at first exposure to football Is

associated with altered corpus callosum white matter microstructure in former professional football players. J Neurotrauma. United States; 2015;32(22):1768-76.

58. Stamm JM, Bourlas AP, Baugh CM, Fritts NG, Daneshvar DH, Martin BM, McClean MD, Tripodis Y, and Stern RA. Age of first exposure to football and later-life cognitive impairment in former NFL players. Neurology. United States; 2015;84(11):1114-20.

59. Montenigro PH, Bernick C, and Cantu RC. Clinical features of repetitive traumatic brain injury and chronic traumatic encephalopathy. Brain Pathol. Switzerland; 2015;25(3):304-17.

60. Gysland SM, Mihalik JP, Register-Mihalik JK, Trulock SC, Shields EW, and Guskiewicz KM. The relationship between subconcussive impacts and concussion history on clinical measures of neurologic function in collegiate football players. Ann Biomed Eng. United States; 2012;40(1):14-22.

61. Guskiewicz KM, Marshall SW, Bailes J, McCrea M, Cantu RC, Randolph C, and Jordan BD. Association between recurrent concussion and late-life cognitive impairment in retired professional football players. Neurosurgery. United States; 2005;57(4):719-26; discussion 719-26.

62. Guskiewicz KM, Marshall SW, Bailes J, McCrea M, Harding HP, Matthews A, Mihalik JR, and Cantu RC. Recurrent concussion and risk of depression in retired professional football players. Med Sci Sports Exerc. United States; 2007;39(6):903-9.

63. Singh R, Meier TB, Kuplicki R, Savitz J, Mukai I, Cavanagh L, Allen T, Teague TK, Nerio C, Polanski D, and Bellgowan PS. Relationship of collegiate football experience and concussion with hippocampal volume and cognitive outcomes. JAMA. United States; 2014;311(18):1883-8.

64. De Beaumont L, Lassonde M, Leclerc S, and Théoret H. Long-term and cumulative effects of sports concussion on motor cortex inhibition. Neurosurgery. United States; 2007;61(2):329-36; discussion 336-7.

65. Koerte IK, Ertl-Wagner B, Reiser M, Zafonte R, and Shenton ME. White matter integrity in the brains of professional soccer players without a symptomatic concussion. JAMA. United States; 2012;308(18):1859-61.

66. Marchi N, Bazarian JJ, Puvenna V, Janigro M, Ghosh C, Zhong J, Zhu T, Blackman E, Stewart D, Ellis J, Butler R, and Janigro D. Consequences of repeated blood-brain barrier disruption in football players. PLoS One. United States; 2013;8(3):e56805.

67. Mayer AR, Bellgowan PS, and Hanlon FM. Functional magnetic resonance imaging of mild traumatic brain injury. Neurosci Biobehav Rev. United States; 2015;49:8-18.

68. Koerte IK, Lin AP, Muehlmann M, Merugumala S, Liao H, Starr T, Kaufmann D, Mayinger M, Steffinger D, Fisch B, Karch S, Heinen F, Ertl-Wagner B, Reiser M, Stern RA, Zafonte R, and Shenton ME. Altered neurochemistry in former professional soccer players without a history of concussion. J Neurotrauma. United States; 2015;32(17):1287-93.

69. Shenk TE, Robinson ME, Svaldi DO, Abbas K, Breedlove KM, Leverenz LJ, Nauman EA, and Talavage TM. FMRI of visual working memory in high school football players. Dev Neuropsychol. England; 2015;40(2):63-8.

70. Poole VN, Breedlove EL, Shenk TE, Abbas K, Robinson ME, Leverenz LJ, Nauman EA, Dydak U, and Talavage TM. Sub-concussive hit characteristics predict deviant brain metabolism in football athletes. Dev Neuropsychol. England; 2015;40(1):12-7.

71. Abbas K, Shenk TE, Poole VN, Breedlove EL, Leverenz LJ, Nauman EA, Talavage TM, and Robinson ME. Alteration of default mode network in high school football athletes due to

repetitive subconcussive mild traumatic brain injury: a resting-state functional magnetic resonance imaging study. Brain Connect. United States; 2015;5(2):91-101.

72. Montenigro PH, Alosco ML, Martin BM, Daneshvar DH, Mez J, Chaisson CE, Nowinski CJ, Au R, McKee AC, Cantu RC, McClean MD, Stern RA, and Tripodis Y. Cumulative head impact exposure predicts later-life depression, apathy, executive dysfunction, and cognitive impairment in former high school and college football players. J Neurotrauma. United States; 2016;.

73. Slemmer JE, and Weber JT. The extent of damage following repeated injury to cultured hippocampal cells is dependent on the severity of insult and inter-injury interval. Neurobiol Dis. United States; 2005;18(3):421-31.

74. Davenport EM, Whitlow CT, Urban JE, Espeland MA, Jung Y, Rosenbaum DA, Gioia GA, Powers AK, Stitzel JD, and Maldjian JA. Abnormal white matter integrity related to head impact exposure in a season of high school varsity football. J Neurotrauma. United States; 2014;31(19):1617-24.

75. Goldstein LE, Fisher AM, Tagge CA, Zhang XL, Velisek L, Sullivan JA, Upreti C, Kracht JM, Ericsson M, Wojnarowicz MW, Goletiani CJ, Maglakelidze GM, Casey N, Moncaster JA, Minaeva O, Moir RD, Nowinski CJ, Stern RA, Cantu RC, Geiling J, Blusztajn JK, Wolozin BL, Ikezu T, Stein TD, Budson AE, Kowall NW, Chargin D, Sharon A, Saman S, Hall GF, Moss WC, Cleveland RO, Tanzi RE, Stanton PK, and McKee AC. Chronic traumatic encephalopathy in blast-exposed military veterans and a blast neurotrauma mouse model. Sci Transl Med. United States; 2012;4(134):134ra60.

76. Robinson ME, Shenk TE, Breedlove EL, Leverenz LJ, Nauman EA, and Talavage TM. The role of location of subconcussive head impacts in FMRI brain activation change. Dev Neuropsychol. England; 2015;40(2):74-9.

77. Weissberg I, Veksler R, Kamintsky L, Saar-Ashkenazy R, Milikovsky DZ, Shelef I, and Friedman A. Imaging blood-brain barrier dysfunction in football players. JAMA Neurol. United States; 2014;71(11):1453-5.

78. Smith DH, and Meaney DF. Axonal damage in traumatic brain injury. Neuroscientist. 2000;6(6):483-495.

79. Giza CC, and Hovda DA. The new neurometabolic cascade of concussion. Neurosurgery. United States; 2014;75 Suppl 4:S24-33.

80. Christman CW, Grady MS, Walker SA, Holloway KL, and Povlishock JT. Ultrastructural studies of diffuse axonal injury in humans. J Neurotrauma. United States; 1994;11(2):173-86.

81. Maxwell WL, Bartlett E, and Morgan H. Wallerian degeneration in the optic nerve stretch-injury model of traumatic brain injury: a stereological analysis. J Neurotrauma. 2015.

82. Mierzwa AJ, Marion CM, Sullivan GM, McDaniel DP, and Armstrong RC. Components of myelin damage and repair in the progression of white matter pathology after mild traumatic brain injury. J Neuropathol Exp Neurol. United States; 2015;74(3):218-32.

83. Armstrong RC, Mierzwa AJ, Marion CM, and Sullivan GM. White matter involvement after TBI: Clues to axon and myelin repair capacity. Exp Neurol. 2015.

84. Shi H, Hu X, Leak RK, Shi Y, An C, Suenaga J, Chen J, and Gao Y. Demyelination as a rational therapeutic target for ischemic or traumatic brain injury. Exp Neurol. 2015.

85. Neishabouri AM, and Faisal AA. The metabolic efficiency of myelinated vs unmyelinated axons. BMC Neurosci. Springer Science + Business Media; 2011;12(Suppl 1):P100.

86. Seo JH, Maki T, Maeda M, Miyamoto N, Liang AC, Hayakawa K, Pham LD, Suwa F, Taguchi A, Matsuyama T, Ihara M, Kim KW, Lo EH, and Arai K. Oligodendrocyte precursor cells support blood-brain barrier integrity via TGF-β signaling. PLoS One. United States; 2014;9(7):e103174.

87. Benarroch EE. Oligodendrocytes: Susceptibility to injury and involvement in neurologic disease. Neurology. United States; 2009;72(20):1779-85.

88. Tsunoda I, and Fujinami RS. Inside-Out versus Outside-In models for virus induced demyelination: axonal damage triggering demyelination. Springer Semin Immunopathol. Germany; 2002;24(2):105-25.

89. Bailes JE, Dashnaw ML, Petraglia AL, and Turner RC. Cumulative effects of repetitive mild traumatic brain injury. Prog Neurol Surg. Switzerland; 2014;28:50-62.

90. Ng HK, Mahaliyana RD, and Poon WS. The pathological spectrum of diffuse axonal injury in blunt head trauma: assessment with axon and myelin strains. Clin Neurol Neurosurg. NETHERLANDS; 1994;96(1):24-31.

91. Tang-Schomer MD, Johnson VE, Baas PW, Stewart W, and Smith DH. Partial interruption of axonal transport due to microtubule breakage accounts for the formation of periodic varicosities after traumatic axonal injury. Exp Neurol. United States; 2012;233(1):364-72.

92. Miyamoto N, Maki T, Pham LD, Hayakawa K, Seo JH, Mandeville ET, Mandeville JB, Kim KW, Lo EH, and Arai K. Oxidative stress interferes with white matter renewal after prolonged cerebral hypoperfusion in mice. Stroke. United States; 2013;44(12):3516-21.

93. Flygt J, Djupsjö A, Lenne F, and Marklund N. Myelin loss and oligodendrocyte pathology in white matter tracts following traumatic brain injury in the rat. Eur J Neurosci. France; 2013;38(1):2153-65.

94. Chatelin S, Deck C, Renard F, Kremer S, Heinrich C, Armspach JP, and Willinger R. Computation of axonal elongation in head trauma finite element simulation. J Mech Behav Biomed Mater. Netherlands; 2011;4(8):1905-19.

95. Sullivan GM, Mierzwa AJ, Kijpaisalratana N, Tang H, Wang Y, Song SK, Selwyn R, and Armstrong RC. Oligodendrocyte lineage and subventricular zone response to traumatic axonal injury in the corpus callosum. J Neuropathol Exp Neurol. United States; 2013;72(12):1106-25.

96. Beaulieu C. The basis of anisotropic water diffusion in the nervous system - a technical review. NMR Biomed. England; 2002;15(7-8):435-55.

97. Shenton ME, Hamoda HM, Schneiderman JS, Bouix S, Pasternak O, Rathi Y, Vu MA, Purohit MP, Helmer K, Koerte I, Lin AP, Westin CF, Kikinis R, Kubicki M, Stern RA, and Zafonte R. A review of magnetic resonance imaging and diffusion tensor imaging findings in mild traumatic brain injury. Brain Imaging Behav. United States; 2012;6(2):137-92.

98. Chong CD, and Schwedt TJ. White matter damage and brain network alterations in concussed patients: a review of recent diffusion tensor imaging and resting-state functional connectivity data. Curr Pain Headache Rep. United States; 2015;19(5):485.

99. Gardner A, Kay-Lambkin F, Stanwell P, Donnelly J, Williams WH, Hiles A, Schofield P, Levi C, and Jones DK. A systematic review of diffusion tensor imaging findings in sports-related concussion. J Neurotrauma. United States; 2012;29(16):2521-38.

100. Kraus MF, Susmaras T, Caughlin BP, Walker CJ, Sweeney JA, and Little DM. White matter integrity and cognition in chronic traumatic brain injury: a diffusion tensor imaging study. Brain. England; 2007;130(Pt 10):2508-19.

101. Niogi SN, Mukherjee P, Ghajar J, Johnson CE, Kolster R, Lee H, Suh M, Zimmerman RD, Manley GT, and McCandliss BD. Structural dissociation of attentional control and memory in adults with and without mild traumatic brain injury. Brain. England; 2008;131(Pt 12):3209-21.

102. Lipton ML, Gulko E, Zimmerman ME, Friedman BW, Kim M, Gellella E, Gold T, Shifteh K, Ardekani BA, and Branch CA. Diffusion-tensor imaging implicates prefrontal axonal injury in executive function impairment following very mild traumatic brain injury. Radiology. United States; 2009;252(3):816-24.

103. Virji-Babul N, Borich MR, Makan N, Moore T, Frew K, Emery CA, and Boyd LA. Diffusion tensor imaging of sports-related concussion in adolescents. Pediatr Neurol. United States; 2013;48(1):24-9.

104. Henry LC, Tremblay J, Tremblay S, Lee A, Brun C, Lepore N, Theoret H, Ellemberg D, and Lassonde M. Acute and chronic changes in diffusivity measures after sports concussion. J Neurotrauma. United States; 2011;28(10):2049-59.

105. Lo C, Shifteh K, Gold T, Bello JA, and Lipton ML. Diffusion tensor imaging abnormalities in patients with mild traumatic brain injury and neurocognitive impairment. J Comput Assist Tomogr. United States; 2009;33(2):293-7.

106. Zhang K, Johnson B, Pennell D, Ray W, Sebastianelli W, and Slobounov S. Are functional deficits in concussed individuals consistent with white matter structural alterations: combined FMRI & DTI study. Exp Brain Res. Germany; 2010;204(1):57-70.

107. Lange RT, Iverson GL, Brubacher JR, Mädler B, and Heran MK. Diffusion tensor imaging findings are not strongly associated with postconcussional disorder 2 months following mild traumatic brain injury. J Head Trauma Rehabil. United States; 2012;27(3):188-98.

108. Dennis EL, Ellis MU, Marion SD, Jin Y, Moran L, Olsen A, Kernan C, Babikian T, Mink R, Babbitt C, Johnson J, Giza CC, Thompson PM, and Asarnow RF. Callosal function in pediatric traumatic brain injury linked to disrupted white matter integrity. J Neurosci. United States; 2015;35(28):10202-11.

109. Hill RA. Do short-term changes in white matter structure indicate learning-induced myelin plasticity? J Neurosci. United States; 2013;33(50):19393-5.

110. Jones DK, Knösche TR, and Turner R. White matter integrity, fiber count, and other fallacies: the do's and don'ts of diffusion MRI. Neuroimage. United States; 2013;73:239-54.

111. Laule C, Leung E, Li DK, Traboulsee AL, Paty DW, MacKay AL, and Moore GR. Myelin water imaging in multiple sclerosis: quantitative correlations with histopathology. Multiple Sclerosis. SAGE Publications; 2006;12(6):747-753.

112. Laule C, Vavasour IM, Kolind SH, Li DK, Traboulsee TL, Moore GR, and MacKay AL. Magnetic resonance imaging of myelin. Neurotherapeutics. United States; 2007;4(3):460-84.

113. Kozlowski P, Liu J, Yung AC, and Tetzlaff W. High-resolution myelin water measurements in rat spinal cord. Magn Reson Med. United States; 2008;59(4):796-802.

114. MacKay AL, and Laule C. Magnetic resonance of myelin water: an in vivo marker for myelin. Brain Plasticity. IOS Press; 2016;2(1):71-91.

115. Prasloski T, Rauscher A, MacKay AL, Hodgson M, Vavasour IM, Laule C, and Mädler B. Rapid whole cerebrum myelin water imaging using a 3D GRASE sequence. Neuroimage. United States; 2012;63(1):533-9.

116. Stewart WA, MacKay AL, Whittall KP, Moore GR, and Paty DW. Spin-spin relaxation in experimental allergic encephalomyelitis. Analysis of CPMG data using a non-linear least squares method and linear inverse theory. Magn Reson Med. United States; 1993;29(6):767-75.

117. Gareau PJ, Rutt BK, Karlik SJ, and Mitchell JR. Magnetization transfer and multicomponent T2 relaxation measurements with histopathologic correlation in an experimental model of MS. J Magn Reson Imaging. United States; 2000;11(6):586-95.

118. Stanisz GJ, Webb S, Munro CA, Pun T, and Midha R. MR properties of excised neural tissue following experimentally induced inflammation. Magn Reson Med. United States; 2004;51(3):473-9.

119. Webb S, Munro CA, Midha R, and Stanisz GJ. Is multicomponent T2 a good measure of myelin content in peripheral nerve? Magn Reson Med. United States; 2003;49(4):638-45.

120. Meyers SM, Laule C, Vavasour IM, Kolind SH, Mädler B, Tam R, Traboulsee AL, Lee J, Li DK, and MacKay AL. Reproducibility of myelin water fraction analysis: a comparison of region of interest and voxel-based analysis methods. Magn Reson Imaging. Netherlands; 2009;27(8):1096-103.

121. Meyers SM, Vavasour IM, Mädler B, Harris T, Fu E, Li DK, Traboulsee AL, MacKay AL, and Laule C. Multicenter measurements of myelin water fraction and geometric mean T2 : intra- and intersite reproducibility. J Magn Reson Imaging. United States; 2013;38(6):1445-53.

122. Liang AL, Vavasour IM, Mädler B, Traboulsee AL, Lang DJ, Li DK, MacKay AL, and Laule C. Short-term stability of T1 and T2 relaxation measures in multiple sclerosis normal appearing white matter. J Neurol. Germany; 2012;259(6):1151-8.

123. Tozer DJ, Davies GR, Altmann DR, Miller DH, and Tofts PS. Correlation of apparent myelin measures obtained in multiple sclerosis patients and controls from magnetization transfer and multicompartmental T2 analysis. Magn Reson Med. United States; 2005;53(6):1415-22.

124. Kolind S, Matthews L, Johansen-Berg H, Leite MI, Williams SC, Deoni S, and Palace J. Myelin water imaging reflects clinical variability in multiple sclerosis. Neuroimage. United States; 2012;60(1):263-70.

125. Vavasour IM, Li DK, Laule C, Traboulsee AL, Moore GR, and Mackay AL. Multi-parametric MR assessment of T(1) black holes in multiple sclerosis : evidence that myelin loss is not greater in hypointense versus isointense T(1) lesions. J Neurol. Germany; 2007;254(12):1653-9.

126. Faizy TD, Thaler C, Kumar D, Sedlacik J, Broocks G, Grosser M, Stellmann JP, Heesen C, Fiehler J, and Siemonsen S. Heterogeneity of multiple sclerosis lesions in multislice myelin water imaging. PLoS One. United States; 2016;11(3):e0151496.

127. Vavasour IM, Laule C, Li DK, Oger J, Moore GR, Traboulsee A, and MacKay AL. Longitudinal changes in myelin water fraction in two MS patients with active disease. J Neurol Sci. Netherlands; 2009;276(1-2):49-53.

128. Manogaran P, Vavasour I, Borich M, Kolind SH, Lange AP, Rauscher A, Boyd L, Li DK, and Traboulsee A. Corticospinal tract integrity measured using transcranial magnetic stimulation and magnetic resonance imaging in neuromyelitis optica and multiple sclerosis. Mult Scler. England; 2016;22(1):43-50.

129. Deoni SC, Zinkstok JR, Daly E, Ecker C, Williams SC, and Murphy DG. White-matter relaxation time and myelin water fraction differences in young adults with autism. Psychol Med. England; 2015;45(4):795-805.

130. Borich MR, Mackay AL, Vavasour IM, Rauscher A, and Boyd LA. Evaluation of white matter myelin water fraction in chronic stroke. Neuroimage Clin. Netherlands; 2013;2:569-80.

131. Tan CO, Meehan WP, Iverson GL, and Taylor JA. Cerebrovascular regulation, exercise, and mild traumatic brain injury. Neurology. United States; 2014;83(18):1665-72.

132. Ellis MJ, Ryner LN, Sobczyk O, Fierstra J, Mikulis DJ, Fisher JA, Duffin J, and Mutch WA. Neuroimaging assessment of cerebrovascular reactivity in concussion: current concepts, methodological considerations, and review of the literature. Front Neurol. Switzerland; 2016;7:61.

133. Ellis MJ, Leddy J, and Willer B. Multi-disciplinary management of athletes with post-concussion syndrome: an evolving pathophysiological approach. Front Neurol. Switzerland; 2016;7:136.

134. Dienel GA, and Hertz L. Glucose and lactate metabolism during brain activation. J Neurosci Res. United States; 2001;66(5):824-38.

135. Brown CN, Guskiewicz KM, and Bleiberg J. Athlete characteristics and outcome scores for computerized neuropsychological assessment: a preliminary analysis. J Athl Train. United States; 2007;42(4):515-23.

136. Wang Y, West JD, Bailey JN, Westfall DR, Xiao H, Arnold TW, Kersey PA, Saykin AJ, and McDonald BC. Decreased cerebral blood flow in chronic pediatric mild TBI: an MRI perfusion study. Dev Neuropsychol. England; 2015;40(1):40-4.

137. Barlow K, Marcil LD, Carlson HL, Dewey D, MacMaster FP, Brooks BL, and Lebel M. Cerebral perfusion changes in post-concussion syndrome: a prospective controlled cohort study. J Neurotrauma. United States; 2016.

138. Doshi H, Wiseman N, Liu J, Wang W, Welch RD, O'Neil BJ, Zuk C, Wang X, Mika V, Szaflarski JP, Haacke EM, and Kou Z. Cerebral hemodynamic changes of mild traumatic brain injury at the acute stage. PLoS One. United States; 2015;10(2):e0118061.

139. Becelewski J, and Pierzchała K. [Cerebrovascular reactivity in patients with mild head injury]. Neurol Neurochir Pol. Poland; 2003;37(2):339-50.

140. Mandera M, Larysz D, and Wojtacha M. Changes in cerebral hemodynamics assessed by transcranial Doppler ultrasonography in children after head injury. Childs Nerv Syst. Germany; 2002;18(3-4):124-8.

141. Wang Y, Nelson LD, LaRoche AA, Pfaller AY, Nencka AS, Koch KM, and McCrea MA. Cerebral blood flow alterations in acute sport-related concussion. J Neurotrauma. 2015.

142. Ge Y, Patel MB, Chen Q, Grossman EJ, Zhang K, Miles L, Babb JS, Reaume J, and Grossman RI. Assessment of thalamic perfusion in patients with mild traumatic brain injury by true FISP arterial spin labeling MR imaging at 3T. Brain Inj. England; 2009;23(7):666-74.

143. Liu W, Wang B, Wolfowitz R, Yeh PH, Nathan DE, Graner J, Tang H, Pan H, Harper J, Pham D, Oakes TR, French LM, and Riedy G. Perfusion deficits in patients with mild traumatic brain injury characterized by dynamic susceptibility contrast MRI. NMR Biomed. England; 2013;26(6):651-63.

144. Churchill N, Hutchison M, Richards D, Leung G, Graham S, and Schweizer TA. Brain structure and function associated with a history of sport concussion: a multi-modal magnetic resonance imaging study. J Neurotrauma. United States; 2016.

145. Willie CK, Tzeng YC, Fisher JA, and Ainslie PN. Integrative regulation of human brain blood flow. J Physiol. England; 2014;592(Pt 5):841-59.

146. Lin CM, Tseng YC, Hsu HL, Chen CJ, Chen DY, Yan FX, and Chiu WT. Arterial spin labeling perfusion study in the patients with subacute mild traumatic brain injury. PLoS One. United States; 2016;11(2):e0149109.

147. Peng SP, Li YN, Liu J, Wang ZY, Zhang ZS, Zhou SK, Tao FX, and Zhang ZX. Pulsed arterial spin labeling effectively and dynamically observes changes in cerebral blood flow after mild traumatic brain injury. Neural Regen Res. India; 2016;11(2):257-61.

148. Chan ST, Evans KC, Rosen BR, Song TY, and Kwong KK. A case study of magnetic resonance imaging of cerebrovascular reactivity: a powerful imaging marker for mild traumatic brain injury. Brain Inj. England; 2015;29(3):403-7.

149. Mutch WA, Ellis MJ, Ryner LN, Ruth Graham M, Dufault B, Gregson B, Hall T, Bunge M, Essig M, Fisher JA, Duffin J, Mikulis DJ, and for The University Health Network Cerebrovascular Reactivity Research Group. Brain magnetic resonance imaging CO2 stress testing in adolescent postconcussion syndrome. J Neurosurg. United States; 2016;125(3):648-60.

150. Willie CK, Colino FL, Bailey DM, Tzeng YC, Binsted G, Jones LW, Haykowsky MJ, Bellapart J, Ogoh S, Smith KJ, Smirl JD, Day TA, Lucas SJ, Eller LK, and Ainslie PN. Utility of transcranial Doppler ultrasound for the integrative assessment of cerebrovascular function. J Neurosci Methods. Netherlands; 2011;196(2):221-37.

151. Cartwright MS, Dupuis JE, Bargoil JM, and Foster DC. Can a combination of ultrasonographic parameters accurately evaluate concussion and guide return-to-play decisions? Med Hypotheses. United States; 2015;85(3):262-5.

152. Johnson MH, Thorisson HM, and Diluna ML. Vascular anatomy: the head, neck, and skull base. Neurosurg Clin N Am. United States; 2009;20(3):239-58.

153. MacDonald ME, and Frayne R. Cerebrovascular MRI: a review of state-of-the-art approaches, methods and techniques. NMR Biomed. England; 2015;28(7):767-91.

154. Tsivgoulis G, Alexandrov AV, and Sloan MA. Advances in transcranial Doppler ultrasonography. Curr Neurol Neurosci Rep. United States; 2009;9(1):46-54.

155. Phillips AA, Krassioukov AV, Zheng MM, and Warburton DE. Neurovascular coupling of the posterior cerebral artery in spinal cord injury: a pilot study. Brain Sci. Switzerland; 2013;3(2):781-9.

156. Phillips AA, Warburton DE, Ainslie PN, and Krassioukov AV. Regional neurovascular coupling and cognitive performance in those with low blood pressure secondary to high-level spinal cord injury: improved by alpha-1 agonist midodrine hydrochloride. J Cereb Blood Flow Metab. United States; 2014;34(5):794-801.

157. Claassen JA, and Zhang R. Cerebral autoregulation in Alzheimer's disease. J Cereb Blood Flow Metab. United States; 2011;31(7):1572-7.

158. Madureira J, Castro P, and Azevedo E. Demographic and systemic hemodynamic influences in mechanisms of cerebrovascular regulation in healthy adults. J Stroke Cerebrovasc Dis. United States; 2016.

159. Aries MJ, Elting JW, De Keyser J, Kremer BP, and Vroomen PC. Cerebral autoregulation in stroke: a review of transcranial Doppler studies. Stroke. United States; 2010;41(11):2697-704.

160. Budohoski KP, Reinhard M, Aries MJ, Czosnyka Z, Smielewski P, Pickard JD, Kirkpatrick PJ, and Czosnyka M. Monitoring cerebral autoregulation after head injury. Which component of transcranial Doppler flow velocity is optimal? Neurocrit Care. United States; 2012;17(2):211-8.

161. Ainslie PN, Ashmead JC, Ide K, Morgan BJ, and Poulin MJ. Differential responses to CO2 and sympathetic stimulation in the cerebral and femoral circulations in humans. J Physiol. England; 2005;566(Pt 2):613-24.

162. Willie CK, Macleod DB, Shaw AD, Smith KJ, Tzeng YC, Eves ND, Ikeda K, Graham J, Lewis NC, Day TA, and Ainslie PN. Regional brain blood flow in man during acute changes in arterial blood gases. J Physiol. England; 2012;590(Pt 14):3261-75.

163. Wolff HG, and Lennox WG. Cerebral Circulation. XII. The effect on pial vessels of variations in the oxygen and carbon dioxide content of the blood. Arch Neuro Psychiat. 1930;23(6):1097-1129.

164. Binks AP, Cunningham VJ, Adams L, and Banzett RB. Gray matter blood flow change is unevenly distributed during moderate isocapnic hypoxia in humans. J Appl Physiol (1985). United States; 2008;104(1):212-7.

165. Lambertsen CJ, Semple SJ, Smyth MG, and Gelfand R. H and pCO2 as chemical factors in respiratory and cerebral circulatory control. J Appl Physiol. United States; 1961;16:473-84.

166. Kontos HA, Raper AJ, and Patterson JL. Analysis of vasoactivity of local pH, PCO2 and bicarbonate on pial vessels. Stroke. United States; 1977;8(3):358-60.

167. Kontos HA, Wei EP, Raper AJ, and Patterson JL. Local mechanism of CO2 action of cat pial arterioles. Stroke. United States; 1977;8(2):226-9.

168. Ainslie PN, and Duffin J. Integration of cerebrovascular CO2 reactivity and chemoreflex control of breathing: mechanisms of regulation, measurement, and interpretation. Am J Physiol Regul Integr Comp Physiol. United States; 2009;296(5):R1473-95.

169. Ide K, Eliasziw M, and Poulin MJ. Relationship between middle cerebral artery blood velocity and end-tidal PCO2 in the hypocapnic-hypercapnic range in humans. J Appl Physiol (1985). United States; 2003;95(1):129-37.

170. Kety SS, and Schmidt CF. The effects of altered arterial tensions of carbon dioxide and oxygen on cerebral blood flow and cerebral oxygen consumption of normal young men. J Clin Invest. United States; 1948;27(4):484-92.

171. Coverdale NS, Gati JS, Opalevych O, Perrotta A, and Shoemaker JK. Cerebral blood flow velocity underestimates cerebral blood flow during modest hypercapnia and hypocapnia. J Appl Physiol (1985). United States; 2014;117(10):1090-6.

172. Mandell DM, Han JS, Poublanc J, Crawley AP, Kassner A, Fisher JA, and Mikulis DJ. Selective reduction of blood flow to white matter during hypercapnia corresponds with leukoaraiosis. Stroke. United States; 2008;39(7):1993-8.

173. Nöth U, Kotajima F, Deichmann R, Turner R, and Corfield DR. Mapping of the cerebral vascular response to hypoxia and hypercapnia using quantitative perfusion MRI at 3 T. NMR Biomed. England; 2008;21(5):464-72.

174. Mardimae A, Balaban DY, Machina MA, Battisti-Charbonney A, Han JS, Katznelson R, Minkovich LL, Fedorko L, Murphy PM, Wasowicz M, Naughton F, Meineri M, Fisher JA,

and Duffin J. The interaction of carbon dioxide and hypoxia in the control of cerebral blood flow. Pflugers Arch. Germany; 2012;464(4):345-51.

175. Gardner AJ, Tan CO, Ainslie PN, van Donkelaar P, Stanwell P, Levi CR, and Iverson GL. Cerebrovascular reactivity assessed by transcranial Doppler ultrasound in sport-related concussion: a systematic review. Br J Sports Med. 2015;49(16):1050-5.

176. Len TK, and Neary JP. Cerebrovascular pathophysiology following mild traumatic brain injury. Clin Physiol Funct Imaging. England; 2011;31(2):85-93.

177. Len TK, Neary JP, Asmundson GJ, Candow DG, Goodman DG, Bjornson B, and Bhambhani YN. Serial monitoring of CO2 reactivity following sport concussion using hypocapnia and hypercapnia. Brain Inj. England; 2013;27(3):346-53.

178. Mutch WA, Ellis MJ, Graham MR, Wourms V, Raban R, Fisher JA, Mikulis D, Leiter J, and Ryner L. Brain MRI CO2 stress testing: a pilot study in patients with concussion. PLoS One. United States; 2014;9(7):e102181.

179. Militana AR, Donahue MJ, Sills AK, Solomon GS, Gregory AJ, Strother MK, and Morgan VL. Alterations in default-mode network connectivity may be influenced by cerebrovascular changes within 1 week of sports related concussion in college varsity athletes: a pilot study. Brain Imaging Behav. 2015;.

180. Bayliss WM, Hill L, and Gulland GL. On Intra-cranial pressure and the cerebral circulation: Part I. Physiological; Part II. Histological. J Physiol. England; 1895;18(4):334-62.

181. Lassen NA. Cerebral blood flow and oxygen consumption in man. Physiol Rev. United States; 1959;39(2):183-238.

182. Paulson OB, Strandgaard S, and Edvinsson L. Cerebral autoregulation. Cerebrovasc Brain Metab Rev. United States; 1990;2(2):161-92.

183. Faraci FM, Heistad DD, and Mayhan WG. Role of large arteries in regulation of blood flow to brain stem in cats. J Physiol. England; 1987;387:115-23.

184. Heistad DD, Marcus ML, and Abboud FM. Role of large arteries in regulation of cerebral blood flow in dogs. J Clin Invest. United States; 1978;62(4):761-8.

185. Kontos HA, Wei EP, Navari RM, Levasseur JE, Rosenblum WI, and Patterson JL. Responses of cerebral arteries and arterioles to acute hypotension and hypertension. Am J Physiol. United States; 1978;234(4):H371-83.

186. Numan T, Bain AR, Hoiland RL, Smirl JD, Lewis NC, and Ainslie PN. Static autoregulation in humans: a review and reanalysis. Med Eng Phys. England; 2014;36(11):1487-95.

187. Aaslid R, Lindegaard KF, Sorteberg W, and Nornes H. Cerebral autoregulation dynamics in humans. Stroke. United States; 1989;20(1):45-52.

188. Birch AA, Dirnhuber MJ, Hartley-Davies R, Iannotti F, and Neil-Dwyer G. Assessment of autoregulation by means of periodic changes in blood pressure. Stroke. United States; 1995;26(5):834-7.

189. Zhang R, Zuckerman JH, Giller CA, and Levine BD. Transfer function analysis of dynamic cerebral autoregulation in humans. Am J Physiol. United States; 1998;274(1 Pt 2):H233-41.

190. Tan CO, and Taylor JA. Integrative physiological and computational approaches to understand autonomic control of cerebral autoregulation. Exp Physiol. England; 2014;99(1):3-15.

191. Hamner JW, Tan CO, Lee K, Cohen MA, and Taylor JA. Sympathetic control of the cerebral vasculature in humans. Stroke. United States; 2010;41(1):102-9.

192. Hamner JW, Tan CO, Tzeng YC, and Taylor JA. Cholinergic control of the cerebral vasculature in humans. J Physiol. England; 2012;590(24):6343-52.

193. Tzeng YC, Chan GS, Willie CK, and Ainslie PN. Determinants of human cerebral pressure-flow velocity relationships: new insights from vascular modelling and Ca^{2}□ channel blockade. J Physiol. England; 2011;589(Pt 13):3263-74.

194. Hamner JW, and Tan CO. Relative contributions of sympathetic, cholinergic, and myogenic mechanisms to cerebral autoregulation. Stroke. United States; 2014;45(6):1771-7.

195. Tiecks FP, Lam AM, Aaslid R, and Newell DW. Comparison of static and dynamic cerebral autoregulation measurements. Stroke. UNITED STATES; 1995;26(6):1014-9.

196. Meel-van den Abeelen AS, van Beek AH, Slump CH, Panerai RB, and Claassen JA. Transfer function analysis for the assessment of cerebral autoregulation using spontaneous oscillations in blood pressure and cerebral blood flow. Med Eng Phys. England; 2014;36(5):563-75.

197. Bouma GJ, Muizelaar JP, Bandoh K, and Marmarou A. Blood pressure and intracranial pressure-volume dynamics in severe head injury: relationship with cerebral blood flow. J Neurosurg. United States; 1992;77(1):15-9.

198. Hlatky R, Valadka AB, Gopinath SP, and Robertson CS. Brain tissue oxygen tension response to induced hyperoxia reduced in hypoperfused brain. J Neurosurg. United States; 2008;108(1):53-8.

199. Sviri GE, Aaslid R, Douville CM, Moore A, and Newell DW. Time course for autoregulation recovery following severe traumatic brain injury. J Neurosurg. United States; 2009;111(4):695-700.

200. Lam JM, Hsiang JN, and Poon WS. Monitoring of autoregulation using laser Doppler flowmetry in patients with head injury. J Neurosurg. United States; 1997;86(3):438-45.

201. Liu X, Czosnyka M, Donnelly J, Budohoski KP, Varsos GV, Nasr N, Brady KM, Reinhard M, Hutchinson PJ, and Smielewski P. Comparison of frequency and time domain methods of assessment of cerebral autoregulation in traumatic brain injury. J Cereb Blood Flow Metab. United States; 2015;35(2):248-56.

202. Hilz MJ, Kolodny EH, Brys M, Stemper B, Haendl T, and Marthol H. Reduced cerebral blood flow velocity and impaired cerebral autoregulation in patients with Fabry disease. J Neurol. Germany; 2004;251(5):564-70.

203. Santos GA, Petersen N, Zamani AA, Du R, LaRose S, Monk A, Sorond FA, and Tan CO. Pathophysiologic differences in cerebral autoregulation after subarachnoid hemorrhage. Neurology. United States; 2016;86(21):1950-6.

204. Budohoski KP, Czosnyka M, Kirkpatrick PJ, Reinhard M, Varsos GV, Kasprowicz M, Ząbek M, Pickard JD, and Smielewski P. Bilateral failure of cerebral autoregulation is related to unfavorable outcome after subarachnoid hemorrhage. Neurocrit Care. United States; 2015;22(1):65-73.

205. Otite F, Mink S, Tan CO, Puri A, Zamani AA, Mehregan A, Chou S, Orzell S, Purkayastha S, Du R, and Sorond FA. Impaired cerebral autoregulation is associated with vasospasm and delayed cerebral ischemia in subarachnoid hemorrhage. Stroke. United States; 2014;45(3):677-82.

206. Leddy JJ, Kozlowski K, Fung M, Pendergast DR, and Willer B. Regulatory and autoregulatory physiological dysfunction as a primary characteristic of post concussion

syndrome: implications for treatment. NeuroRehabilitation. Netherlands; 2007;22(3):199-205.

207. Choe MC, Babikian T, DiFiori J, Hovda DA, and Giza CC. A pediatric perspective on concussion pathophysiology. Curr Opin Pediatr. United States; 2012;24(6):689-95.

208. Jünger EC, Newell DW, Grant GA, Avellino AM, Ghatan S, Douville CM, Lam AM, Aaslid R, and Winn HR. Cerebral autoregulation following minor head injury. J Neurosurg. UNITED STATES; 1997;86(3):425-32.

209. Strebel S, Lam AM, Matta BF, and Newell DW. Impaired cerebral autoregulation after mild brain injury. Surg Neurol. UNITED STATES; 1997;47(2):128-31.

210. Smirl JD, Hoffman K, Tzeng YC, Hansen A, and Ainslie PN. Methodological comparison of active and passive driven oscillations in blood pressure; implications for the assessment of cerebral-pressure flow relationships. J Appl Physiol (1985). 2015;:jap.00264.2015.

211. Panerai RB, Eames PJ, and Potter JF. Variability of time-domain indices of dynamic cerebral autoregulation. Physiol Meas. England; 2003;24(2):367-81.

212. Claassen JA, Meel-van den Abeelen AS, Simpson DM, Panerai RB, and international Cerebral Autoregulation Research Network (CARNet). Transfer function analysis of dynamic cerebral autoregulation: A white paper from the International Cerebral Autoregulation Research Network. J Cereb Blood Flow Metab. United States; 2016;36(4):665-80.

213. Smirl JD, Wright AD, Bryk K, and van Donkelaar P. Where's Waldo? The utility of a complicated visual search paradigm for transcranial Doppler-based assessments of neurovascular coupling. J Neurosci Methods. 2016;270:92-101.

214. Blaber AP, Bondar RL, Stein F, Dunphy PT, Moradshahi P, Kassam MS, and Freeman R. Transfer function analysis of cerebral autoregulation dynamics in autonomic failure patients. Stroke. UNITED STATES; 1997;28(9):1686-92.

215. Claassen JA, Diaz-Arrastia R, Martin-Cook K, Levine BD, and Zhang R. Altered cerebral hemodynamics in early Alzheimer disease: a pilot study using transcranial Doppler. J Alzheimers Dis. Netherlands; 2009;17(3):621-9.

216. Smirl JD, Haykowsky MJ, Nelson MD, Tzeng YC, Marsden KR, Jones H, and Ainslie PN. Relationship between cerebral blood flow and blood pressure in long-term heart transplant recipients. Hypertension. United States; 2014;64(6):1314-20.

217. Ku HL, Wang JK, Lee HC, Lane TJ, Liu IC, Chen YC, Lee YT, Lin IC, Lin CP, Hu CJ, and Chi NF. Cerebral blood flow autoregulation is impaired in schizophrenia: A pilot study. Schizophr Res. Netherlands; 2017;.

218. Castro P, Azevedo E, Serrador J, Rocha I, and Sorond F. Hemorrhagic transformation and cerebral edema in acute ischemic stroke: link to cerebral autoregulation. J Neurol Sci. Netherlands; 2017;372:256-261.

219. Roy CS, and Sherrington CS. On the regulation of the blood-supply of the brain. J Physiol. England; 1890;11(1-2):85-158.17.

220. Phillips AA, Chan FH, Zheng MM, Krassioukov AV, and Ainslie PN. Neurovascular coupling in humans: Physiology, methodological advances and clinical implications. J Cereb Blood Flow Metab. United States; 2016;36(4):647-64.

221. Kawamura H, Sugiyama T, Wu DM, Kobayashi M, Yamanishi S, Katsumura K, and Puro DG. ATP: a vasoactive signal in the pericyte-containing microvasculature of the rat retina. J Physiol. England; 2003;551(Pt 3):787-99.

222. Lagaud G, Karicheti V, Knot HJ, Christ GJ, and Laher I. Inhibitors of gap junctions attenuate myogenic tone in cerebral arteries. Am J Physiol Heart Circ Physiol. United States; 2002;283(6):H2177-86.

223. Iadecola C. Neurovascular regulation in the normal brain and in Alzheimer's disease. Nat Rev Neurosci. England; 2004;5(5):347-60.

224. Filosa JA, Morrison HW, Iddings JA, Du W, and Kim KJ. Beyond neurovascular coupling, role of astrocytes in the regulation of vascular tone. Neuroscience. 2015;.

225. Hamel E. Perivascular nerves and the regulation of cerebrovascular tone. J Appl Physiol (1985). United States; 2006;100(3):1059-64.

226. Peterson EC, Wang Z, and Britz G. Regulation of cerebral blood flow. Int J Vasc Med. United States; 2011;2011:823525.

227. Attwell D, Buchan AM, Charpak S, Lauritzen M, Macvicar BA, and Newman EA. Glial and neuronal control of brain blood flow. Nature. England; 2010;468(7321):232-43.

228. Ko KR, Ngai AC, and Winn HR. Role of adenosine in regulation of regional cerebral blood flow in sensory cortex. Am J Physiol. United States; 1990;259(6 Pt 2):H1703-8.

229. Chen BR, Bouchard MB, McCaslin AF, Burgess SA, and Hillman EM. High-speed vascular dynamics of the hemodynamic response. Neuroimage. United States; 2011;54(2):1021-30.

230. Peppiatt CM, Howarth C, Mobbs P, and Attwell D. Bidirectional control of CNS capillary diameter by pericytes. Nature. England; 2006;443(7112):700-4.

231. Boas DA, Jones SR, Devor A, Huppert TJ, and Dale AM. A vascular anatomical network model of the spatio-temporal response to brain activation. Neuroimage. United States; 2008;40(3):1116-29.

232. Rosenegger DG, and Gordon GR. A slow or modulatory role of astrocytes in neurovascular coupling. Microcirculation. United States; 2015;22(3):197-203.

233. Gordon GR, Choi HB, Rungta RL, Ellis-Davies GC, and MacVicar BA. Brain metabolism dictates the polarity of astrocyte control over arterioles. Nature. England; 2008;456(7223):745-9.

234. Persidsky Y, Ramirez SH, Haorah J, and Kanmogne GD. Blood-brain barrier: structural components and function under physiologic and pathologic conditions. J Neuroimmune Pharmacol. United States; 2006;1(3):223-36.

235. Howarth C. The contribution of astrocytes to the regulation of cerebral blood flow. Front Neurosci. Switzerland; 2014;8:103.

236. Chen BR, Kozberg MG, Bouchard MB, Shaik MA, and Hillman EM. A critical role for the vascular endothelium in functional neurovascular coupling in the brain. J Am Heart Assoc. England; 2014;3(3):e000787.

237. Lecrux C, and Hamel E. The neurovascular unit in brain function and disease. Acta Physiol (Oxf). England; 2011;203(1):47-59.

238. Cauli B, Tong XK, Rancillac A, Serluca N, Lambolez B, Rossier J, and Hamel E. Cortical GABA interneurons in neurovascular coupling: relays for subcortical vasoactive pathways. J Neurosci. United States; 2004;24(41):8940-9.

239. Kim KJ, Ramiro Diaz J, Iddings JA, and Filosa JA. Vasculo-neuronal coupling: retrograde vascular communication to brain neurons. J Neurosci. United States; 2016;36(50):12624-12639.

240. Rosengarten B, Huwendiek O, and Kaps M. Neurovascular coupling in terms of a control system: validation of a second-order linear system model. Ultrasound Med Biol. England; 2001;27(5):631-5.

241. Azevedo E, Rosengarten B, Santos R, Freitas J, and Kaps M. Interplay of cerebral autoregulation and neurovascular coupling evaluated by functional TCD in different orthostatic conditions. J Neurol. Germany; 2007;254(2):236-41.

242. Willie CK, Cowan EC, Ainslie PN, Taylor CE, Smith KJ, Sin PY, and Tzeng YC. Neurovascular coupling and distribution of cerebral blood flow during exercise. J Neurosci Methods. Netherlands; 2011;198(2):270-3.

243. Zaletel M, Strucl M, and Zvan B. The influence of visual contrast on visually evoked cerebral blood flow responses. Ultrasound Med Biol. England; 2004;30(8):1029-34.

244. Gommer ED, Bogaarts G, Martens EG, Mess WH, and Reulen JP. Visually evoked blood flow responses and interaction with dynamic cerebral autoregulation: correction for blood pressure variation. Med Eng Phys. England; 2014;36(5):613-9.

245. Aaslid R. Visually evoked dynamic blood flow response of the human cerebral circulation. Stroke. 1987;18(4):771-775.

246. Rosengarten B, Osthaus S, and Kaps M. Influence of stimulus duration on the neurovascular coupling response. Ultraschall Med. Germany; 2004;25(2):116-9.

247. Rosengarten B, Aldinger C, Spiller A, and Kaps M. Neurovascular coupling remains unaffected during normal aging. Journal of Neuroimaging. 2003;13(1):43-47.

248. Fabiani M, Gordon BA, Maclin EL, Pearson MA, Brumback-Peltz CR, Low KA, McAuley E, Sutton BP, Kramer AF, and Gratton G. Neurovascular coupling in normal aging: a combined optical, ERP and fMRI study. Neuroimage. United States; 2014;85 Pt 1:592-607.

249. Azevedo E, Castro P, Santos R, Freitas J, Coelho T, Rosengarten B, and Panerai R. Autonomic dysfunction affects cerebral neurovascular coupling. Clin Auton Res. Germany; 2011;21(6):395-403.

250. Nicolakakis N, and Hamel E. Neurovascular function in Alzheimer's disease patients and experimental models. J Cereb Blood Flow Metab. United States; 2011;31(6):1354-70.

251. Zlokovic BV. Neurovascular mechanisms of Alzheimer's neurodegeneration. Trends Neurosci. England; 2005;28(4):202-8.

252. Hinzman JM, Andaluz N, Shutter LA, Okonkwo DO, Pahl C, Strong AJ, Dreier JP, and Hartings JA. Inverse neurovascular coupling to cortical spreading depolarizations in severe brain trauma. Brain. England; 2014;137(Pt 11):2960-72.

253. Lok J, Wang XS, Xing CH, Maki TK, Wu LM, Guo SZ, Noviski N, Arai K, Whalen MJ, Lo EH, and Wang XY. Targeting the neurovascular unit in brain trauma. CNS Neurosci Ther. 2014.

254. Østergaard L, Engedal TS, Aamand R, Mikkelsen R, Iversen NK, Anzabi M, Næss-Schmidt ET, Drasbek KR, Bay V, Blicher JU, Tietze A, Mikkelsen IK, Hansen B, Jespersen SN, Juul N, Sørensen JC, and Rasmussen M. Capillary transit time heterogeneity and flow-metabolism coupling after traumatic brain injury. J Cereb Blood Flow Metab. United States; 2014;34(10):1585-98.

255. Jantzen KJ, Anderson B, Steinberg FL, and Kelso JA. A prospective functional MR imaging study of mild traumatic brain injury in college football players. AJNR Am J Neuroradiol. United States; 2004;25(5):738-45.

256. Slobounov SM, Zhang K, Pennell D, Ray W, Johnson B, and Sebastianelli W. Functional abnormalities in normally appearing athletes following mild traumatic brain injury: a functional MRI study. Exp Brain Res. Germany; 2010;202(2):341-54.

257. Dettwiler A, Murugavel M, Putukian M, Cubon V, Furtado J, and Osherson D. Persistent differences in patterns of brain activation after sports-related concussion: a longitudinal functional magnetic resonance imaging study. J Neurotrauma. United States; 2014;31(2):180-8.

258. Chen XH, Siman R, Iwata A, Meaney DF, Trojanowski JQ, and Smith DH. Long-term accumulation of amyloid-beta, beta-secretase, presenilin-1, and caspase-3 in damaged axons following brain trauma. Am J Pathol. United States; 2004;165(2):357-71.

259. Chen JK, Johnston KM, Petrides M, and Ptito A. Recovery from mild head injury in sports: evidence from serial functional magnetic resonance imaging studies in male athletes. Clin J Sport Med. United States; 2008;18(3):241-7.

260. Gosselin N, Bottari C, Chen JK, Petrides M, Tinawi S, de Guise E, and Ptito A. Electrophysiology and functional MRI in post-acute mild traumatic brain injury. J Neurotrauma. United States; 2011;28(3):329-41.

261. Keightley ML, Saluja RS, Chen JK, Gagnon I, Leonard G, Petrides M, and Ptito A. A functional magnetic resonance imaging study of working memory in youth after sports-related concussion: is it still working? J Neurotrauma. United States; 2014;31(5):437-51.

262. Lovell MR, Pardini JE, Welling J, Collins MW, Bakal J, Lazar N, Roush R, Eddy WF, and Becker JT. Functional brain abnormalities are related to clinical recovery and time to return-to-play in athletes. Neurosurgery. United States; 2007;61(2):352-9; discussion 359-60.

263. Dean PJ, Sato JR, Vieira G, McNamara A, and Sterr A. Multimodal imaging of mild traumatic brain injury and persistent postconcussion syndrome. Brain Behav. United States; 2015;5(1):45-61.

264. Yuh EL, Cooper SR, Mukherjee P, Yue JK, Lingsma HF, Gordon WA, Valadka AB, Okonkwo DO, Schnyer DM, Vassar MJ, Maas AI, Manley GT, Casey SS, Cheong M, Dams-O'Connor K, Hricik AJ, Inoue T, Menon DK, Morabito DJ, Pacheco JL, Puccio AM, and Sinha TK. Diffusion tensor imaging for outcome prediction in mild traumatic brain injury: a TRACK-TBI study. J Neurotrauma. United States; 2014;31(17):1457-77.

265. Mayer AR, Toulouse T, Klimaj S, Ling JM, Pena A, and Bellgowan PS. Investigating the properties of the hemodynamic response function after mild traumatic brain injury. J Neurotrauma. United States; 2014;31(2):189-97.

266. Rosenbaum SB, and Lipton ML. Embracing chaos: the scope and importance of clinical and pathological heterogeneity in mTBI. Brain Imaging Behav. United States; 2012;6(2):255-82.

267. Bryer EJ, Medaglia JD, Rostami S, and Hillary FG. Neural recruitment after mild traumatic brain injury is task dependent: a meta-analysis. J Int Neuropsychol Soc. England; 2013;19(7):751-62.

268. Nauman EA, Breedlove KM, Breedlove EL, Talavage TM, Robinson ME, and Leverenz LJ. Post-season neurophysiological deficits assessed by ImPACT and fMRI in athletes competing in american football. Dev Neuropsychol. England; 2015;40(2):85-91.

269. Elbin RJ, Covassin T, Hakun J, Kontos AP, Berger K, Pfeiffer K, and Ravizza S. Do brain activation changes persist in athletes with a history of multiple concussions who are asymptomatic? Brain Inj. England; 2012;26(10):1217-25.

270. Terry DP, Faraco CC, Smith D, Diddams MJ, Puente AN, and Miller LS. Lack of long-term fMRI differences after multiple sports-related concussions. Brain Inj. England; 2012;26(13-14):1684-96.

271. Ford JH, Giovanello KS, and Guskiewicz KM. Episodic memory in former professional football players with a history of concussion: an event-related functional neuroimaging study. J Neurotrauma. United States; 2013;30(20):1683-701.

272. Hamel E. Perivascular nerves and the regulation of cerebrovascular tone. J Appl Physiol (1985). United States; 2006;100(3):1059-64.

273. Cipolla MJ, Li R, and Vitullo L. Perivascular innervation of penetrating brain parenchymal arterioles. J Cardiovasc Pharmacol. United States; 2004;44(1):1-8.

274. Suzuki J, Iwabuchi T, and Hori S. Cervical sympathectomy for cerebral vasospasm after aneurysm rupture. Neurol Med Chir (Tokyo). Japan; 1975;15 pt 1:41-50.

275. Shenkin HA. Cervical sympathectomy on patients with occlusive cerebrovascular disease. Arch Surg. United States; 1969;98(3):317-20.

276. Shenkin HA, Cabieses F, and Van den Noordt G. The effect of bilateral stellectomy upon the cerebral circulation of man. J Clin Invest. United States; 1951;30(1):90-3.

277. Jeng JS, Yip PK, Huang SJ, and Kao MC. Changes in hemodynamics of the carotid and middle cerebral arteries before and after endoscopic sympathectomy in patients with palmar hyperhidrosis: preliminary results. J Neurosurg. United States; 1999;90(3):463-7.

278. Linden L. The effect of stellate ganglion block on cerebral circulation in cerebrovascular accidents. Acta Med Scand Suppl. Sweden; 1955;301:1-110.

279. Ide K, Boushel R, Sørensen HM, Fernandes A, Cai Y, Pott F, and Secher NH. Middle cerebral artery blood velocity during exercise with beta-1 adrenergic and unilateral stellate ganglion blockade in humans. Acta Physiol Scand. England; 2000;170(1):33-8.

280. Treggiari MM, Romand JA, Martin JB, Reverdin A, Rüfenacht DA, and de Tribolet N. Cervical sympathetic block to reverse delayed ischemic neurological deficits after aneurysmal subarachnoid hemorrhage. Stroke. United States; 2003;34(4):961-7.

281. Yokoyama K, Kishida T, and Sugiyama K. Stellate ganglion block and regional cerebral blood volume and oxygenation. Can J Anaesth. United States; 2004;51(5):515-6.

282. Ainslie PN, and Tzeng YC. On the regulation of the blood supply to the brain: old age concepts and new age ideas. J Appl Physiol (1985). United States; 2010;108(6):1447-9.

283. Tzeng YC, Willie CK, Atkinson G, Lucas SJ, Wong A, and Ainslie PN. Cerebrovascular regulation during transient hypotension and hypertension in humans. Hypertension. United States; 2010;56(2):268-73.

284. Tzeng YC, Lucas SJ, Atkinson G, Willie CK, and Ainslie PN. Fundamental relationships between arterial baroreflex sensitivity and dynamic cerebral autoregulation in humans. J Appl Physiol (1985). United States; 2010;108(5):1162-8.

285. ter Laan M, van Dijk JM, Elting JW, Staal MJ, and Absalom AR. Sympathetic regulation of cerebral blood flow in humans: a review. Br J Anaesth. England; 2013;111(3):361-7.

286. Ainslie PN, and Brassard P. Why is the neural control of cerebral autoregulation so controversial? F1000Prime Rep. England; 2014;6:14.

287. Zhang R, Crandall CG, and Levine BD. Cerebral hemodynamics during the Valsalva maneuver: insights from ganglionic blockade. Stroke. United States; 2004;35(4):843-7.

288. Kimmerly DS, Tutungi E, Wilson TD, Serrador JM, Gelb AW, Hughson RL, and Shoemaker JK. Circulating norepinephrine and cerebrovascular control in conscious humans. Clin Physiol Funct Imaging. England; 2003;23(6):314-9.

289. Zhang R, Zuckerman JH, Iwasaki K, Wilson TE, Crandall CG, and Levine BD. Autonomic neural control of dynamic cerebral autoregulation in humans. Circulation. United States; 2002;106(14):1814-20.

290. Routledge FS, Campbell TS, McFetridge-Durdle JA, and Bacon SL. Improvements in heart rate variability with exercise therapy. Canadian Journal of Cardiology. Elsevier; 2010;26(6):303-312.

291. Papaioannou V, Giannakou M, Maglaveras N, Sofianos E, and Giala M. Investigation of heart rate and blood pressure variability, baroreflex sensitivity, and approximate entropy in acute brain injury patients. J Crit Care. United States; 2008;23(3):380-6.

292. Brown AW, Leibson CL, Malec JF, Perkins PK, Diehl NN, and Larson DR. Long-term survival after traumatic brain injury: a population-based analysis. NeuroRehabilitation. Netherlands; 2004;19(1):37-43.

293. McMillan TM, and Teasdale GM. Death rate is increased for at least 7 years after head injury: a prospective study. Brain. England; 2007;130(Pt 10):2520-7.

294. McMillan TM, Teasdale GM, Weir CJ, and Stewart E. Death after head injury: the 13-year outcome of a case control study. J Neurol Neurosurg Psychiatry. England; 2011;82(8):931-5.

295. Black M, and Graham DI. Sudden unexplained death in adults caused by intracranial pathology. J Clin Pathol. England; 2002;55(1):44-50.

296. Sung CW, Lee HC, Chiang YH, Chiu WT, Chu SF, Ou JC, Tsai SH, Liao KH, Lin CM, Lin JW, Chen GS, Li WJ, and Wang JY. Early dysautonomia detected by heart rate variability

predicts late depression in female patients following mild traumatic brain injury. Psychophysiology. United States; 2016;53(4):455-64.

297. Sung CW, Chen KY, Chiang YH, Chiu WT, Ou JC, Lee HC, Tsai SH, Lin JW, Yang CM, Tsai YR, Liao KH, Chen GS, Li WJ, and Wang JY. Heart rate variability and serum level of insulin-like growth factor-1 are correlated with symptoms of emotional disorders in patients suffering a mild traumatic brain injury. Clin Neurophysiol. Netherlands; 2016;127(2):1629-38.

298. Abaji JP, Curnier D, Moore RD, and Ellemberg D. Persisting effects of concussion on heart rate variability during physical exertion. J Neurotrauma. 2015.

299. Gall B, Parkhouse W, and Goodman D. Heart rate variability of recently concussed athletes at rest and exercise. Medicine & Science in Sports & Exercise. Ovid Technologies (Wolters Kluwer Health); 2004;36(8):1269-1274.

300. La Fountaine MF, Toda M, Testa AJ, and Hill-Lombardi V. Autonomic nervous system responses to concussion: arterial pulse contour analysis. Front Neurol. Switzerland; 2016;7:13.

301. Hilz MJ, DeFina PA, Anders S, Koehn J, Lang CJ, Pauli E, Flanagan SR, Schwab S, and Marthol H. Frequency analysis unveils cardiac autonomic dysfunction after mild traumatic brain injury. J Neurotrauma. United States; 2011;28(9):1727-38.

302. Hilz MJ, Aurnhammer F, Flanagan SR, Intravooth T, Wang R, Hösl KM, Pauli E, and Koehn J. Eyeball pressure stimulation unveils subtle autonomic cardiovascular dysfunction in persons with a history of mild traumatic brain injury. J Neurotrauma. United States; 2015;32(22):1796-804.

303. Hutchison MG, Mainwaring L, Senthinathan A, Churchill N, Thomas S, and Richards D. Psychological and physiological markers of stress in concussed athletes across recovery milestones. J Head Trauma Rehabil. United States; 2016.

304. Senthinathan A, Mainwaring LM, and Hutchison M. Heart rate variability of athletes across concussion recovery milestones: a preliminary study. Clin J Sport Med. United States; 2016.

305. Bishop S, Dech R, Baker T, Butz M, Aravinthan K, and Neary JP. Parasympathetic baroreflexes and heart rate variability during acute stage of sport concussion recovery. Brain Inj. England; 2017:1-13.

306. Blake TA, McKay CD, Meeuwisse WH, and Emery CA. The impact of concussion on cardiac autonomic function: A systematic review. Brain Inj. England; 2016;30(2):132-45.

307. Faul, Xu L, Wald MM, and Coronado VG. Traumatic brain injury in the United States: Emergency department visits, hospitalizations, and deaths, 2002-2006. Atlanta (GA); 2010.

308. Johnson VE, Stewart JE, Begbie FD, Trojanowski JQ, Smith DH, and Stewart W. Inflammation and white matter degeneration persist for years after a single traumatic brain injury. Brain. England; 2013;136(Pt 1):28-42.

309. Liu MC, Akle V, Zheng W, Kitlen J, O'Steen B, Larner SF, Dave JR, Tortella FC, Hayes RL, and Wang KK. Extensive degradation of myelin basic protein isoforms by calpain following traumatic brain injury. J Neurochem. England; 2006;98(3):700-12.

310. Povlishock JT, and Katz DI. Update of neuropathology and neurological recovery after traumatic brain injury. J Head Trauma Rehabil. United States; 2005;20(1):76-94.

311. O'Connor WT, Smyth A, and Gilchrist MD. Animal models of traumatic brain injury: a critical evaluation. Pharmacol Ther. England; 2011;130(2):106-13.

312. MacKay A, Laule C, Vavasour I, Bjarnason T, Kolind S, and Mädler B. Insights into brain microstructure from the T2 distribution. Magnetic Resonance Imaging. Elsevier BV; 2006;24(4):515-525.

313. Oh J, Han ET, Lee MC, Nelson SJ, and Pelletier D. Multislice brain myelin water fractions at 3T in multiple sclerosis. J Neuroimaging. United States; 2007;17(2):156-63.

314. Jarrett M, Tam R, Hernández-Torres E, Martin N, Perera W, Zhao Y, Shahinfard E, Dadachanji S, Taunton J, Li DK, and Rauscher A. A prospective pilot investigation of brain volume, white matter hyperintensities, and hemorrhagic lesions after mild traumatic brain injury. Front Neurol. Switzerland; 2016;7:11.

315. Smith SM, Jenkinson M, Johansen-Berg H, Rueckert D, Nichols TE, Mackay CE, Watkins KE, Ciccarelli O, Cader MZ, Matthews PM, and Behrens TE. Tract-based spatial statistics: voxelwise analysis of multi-subject diffusion data. Neuroimage. United States; 2006;31(4):1487-505.

316. Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TE, Johansen-Berg H, Bannister PR, De Luca M, Drobnjak I, Flitney DE, Niazy RK, Saunders J, Vickers J, Zhang Y, De Stefano N, Brady JM, and Matthews PM. Advances in functional and structural MR image analysis and implementation as FSL. Neuroimage. United States; 2004;23 Suppl 1:S208-19.

317. Wright AD, Jarrett M, Vavasour I, Shahinfard E, Kolind S, van Donkelaar P, Taunton J, Li D, and Raushcer A. Myelin water fraction is transiently reduced after a single mild traumatic brain injury – a prospective cohort study in collegiate hockey players. PLoS ONE. 2016.

318. Baugh CM, Stamm JM, Riley DO, Gavett BE, Shenton ME, Lin A, Nowinski CJ, Cantu RC, McKee AC, and Stern RA. Chronic traumatic encephalopathy: neurodegeneration

following repetitive concussive and subconcussive brain trauma. Brain Imaging Behav. United States; 2012;6(2):244-54.

319. Berger RP. The use of serum biomarkers to predict outcome after traumatic brain injury in adults and children. J Head Trauma Rehabil. United States; 2006;21(4):315-33.

320. Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mörk S, and Bö L. Axonal transection in the lesions of multiple sclerosis. N Engl J Med. United States; 1998;338(5):278-85.

321. Kelley BJ, Lifshitz J, and Povlishock JT. Neuroinflammatory responses after experimental diffuse traumatic brain injury. J Neuropathol Exp Neurol. United States; 2007;66(11):989-1001.

322. Vavasour IM, Laule C, Li DK, Oger J, Moore GR, Traboulsee A, and MacKay AL. Longitudinal changes in myelin water fraction in two MS patients with active disease. J Neurol Sci. Netherlands; 2009;276(1-2):49-53.

323. Donovan V, Kim C, Anugerah AK, Coats JS, Oyoyo U, Pardo AC, and Obenaus A. Repeated mild traumatic brain injury results in long-term white-matter disruption. J Cereb Blood Flow Metab. United States; 2014;34(4):715-23.

324. Li S, Sun Y, Shan D, Feng B, Xing J, Duan Y, Dai J, Lei H, and Zhou Y. Temporal profiles of axonal injury following impact acceleration traumatic brain injury in rats--a comparative study with diffusion tensor imaging and morphological analysis. Int J Legal Med. Germany; 2013;127(1):159-67.

325. Waxman SG. Conduction in myelinated, unmyelinated, and demyelinated fibers. Arch Neurol. United States; 1977;34(10):585-9.

326. Henry LC, Tremblay S, Boulanger Y, Ellemberg D, and Lassonde M. Neurometabolic changes in the acute phase after sports concussions correlate with symptom severity. J Neurotrauma. United States; 2010;27(1):65-76.

327. Nordengen K, Heuser C, Rinholm JE, Matalon R, and Gundersen V. Localisation of N-acetylaspartate in oligodendrocytes/myelin. Brain Struct Funct. Germany; 2015;220(2):899-917.

328. Narayanan S, Fu L, Pioro E, De Stefano N, Collins DL, Francis GS, Antel JP, Matthews PM, and Arnold DL. Imaging of axonal damage in multiple sclerosis: spatial distribution of magnetic resonance imaging lesions. Ann Neurol. United States; 1997;41(3):385-91.

329. Sánchez I, Hassinger L, Paskevich PA, Shine HD, and Nixon RA. Oligodendroglia regulate the regional expansion of axon caliber and local accumulation of neurofilaments during development independently of myelin formation. J Neurosci. United States; 1996;16(16):5095-105.

330. de Waegh SM, Lee VM, and Brady ST. Local modulation of neurofilament phosphorylation, axonal caliber, and slow axonal transport by myelinating Schwann cells. Cell. United States; 1992;68(3):451-63.

331. Hsieh S-T, Crawford TO, Bouldin TW, and Griffin JW. Influence of demyelination and remyelination on axonal organization. Brain Pathology. 1993;3(3):307.

332. Mehta V, and Namboodiri MA. N-acetylaspartate as an acetyl source in the nervous system. Brain Res Mol Brain Res. NETHERLANDS; 1995;31(1-2):151-7.

333. Reeves TM, Phillips LL, and Povlishock JT. Myelinated and unmyelinated axons of the corpus callosum differ in vulnerability and functional recovery following traumatic brain injury. Exp Neurol. United States; 2005;196(1):126-37.

334. Reeves TM, Smith TL, Williamson JC, and Phillips LL. Unmyelinated axons show selective rostrocaudal pathology in the corpus callosum after traumatic brain injury. J Neuropathol Exp Neurol. United States; 2012;71(3):198-210.

335. Blennow K, Jonsson M, Andreasen N, Rosengren L, Wallin A, Hellström PA, and Zetterberg H. No neurochemical evidence of brain injury after blast overpressure by repeated explosions or firing heavy weapons. Acta Neurol Scand. Denmark; 2011;123(4):245-51.

336. Zetterberg H, Hietala MA, Jonsson M, Andreasen N, Styrud E, Karlsson I, Edman A, Popa C, Rasulzada A, Wahlund LO, Mehta PD, Rosengren L, Blennow K, and Wallin A. Neurochemical aftermath of amateur boxing. Arch Neurol. United States; 2006;63(9):1277-80.

337. Zetterberg H, Smith DH, and Blennow K. Biomarkers of mild traumatic brain injury in cerebrospinal fluid and blood. Nat Rev Neurol. England; 2013;9(4):201-10.

338. Berger RP, Adelson PD, Pierce MC, Dulani T, Cassidy LD, and Kochanek PM. Serum neuron-specific enolase, S100B, and myelin basic protein concentrations after inflicted and noninflicted traumatic brain injury in children. J Neurosurg. United States; 2005;103(1 Suppl):61-8.

339. Povlishock JT. The window of risk in repeated head injury. J Neurotrauma. United States; 2013;30(1):1.

340. Zhu DC, Covassin T, Nogle S, Doyle S, Russell D, Pearson RL, Monroe J, Liszewski CM, DeMarco JK, and Kaufman DI. A Potential Biomarker in Sports-Related Concussion: Brain Functional Connectivity Alteration of the Default-Mode Network Measured with

Longitudinal Resting-State fMRI over Thirty Days. J Neurotrauma. United States; 2015;32(5):327-41.

341. Rangel-Castilla L, Gasco J, Nauta HJ, Okonkwo DO, and Robertson CS. Cerebral pressure autoregulation in traumatic brain injury. Neurosurg Focus. United States; 2008;25(4):E7.

342. DeWitt DS, and Prough DS. Traumatic cerebral vascular injury: the effects of concussive brain injury on the cerebral vasculature. J Neurotrauma. United States; 2003;20(9):795-825.

343. Kirkness CJ, Mitchell PH, Burr RL, and Newell DW. Cerebral autoregulation and outcome in acute brain injury. Biol Res Nurs. United States; 2001;2(3):175-85.

344. Ainslie PN, Murrell C, Peebles K, Swart M, Skinner MA, Williams MJ, and Taylor RD. Early morning impairment in cerebral autoregulation and cerebrovascular CO2 reactivity in healthy humans: relation to endothelial function. Exp Physiol. England; 2007;92(4):769-77.

345. Hilz MJ, Wang R, Marthol H, Liu M, Tillmann A, Riss S, Hauck P, Hösl KM, Wasmeier G, Stemper B, and Köhrmann M. Partial pharmacologic blockade shows sympathetic connection between blood pressure and cerebral blood flow velocity fluctuations. J Neurol Sci. Netherlands; 2016;365:181-7.

346. Doshi H, Wiseman N, Liu J, Wang W, Welch RD, O'Neil BJ, Zuk C, Wang X, Mika V, Szaflarski JP, Haacke EM, and Kou Z. Cerebral hemodynamic changes of mild traumatic brain injury at the acute stage. PLoS One. United States; 2015;10(2):e0118061.

347. Allen B, Jennings JR, Gianaros PJ, Thayer JF, and Manuck SB. Resting high-frequency heart rate variability is related to resting brain perfusion. Psychophysiology. United States; 2015;52(2):277-87.

348. Kenney K, Amyot F, Haber M, Pronger A, Bogoslovsky T, Moore C, and Diaz-Arrastia R. Cerebral vascular injury in traumatic brain injury. Exp Neurol. United States; 2016;275 Pt 3:353-66.

349. Park E, Bell JD, Siddiq IP, and Baker AJ. An analysis of regional microvascular loss and recovery following two grades of fluid percussion trauma: a role for hypoxia-inducible factors in traumatic brain injury. J Cereb Blood Flow Metab. United States; 2009;29(3):575-84.

350. Purkayastha S, Fadar O, Mehregan A, Salat DH, Moscufo N, Meier DS, Guttmann CR, Fisher ND, Lipsitz LA, and Sorond FA. Impaired cerebrovascular hemodynamics are associated with cerebral white matter damage. J Cereb Blood Flow Metab. United States; 2014;34(2):228-34.

351. Oppenheimer SM, Gelb A, Girvin JP, and Hachinski VC. Cardiovascular effects of human insular cortex stimulation. Neurology. United States; 1992;42(9):1727-32.

352. Oppenheimer S. The anatomy and physiology of cortical mechanisms of cardiac control. Stroke. United States; 1993;24(12 Suppl):I3-5.

353. Zamrini EY, Meador KJ, Loring DW, Nichols FT, Lee GP, Figueroa RE, and Thompson WO. Unilateral cerebral inactivation produces differential left/right heart rate responses. Neurology. United States; 1990;40(9):1408-11.

354. Thayer JF, Hansen AL, Saus-Rose E, and Johnsen BH. Heart rate variability, prefrontal neural function, and cognitive performance: the neurovisceral integration perspective on self-regulation, adaptation, and health. Ann Behav Med. United States; 2009;37(2):141-53.

355. Jeong SM, Hwang GS, Kim SO, Levine BD, and Zhang R. Dynamic cerebral autoregulation after bed rest: effects of volume loading and exercise countermeasures. J Appl Physiol (1985). United States; 2014;116(1):24-31.

356. Covassin T, Moran R, and Elbin RJ. Sex differences in reported concussion injury rates and time loss from participation: an update of the National Collegiate Athletic Association Injury Surveillance Program from 2004-2005 through 2008-2009. J Athl Train. 2016.

357. Hoiland RL, and Ainslie PN. CrossTalk proposal: The middle cerebral artery diameter does change during alterations in arterial blood gases and blood pressure. J Physiol. England; 2016;594(15):4073-5.

358. Brothers RM, and Zhang R. CrossTalk opposing view: The middle cerebral artery diameter does not change during alterations in arterial blood gases and blood pressure. J Physiol. England; 2016;594(15):4077-9.

359. Ainslie PN, and Hoiland RL. Transcranial Doppler ultrasound: valid, invalid, or both? J Appl Physiol (1985). United States; 2014;117(10):1081-3.

360. Jeong SM, Kim SO, DeLorey DS, Babb TG, Levine BD, and Zhang R. Lack of correlation between cerebral vasomotor reactivity and dynamic cerebral autoregulation during stepwise increases in inspired CO2 concentration. J Appl Physiol (1985). United States; 2016;120(12):1434-41.

361. Militana AR, Donahue MJ, Sills AK, Solomon GS, Gregory AJ, Strother MK, and Morgan VL. Alterations in default-mode network connectivity may be influenced by cerebrovascular changes within 1 week of sports related concussion in college varsity athletes: a pilot study. Brain Imaging Behav. 2015.

362. McCuen E, Svaldi D, Breedlove K, Kraz N, Cummiskey B, Breedlove EL, Traver J, Desmond KF, Hannemann RE, Zanath E, Guerra A, Leverenz L, Talavage TM, and Nauman EA. Collegiate women's soccer players suffer greater cumulative head impacts than their high school counterparts. J Biomech. United States; 2015;48(13):3720-3.

363. Myer GD, Yuan W, Barber Foss KD, Thomas S, Smith D, Leach J, Kiefer AW, Dicesare C, Adams J, Gubanich PJ, Kitchen K, Schneider DK, Braswell D, Krueger D, and Altaye M. Analysis of head impact exposure and brain microstructure response in a season-long application of a jugular vein compression collar: a prospective, neuroimaging investigation in American football. Br J Sports Med. England; 2016;50(20):1276-1285.

364. Chamard E, Théoret H, Skopelja EN, Forwell LA, Johnson AM, and Echlin PS. A prospective study of physician-observed concussion during a varsity university hockey season: metabolic changes in ice hockey players. Part 4 of 4. Neurosurg Focus. United States; 2012;33(6):E4: 1-7.

365. Chrisman SP, Mac Donald CL, Friedman S, Andre J, Rowhani-Rahbar A, Drescher S, Stein E, Holm M, Evans N, Poliakov AV, Ching RP, Schwien CC, Vavilala MS, and Rivara FP. Head impact exposure during a weekend youth soccer tournament. J Child Neurol. United States; 2016;31(8):971-8.

366. Kemp S, Duff A, and Hampson N. The neurological, neuroimaging and neuropsychological effects of playing professional football: Results of the UK five-year follow-up study. Brain Injury. Informa Healthcare; 2016;30(9):1068-1074.

367. Munce TA, Dorman JC, Thompson PA, Valentine VD, and Bergeron MF. Head impact exposure and neurologic function of youth football players. Med Sci Sports Exerc. United States; 2015;47(8):1567-76.

368. Belanger HG, Vanderploeg RD, and McAllister T. Subconcussive blows to the head: a formative review of short-term clinical outcomes. J Head Trauma Rehabil. United States; 2016;31(3):159-66.

369. Miller JR, Adamson GJ, Pink MM, and Sweet JC. Comparison of preseason, midseason, and postseason neurocognitive scores in uninjured collegiate football players. Am J Sports Med. United States; 2007;35(8):1284-8.

370. Koerte IK, Kaufmann D, Hartl E, Bouix S, Pasternak O, Kubicki M, Rauscher A, Li DK, Dadachanji SB, Taunton JA, Forwell LA, Johnson AM, Echlin PS, and Shenton ME. A prospective study of physician-observed concussion during a varsity university hockey season: white matter integrity in ice hockey players. Part 3 of 4. Neurosurg Focus. United States; 2012;33(6):E3: 1-7.

371. Sasaki T, Pasternak O, Mayinger M, Muehlmann M, Savadjiev P, Bouix S, Kubicki M, Fredman E, Dahlben B, Helmer KG, Johnson AM, Holmes JD, Forwell LA, Skopelja EN, Shenton ME, Echlin PS, and Koerte IK. Hockey Concussion Education Project, Part 3. White matter microstructure in ice hockey players with a history of concussion: a diffusion tensor imaging study. J Neurosurg. United States; 2014;120(4):882-90.

372. Chun IY, Mao X, Breedlove EL, Leverenz LJ, Nauman EA, and Talavage TM. DTI detection of longitudinal WM abnormalities due to accumulated head impacts. Dev Neuropsychol. England; 2015;40(2):92-7.

373. Hwang S, Ma L, Kawata K, Tierney R, and Jeka JJ. Vestibular Dysfunction after Subconcussive Head Impact. J Neurotrauma. United States; 2016.

374. Ainslie PN, Celi L, McGrattan K, Peebles K, and Ogoh S. Dynamic cerebral autoregulation and baroreflex sensitivity during modest and severe step changes in arterial PCO2. Brain Res. Netherlands; 2008;1230:115-24.

375. Poole VN, Abbas K, Shenk TE, Breedlove EL, Breedlove KM, Robinson ME, Leverenz LJ, Nauman EA, Talavage TM, and Dydak U. MR spectroscopic evidence of brain injury in the non-diagnosed collision sport athlete. Dev Neuropsychol. England; 2014;39(6):459-73.

376. Bazarian JJ, Zhu T, Zhong J, Janigro D, Rozen E, Roberts A, Javien H, Merchant-Borna K, Abar B, and Blackman EG. Persistent, long-term cerebral white matter changes after sports-related repetitive head impacts. PLoS One. United States; 2014;9(4):e94734.

377. Merchant-Borna K, Lee H, Wang D, Bogner V, van Griensven M, Gill J, and Bazarian JJ. Genome-wide changes in peripheral gene expression following sports-related concussion. J Neurotrauma. United States; 2016;33(17):1576-85.

378. Beckwith JG, Greenwald RM, Chu JJ, Crisco JJ, Rowson S, Duma SM, Broglio SP, McAllister TW, Guskiewicz KM, Mihalik JP, Anderson S, Schnebel B, Brolinson PG, and Collins MW. Head impact exposure sustained by football players on days of diagnosed concussion. Med Sci Sports Exerc. United States; 2013;45(4):737-46.

379. Broglio SP, Sosnoff JJ, Shin S, He X, Alcaraz C, and Zimmerman J. Head impacts during high school football: a biomechanical assessment. J Athl Train. United States; 2009;44(4):342-9.

380. Wu LC, Nangia V, Bui K, Hammoor B, Kurt M, Hernandez F, Kuo C, and Camarillo DB. In vivo evaluation of wearable head impact sensors. Ann Biomed Eng. United States; 2016;44(4):1234-45.

381. Rosengarten B, Aldinger C, Kaufmann A, and Kaps M. Comparison of visually evoked peak systolic and end diastolic blood flow velocity using a control system approach. Ultrasound Med Biol. England; 2001;27(11):1499-503.

382. Saluja RS, Chen JK, Gagnon IJ, Keightley M, and Ptito A. Navigational memory functional magnetic resonance imaging: a test for concussion in children. J Neurotrauma. 2015.

383. Szmydynger-Chodobska J, Shan R, Thomasian N, and Chodobski A. The involvement of pial microvessels in leukocyte invasion after mild traumatic brain injury. PLoS One. United States; 2016;11(12):e0167677.

384. Mihalik JP, Bell DR, Marshall SW, and Guskiewicz KM. Measurement of head impacts in collegiate football players: an investigation of positional and event-type differences. Neurosurgery. United States; 2007;61(6):1229-35; discussion 1235.

385. Schnebel B, Gwin JT, Anderson S, and Gatlin R. In vivo study of head impacts in football: a comparison of National Collegiate Athletic Association Division I versus high school impacts. Neurosurgery. United States; 2007;60(3):490-5; discussion 495-6.

386. Rowson S, Brolinson G, Goforth M, Dietter D, and Duma S. Linear and angular head acceleration measurements in collegiate football. J Biomech Eng. United States; 2009;131(6):061016.

387. Broglio SP, Martini D, Kasper L, Eckner JT, and Kutcher JS. Estimation of head impact exposure in high school football: implications for regulating contact practices. Am J Sports Med. United States; 2013;41(12):2877-84.

388. Cubon VA, Putukian M, Boyer C, and Dettwiler A. A diffusion tensor imaging study on the white matter skeleton in individuals with sports-related concussion. J Neurotrauma. United States; 2011;28(2):189-201.

389. Borich M, Makan N, Boyd L, and Virji-Babul N. Combining whole-brain voxel-wise analysis with in vivo tractography of diffusion behavior after sports-related concussion in adolescents: a preliminary report. J Neurotrauma. United States; 2013;30(14):1243-9.

390. Mayer AR, Ling J, Mannell MV, Gasparovic C, Phillips JP, Doezema D, Reichard R, and Yeo RA. A prospective diffusion tensor imaging study in mild traumatic brain injury. Neurology. United States; 2010;74(8):643-50.

391. Panerai RB. Nonstationarity of dynamic cerebral autoregulation. Med Eng Phys. England; 2014;38(5):576-84.

392. Hinson HE, Puybasset L, Weiss N, Perlbarg V, Benali H, Galanaud D, Lasarev M, Stevens RD, and Neuro Imaging for Coma Emergence, Recovery (NICER) Consortium. Neuroanatomical basis of paroxysmal sympathetic hyperactivity: a diffusion tensor imaging analysis. Brain Inj. England; 2015;29(4):455-61.

393. Tomoto T, Sugawara J, Nogami Y, Aonuma K, and Maeda S. The influence of central arterial compliance on cerebrovascular hemodynamics: insights from endurance training intervention. J Appl Physiol (1985). United States; 2015;119(5):445-51.

394. Al-Qudah ZA, Yacoub HA, and Souayah N. Disorders of the autonomic nervous system after hemispheric cerebrovascular disorders: an update. J Vasc Interv Neurol. United States; 2015;8(4):43-52.

395. Cheyuo C, Jacob A, Wu R, Zhou M, Coppa GF, and Wang P. The parasympathetic nervous system in the quest for stroke therapeutics. J Cereb Blood Flow Metab. United States; 2011;31(5):1187-95.

396. Thygesen KS, and Sabers A. Promising effect of vagal stimulation in Danish patients with epilepsy. Dan Med J. Denmark; 2013;60(3):A4597.

397. García-Navarrete E, Torres CV, Gallego I, Navas M, Pastor J, and Sola RG. Long-term results of vagal nerve stimulation for adults with medication-resistant epilepsy who have been on unchanged antiepileptic medication. Seizure. England; 2013;22(1):9-13.

398. Miyamoto O, Pang J, Sumitani K, Negi T, Hayashida Y, and Itano T. Mechanisms of the anti-ischemic effect of vagus nerve stimulation in the gerbil hippocampus. Neuroreport. England; 2003;14(15):1971-4.

399. Narouze S. Neurostimulation at pterygopalatine fossa for cluster headaches and cerebrovascular disorders. Curr Pain Headache Rep. United States; 2014;18(7):432.

400. Sussman WI, Mautner K, Mason RA, Bonecutter K, and Shealy AK. Sphenopalatine ganglion block for management of refractory chronic posttraumatic headaches after a sport-related concussion. Clin J Sport Med. United States; 2016.

401. Bruinvels G, Burden RJ, McGregor AJ, Ackerman KE, Dooley M, Richards T, and Pedlar C. Sport, exercise and the menstrual cycle: where is the research? Br J Sports Med. England; 2016.

402. Barlow KM, Crawford S, Stevenson A, Sandhu SS, Belanger F, and Dewey D. Epidemiology of postconcussion syndrome in pediatric mild traumatic brain injury. Pediatrics. United States; 2010;126(2):e374-81.

403. Hung R, Carroll LJ, Cancelliere C, Côté P, Rumney P, Keightley M, Donovan J, Stålnacke BM, and Cassidy JD. Systematic review of the clinical course, natural history, and prognosis for pediatric mild traumatic brain injury: results of the International Collaboration on Mild Traumatic Brain Injury Prognosis. Arch Phys Med Rehabil. United States; 2014;95(3 Suppl):S174-91.