THE EFFECT OF REPETITIVE HEAD IMPACT EXPOSURE ON CEREBROVASCULAR FUNCTION IN HIGH SCHOOL ATHLETES

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A dissertation submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Human Movement Science Curriculum in the School of Medicine.

Chapel Hill
2020

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ABSTRACT

Patricia Rhys Roby: The Effect of Repetitive Head Impact Exposure on Cerebrovascular Function in High School Athletes
(Under the direction of Jason P. Mihalik)

Recent data suggest subconcussive head impacts may contribute to short- and long-term neurophysiological deficits. It is important to characterize the neurophysiological effects of these impacts early in the athletic career. The purpose of this study was to determine how subconcussive head impact exposure in high school collision sport student-athletes influenced cerebrovascular function (as measured using transcranial Doppler). Transcranial Doppler was used to assess: 1) resting middle (MCA) and posterior cerebral arteries (PCA), 2) cerebrovascular reactivity (CVR) via breath-holding index, vasomotor reactivity response (VMRr) and overall MCA response curve, and 3) neurovascular coupling (NVC) via NVC response magnitude and overall PCA response curve. Fifty-three high school-aged athletes (age=15.8±1.2yrs, height=175.8±8.1cm, mass=69.4±13.5kg) were recruited into two groups based on sport participation (collision vs. non-collision sport). Prior to the start of their respective seasons, all participants completed a preseason cerebrovascular function assessment using transcranial Doppler. Following a 4- to 5-month window (median=113 days, IQR=23 days), 48 athletes (age=16.0±1.2yrs, height=175.5±8.1cm, mass=68.6± 4.0kg) repeated the cerebrovascular assessment. At pre-season testing, there were no group differences in resting MCA (t_{50}=1.70, p=0.10) or PCA velocities (t_{50}=1.70, p=0.10), CVR as measured by breath-holding index (BHI) (t_{50}=0.68, p=0.50), vasomotor reactivity response (VMRr) (t_{50}=1.70, p=0.10), and overall MCA response curve during breath-holding ($F_{1,2594}$=0.20, p=0.66) or hyperventilation ($F_{1,2594}$=0.00, p=0.99), or NVC as measured by NVC response magnitude during the reading

task (t_{48}=-0.91, p=0.37) or the visual search task (t_{49}=0.50, p=0.62), and overall PCA response curve during the reading task ($F_{1,1448}$=0.82, p=0.36) or the visual search task ($F_{1,1448}$=0.08, p=0.78). At post-season testing, there were significant group differences in pre- to post season BHI changes (t_{44}=-2.21, p=0.03) and overall PCA response curve to the reading task ($F_{1,2710}$=101.54, p<0.001). All other pre- to post-season change values were non-significant (P>0.05). Though the clinical meaningfulness of our results are still unclear, our study demonstrates that CVR and NVC assessments may be sensitive to the dynamic cerebrovascular changes occurring in adolescent athletes. Future research should continue to assess these outcomes following both subconcussive head impact exposure and throughout the recovery trajectory following concussion.

To Jimmy, thank you for being the best dog dad while I finished this.

To Mom and Dad, thank you for your unconditional love and support.

To Zac, I wouldn't have done this if you hadn't done it first.

ACKNOWLEDGEMENTS

The completion of this project required the collaborative effort from many people. I'd first like to thank Dr. Jason P. Mihalik for being a great mentor and colleague. His guidance and mentorship allowed me to challenge myself to develop into a skilled researcher. I appreciate the opportunity to build my study from the ground up with the appropriate amount of oversight to guide me through the development of the study.

My next thanks goes out to my committee: Drs. Peter Duquette, Zachary Kerr, Johna Register-Mihalik, and Lee Stoner. Their expertise and insights helped me synthesize the enormous amounts of data to complete this project. I would also like to acknowledge the Athletic Directors and Athletic Training Staff from Chapel Hill High School (Tim Bennett and Merritt Henderson), Carrboro High School (April Ross and Heriberto Zamora), East Chapel Hill High School (Randy Trumbower and Amanda Gerkin), and Orange High School (Mike McCauley and Emily Gaddy) for their support and assistance through the logistics of recruitment and data collection. I truly couldn't have done this without your backing.

Last, I would like to thank my peers and colleagues at the Matthew Gfeller Center. Their encouragement and help powered me through the program and taught me the importance of teamwork, camaraderie, and wine.

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LIST OF ABBREVIATIONS

ACA	Anterior cerebral artery
ADHD	Attention-deficit/hyperactivity disorder
AE	Athlete exposure
ANS	Autonomic nervous system
ATP	Adenosine triphosphate
BESS	Balance Error Scoring System
BHI	Breath-holding index
CA	Cerebral autoregulation
CBF	Cerebral blood flow
CBFv	Cerebral blood flow velocity
cm	Centimeter
CNS	CNS Vital Signs
CPP	Cerebral perfusion pressure
CVR	Cerebrovascular reactivity
dCA	Dynamic Cerebral Autoregulation
GABA	Gamma-Aminobutyric acid
GSC	Graded Symptom Checklist
Hz	Hertz
IPAQ-SF	International Physical Activity Questionnaire – Short Form
MAC	Multimodal Assessment for Cognition and Symptoms
MAP	Mean arterial pressure
MCA	Middle cerebral artery
MCAv	Middle cerebral artery velocity
MHz	Megahertz
NVC	Neurovascular coupling

$PaCO_2$	Partial pressure of carbon dioxide
PCA	Posterior cerebral artery
PCAv	Posterior cerebral artery velocity
$P_{ET}CO_2$	End-tidal carbon dioxide
SAC	Standardized Assessment of Concussion
TBI	Traumatic brain injury
TCD	Transcranial Doppler
TOWRE-2	Test of Word Reading Efficiency – Second Edition
VMRr	Vasomotor reactivity response

CHAPTER 1

INTRODUCTION

Concussion incidence rates in high school sports have consistently remained higher in collision sports such as football, ice hockey, and lacrosse relative to all other sports.[1,2] Due to the collision nature of these sports, athletes are more likely to be exposed to concussive and subconcussive head impacts. This is concerning given recent evidence linking sport-related concussion and cumulative subconcussive head impacts to late-life cognitive and psychological dysfunction in retired athletes. Compounding this growing concern for youth sport safety are the previously published data demonstrating younger athletes may be more physiologically vulnerable to the effects of brain injury[3] and take longer to recover.[4]

Physiologically, the adolescent brain is undergoing maximal synaptic remodeling, eventually leading to stronger networks with frontal systems.[5] These reorganizational processes are associated with improved cognitive and social-affective abilities at this age.[5] Within the cerebrovascular system, cerebral blood flow perfusion rates rapidly increase through childhood, peaking between the ages of 6-9 then slowly decreasing toward adult rates, leveling out around the age of 20-25 years old.[6] Following concussion in patients aged 11-15 years old, cerebral blood flow immediately decreases and remains low even after symptom resolution.[7] This may be due to dysfunction in the regulatory mechanisms responsible for maintaining adequate cerebral blood flow, including cerebrovascular reactivity and neurovascular coupling. Cerebrovascular reactivity describes the ability for cerebral blood flow to adapt to variations in partial pressure of carbon dioxide in the blood. This response has shown to be significantly reduced acutely following concussion,[8,9] suggesting the concussed brain may be more vulnerable to manipulations in oxygen delivery.

Neurovascular coupling is the cerebrovascular response to a neural activation. Acutely following injury, this response is significantly elevated relative to baseline, suggesting a period of over-compensation where the brain is working harder to complete a task than it was prior to injury.[10] Previous literature studying these regulatory mechanisms have largely focused on older athlete populations.[8–10] This is concerning as the adolescent brain may be more vulnerable to shear stress and cerebrovascular dysregulation.[3,11] Additionally, these cerebrovascular responses have primarily been examined in the acute phase following concussion.[8–10] While investigating cumulative head impact burden in high school football athletes, Broglio et al.[12] reported 20 concussions out of 101,994 recorded head impacts suggesting that a majority of head impact exposures in this age group are subconcussive. Concurrently, recent studies have linked cumulative subconcussive head impact exposure and impaired long-term cognitive and neuropsychological function.[13–15] Currently, there is a dearth of literature investigating cerebrovascular function in adolescent athletes following subconcussive head impact exposure. Advanced cerebrovascular assessments looking at functional responses in physiological outcomes can provide further insight into subtle changes following subconcussive head impacts. Novel neurophysiological measures such as transcranial Doppler (TCD) may provide further insight into the physiological consequences of subconcussive head impact exposure on pediatric neurophysiology.

Our *long-term goal* is to determine how youth contact and collision sport participation effects neurophysiological function by investigating the short and long-term effects subconcussive head impacts. Our *central hypothesis* in the proposed dissertation is that athletes who participate in collision sports are exposed to repetitive head impact exposure and will demonstrate significantly worse cerebrovascular assessment changes following one competitive season relative to non-collision sports athletes. Our general *approach* will be to have 30 healthy high school collision sport (e.g., football, ice hockey) athletes and 30 non-collision sport control athletes complete pre- and post-season cerebrovascular assessments to

determine the changes in outcomes after one competitive season. Our *rationale* for examining the effect of subconcussive head impact exposure on cerebrovascular function in high school collision sport athletes is that head impact exposure has been associated with late-life cognitive and psychological dysfunction, but it's currently unclear what, if any, short-term affects these head impact exposures have. Additionally, high school athlete are experiencing on-going neurodevelopmental changes which may make them more susceptible to shear stress and cerebrovascular dysfunction. With this project, we aim to investigate short-term cerebrovascular effects of cumulative subconcussive head impacts experienced by young athletes participating in collision sports.

Specific Aims and Hypotheses

Specific Aim 1. To investigate baseline differences in cerebrovascular function between high school collision and non-collision athletes.

Hypothesis: High school collision sport athletes would demonstrate similar cerebrovascular function relative to non-collision sport control athletes at baseline.

Significance: Developments in advanced physiological assessments have largely focused on collegiate, professional, and retired athletes. Adolescent athletes are undergoing neurodevelopmental changes, including cerebral blood flow perfusion rates. The cerebrovascular function of high school athletes at baseline is relatively unknown.

Innovation: Technological advancements allow noninvasive neurophysiological measurements in populations not previously possible.

Approach: Fifty-three high school athletes (30 collision sport and 23 non-collision sport controls) completed pre-season cerebrovascular TCD measures (cerebral blood flow velocity, cerebral vasoreactivity, and neurovascular coupling response).

Specific Aim 2. To compare single-season changes in cerebrovascular function between high school collision sport and non-collision sport control athletes.

Hypothesis: Collision sport athletes would demonstrate greater single-season changes in cerebrovascular function when compared to non-collision sport athletes. Specifically, collision sport athletes will demonstrate significantly reduced cerebrovascular reactivity outcomes and significantly elevated neurovascular coupling outcomes relative to non-collision sport athletes.

Significance: Adolescent athletes are undergoing rapid neurophysiological changes including synaptic pruning, axon myelination, and cerebrovascular perfusion changes, while simultaneously being exposed to repetitive subconcussive head impacts, the physiological effects of which are currently unknown.

Innovation: The use of advanced neurophysiological assessments in adolescent athletes has largely focused on those acutely post-concussion and have used more invasive methods (e.g., arterial spin labeling, BOLD fMRI, etc.). The use of TCD to portably and non-invasively assess cerebrovascular function may provide further information regarding the physiological response to repetitive head impact exposure to which standard concussion assessment may not be sensitive.

Approach: Twenty-five of the collision-sport athletes from Aim 1 repeated the cerebrovascular assessment (TCD) following the end of their competitive season. All non-collision sport athletes repeat the cerebrovascular assessment (TCD) following the same window of time as the collision sport athletes. Single-season changes in cerebrovascular function were examined using change scores (Change score = post-season – pre-season) to control for baseline.

CHAPTER 2

LITERATURE REVIEW

Introduction

Up to 45 million youth athletes, aged 18 or younger, participate in organized sport each year.[16] Though youth athletes make up the largest population of sport participants, legislated concussion management policies largely focus on high school and collegiate athletes. This is concerning given the apparent rise in high school concussion rates in the last decade;[1,17] however, this may be due to increased concussion education and detecting among athletes, coaches, and parents. Consistently, contact sports report higher concussion rates with the highest being football with 9.21 per 10,000 Athlete-Exposures (AEs).[1]

Current consensus statements recommend a multimodal concussion assessment including symptom reporting, postural control assessment, and neurocognitive assessment for adult athletes[18] and age-specific assessment paradigms for both children and adolescent athletes.[4] Youth athletes present with similar signs and symptoms relative to adult athletes following injury, but typically take up to two weeks longer to recover.[4] Additionally, youth athletes may be more vulnerable to injury due to ongoing neurodevelopment occurring at this time.[5] Recent literature has found that physiological deficits following concussion may outlast clinical recovery[19,20] and may be present in asymptomatic athletes with no reported concussion.[21–23] For many athletes, high school is the first exposure to American football and coincides with this vulnerable time in neurodevelopment. Understanding how head impact exposure may influence neurophysiological outcomes in youth athletes is imperative to designing health and safety policies mitigating concussion risk and improving long-term health outcomes in this understudied population. This literature review will focus on the pathophysiology behind

concussion and head impact exposure, how this pathophysiology may relate to some standard concussion assessments, the biomechanics underlying concussive and subconcussive head impacts, and considerations needed when studying each of these constructs in a pediatric population.

Pathophysiology of concussion

Following concussion, a complex neurometabolic cascade begins leading to physiological changes that are not seen using structural neuroimaging techniques.[24] Because of this, concussion is largely considered a functional, rather than a structural injury.[24] Recent evidence states that these physiological consequences of injury may persist beyond clinical recovery, suggesting a need for further understanding of the regulatory processes maintaining healthy neurophysiological function and how injury may affect these processes.

Autonomic Nervous System

The autonomic nervous system (ANS) is responsible for retaining homeostasis through balancing the sympathetic and parasympathetic nervous systems. Regarding cerebral blood flow regulation, sympathetic activation leads to blood vessel constriction and parasympathetic activation leads to blood vessel dilation.[25] More specifically, the ANS controls the baroreflex, a feedback loop that maintains stable blood flow to the brain.[25] The baroreceptors sense changes in carotid artery pressure, then signals the heart (sinus node, heart muscle, and heart vasculature) to increase or decrease heart rate and contractility based on these changes in pressure.[25]

Traumatic brain injury is known to create imbalance such that there is greater sympathetic and less parasympathetic activation relative to uninjured controls.[26] This imbalance can lead to an uncoupling of the ANS and cardiovascular system.[26] Specifically, heart rate variability and blood pressure are inversely associated with TBI severity.[26] There is limited evidence that these effects are paralleled in mild TBI/concussion.[27] Griesburg et al.[28] showed that heart rate is significantly elevated during exercise in rats with mTBI relative to healthy,

uninjured rats 7 days post-injury. Currently, there is conflicting, limited, or no evidence to support heart rate or heart rate variability differences in healthy, uninjured human subjects relative to those with a concussion history[29] and post-concussion.[30] In a recent systematic review, Blake et al.[31] calls for future research to include more animal models and prospective cohorts in order to improve the validity of post-concussion cardiac autonomic dysfunction studies. In doing so, cardiac autonomic dysfunction research may shed light on the more subtle cerebrovascular impairments following concussion.

Neurometabolic

In a healthy brain, information is transmitted electrochemically via neuronal activation. This transmission relies on stable cell membrane potential, which is maintained by the sodium potassium pump.[32] The sodium potassium pump maintains higher concentrations of sodium outside of the cell and higher concentrations of potassium inside the cell.[32] These concentrations may change due to chemical or physical stimuli, and when enough sodium enters the cell, triggers an action potential ending in the release of neurotransmitters.[32] Glutamate is the most common excitatory neurotransmitter and is important for mental activity.[32]

Animal studies have identified a neurometabolic cascade invoked by concussion that can be broadly summarized as involving primarily 1) excess glutamate release, 2) potassium efflux, and 3) calcium influx. As a neurotransmitter, increased glutamate release seen following concussive injury can lead to cellular swelling and large ionic fluxes.[33] Extreme potassium effluxes out of and calcium influxes in to the neuronal cell body cause an ionic flux that disrupts the cell's sodium potassium pumps. This ionic flux can trigger the opening of ion channels leading to a diffuse "spreading depression-like" state often associated with acute impairments post-injury. While trying to reestablish a homeostatic ionic balance, neuronal cells end up draining its intracellular adenosine triphosphate (ATP) stores. This, combined with a decrease in cerebral blood flow, creates an energy crisis which manifests as the functional signs and symptoms observed acutely following concussion and monitored throughout the patient's

recovery. Additionally, shear stress from biomechanical forces can cause increased intra-axonal calcium excretion which may degrade axon integrity and lead to axonal dysfunction.[34] This ionic flux has been associated with migraine-like symptoms, headaches, dizziness, photophobia, phonophobia, and nausea.[24] The energy crisis contributes to the brain's increased vulnerability following injury and supports the current recommendation of immediately removing concussed athletes from contact risk and allowing for delayed return to sport.

Cerebrovascular

Cerebral regulation is primarily accomplished through adaptations in cerebral perfusion pressure (CPP) and cerebrovascular resistance in healthy brains.[35] Cerebral perfusion pressure is defined as the difference between mean arterial pressure (MAP) and intracranial pressure. Cerebral autoregulation impairment is evident in mild, moderate and severe TBI patients,[36,37] and may contribute to secondary neuronal injury.[37] In moderate to severe TBI, impaired or absent cerebral autoregulation can cause intracranial pressure to become greater than MAP, causing an immediate decrease in cerebral blood flow.[36] This decrease will trigger peripheral vasoconstriction which will lead to an increase in MAP. This increase in MAP will in turn increase blood flow to the brain, potentially worsening any bleed that may be occurring and increasing intracranial pressure. In mild TBI, cerebral autoregulation is less impaired relative to moderate to severe TBI but may still contribute to the brain's vulnerability to secondary ischemic injury.[37] The ability of the brain to make cerebrovascular adaptions to changing MAP and intracranial pressure is essential to maintaining CPP. Additionally, the partial pressure of arterial carbon dioxide ($PaCO_2$) is a major mediator of cerebral blood flow and, specifically, cerebrovascular reactivity (CVR). Cerebrovascular reactivity describes the brain's ability to adapt cerebral blood flow to variations in $PaCO_2$ and may be a more sensitive mediator of cerebral blood flow regulation than CPP. Lang et al.[38] found that with impaired CPP and intact CVR, cerebral blood flow was stable; however, with impaired CVR, cerebral blood flow became highly dependent on CPP.

Cerebral Autoregulation

Cerebral autoregulation (CA) describes the brain's ability to maintain constant cerebral blood flow through a range of mean arterial blood pressures (60mmHg - 150mmHg) by constantly adjusting cerebral vascular resistance.[39] Beyond these ranges of systemic blood pressures, CA failure may lead to ischemic damage or hemorrhage. Static CA measurements reflect cerebrovascular regulation of gradual changes in perfusion pressure, while dynamic CA (dCA) measures rapid CBF regulation in response to changes in arterial blood pressure that occur across a few seconds.[40] Though static CA assessment has been the historically accepted model for cerebral blood flow regulation though a range of mean arterial blood pressures,[41] recent literature suggests that cerebral blood flow changes may parallel changes in blood pressure rather than remain constant.[40] This suggests that dCA may be a more accurate measure of CA. There are several non-invasive methods of assessing dCA using TCD, all of which induce spontaneous fluctuations in mean arterial blood pressure, including suprasystolic thigh cuffs and postural alterations.[39] The suprasystolic thigh cuff technique involves rapidly releasing bilateral thigh cuffs inflated to 20-40mmHg above systolic blood pressure.[37,39] Simple postural alterations such as going from sitting to standing[42] or squating to standing[43] can elicit a drop in mean arterial blood pressure and cerebral blood flow velocity.

Cerebrovascular Reactivity

Cerebrovascular reactivity (CVR) describes the brain's ability to adapt to varying $PaCO_2$. Cerebrovascular responses to changes in $PaCO_2$ are typically greater than autoregulatory mechanisms indicating that CVR is a significant mediator of cerebral blood flow regulation.[35] Because $PaCO_2$ is a significant moderator of vasoconstriction and dilation, CVR is typically assessed by measuring cerebral blood flow velocity differences from resting normal $PaCO_2$ (35-45 mmHg) to either hypocapnia ($PaCO_2$ < 35 mmHg) or hypercapnia ($PaCO_2$ > 45 mmHg).[44] Hypocapnia will lead to vasodilation and should cause decreases in cerebral blood flow velocity, while hypercapnia will lead to vasoconstriction and should cause increases in cerebral blood

flow velocity. Vasoactive stimuli can be administered by injecting an exogenous vasodilatory agent but are more commonly induced by modulating $PaCO_2$ through inhaled carbon dioxide or breath-holding.[45] Inhaled carbon dioxide provides a more controlled environment as well as better short- and long-term reproducibility relative to breath-holding; however, breath-holding and hyperventilation are more clinically applicable.[46] Changes in cerebral blood flow in response to a vasoactive stimulus are commonly measured with TCD or advanced neuroimaging, such as blood oxygen-level dependent MRI or CT. Advanced neuroimaging techniques allow for accurate and reproducible measures of whole-brain and regional cerebral blood flow but are expensive and invasive. While TCD lacks spatial resolution, this measurement has excellent temporal resolution[39] and is an inexpensive, non-invasive, portable alternative to advanced imaging.

Neurovascular Coupling

Neurovascular coupling (NVC) describes the cerebrovascular response to neural activation. During neural activation, there is a demand for both nutrient delivery and by-product removal. The release of the neurotransmitter glutamate activates vasodilators and inhibits vasoconstrictors in both neurons and astrocytes. In a healthy brain, this combined stimulation of neurons and astrocytes creates a four-fold greater increase in cerebral blood flow relative to the energy being consumed to perform the neural task.[47] Due to the functional anatomy of the cerebrovascular system, NVC can be easily and reliably measured through a visual, cognitive, or motor task.[39] Assessing NVC in response to a visual stimulus was first introduced by Aaslid et al.[48] in 1987, who showed a rapid response to light stimulation in the PCA. Since then, several different visual stimuli have been used to provoke NVC response ranging in difficulty from turning on lights[48] to a Where's Waldo visual search task.[49]

Pediatric Considerations for Pathophysiology of Concussion

The physiological implications of concussive and subconcussive head impacts may differ considerably in pediatric patients relative to adults. The ionic flux following concussion that

leads to altered neurochemistry can disrupt brain plasticity in the developing brain.[3] Neural network reorganization and restructuring (i.e., neural plasticity) is driven largely by an adolescent's experiences.[5] These experiences have been associated with drastic emotional and cognitive changes, particularly those that control thought and behavior, allowing the individual to adapt to new environments and tasks.[50] Neural plasticity is heavily influenced by Gamma-Aminobutyric acid (GABA), which is the brain's primary inhibitory neurotransmitter. It is hypothesized that disrupted neural plasticity following concussion may be due to altered $GABA_b$-mediated inhibition in animal models[51] and college football athletes.[52] Axonal damage occurs due to the head impact's shear and tensile forces; however, myelinated axons are known to be better preserved than non-myelinated axons in closed head injuries.[53] Myelination in the developing brain is an ongoing process suggesting the pediatric brain may be more vulnerable to axonal damage following head injury.[3] Throughout childhood, cerebral blood flow perfusion is steadily increasing until puberty where trajectories tend to diverge with females experiencing higher perfusion rates than males.[54] These higher perfusion rates in females continue on through adulthood.[55,56] Following TBI, cerebral blood flow modulation appears to differ between adults and children suggesting that age plays a role in cerebrovascular response.[3] Additionally, the autoregulatory processes used to maintain cerebrovascular function are more impaired in children relative to adults following concussion.[3] Regional cerebral blood flow alterations are observed in male and female children and adolescents with concussion history suggesting that subtle physiological defects remain beyond clinical recovery this population.[57] Ongoing neurodevelopment may make adolescent athletes more vulnerable to the pathophysiological changes that occur following concussion. Further research is needed to understand the short and long-term effect of concussive and subconcussive head impacts in youth athletes.

Cerebrovascular Assessment

Recent studies have suggested that physiological deficits may outlast clinical recovery following concussion.[58–60] These deficits, including cerebrovascular,[58] electrophysiological,[60] and

neurometabolic changes,[59] are commonly measured using expensive and non-portable instruments. Data indicate cerebral blood flow remains reduced in up to 36% of pediatric concussion patients relative to controls 30 days post-injury.[7] Additionally, cerebrovascular deficits have been shown in clinically asymptomatic football and soccer athletes with higher cumulative subconcussive exposure relative to low cumulative exposure.[61,62] Due to the rapid neurodevelopmental changes occurring in youth athletes and the risk for cerebrovascular deficits following both concussive and subconcussive head impacts, non-invasive neurophysiological assessment is needed to comprehensively assess the effect of head impact exposure on pediatric neurophysiology.

Transcranial Doppler

While there is no clear gold standard, advanced neuroimaging techniques such as MRI have been used for measures of whole-brain and regional cerebral blood flow, though these techniques are expensive and invasive. Transcranial Doppler uses the Doppler effect to determine cerebral blood flow velocity (CBFv) and blood flow direction. In order to sufficiently penetrate the bones of the skull, 2 MHz frequency ultrasound probes are typically used over available acoustic windows. There are three commonly used acoustic windows that allow for adequate intracerebral artery insonation through the skull. The orbital window allows ophthalmic artery insonation and is located directly over the eye. The suboccipital window allows for basilar and vertebral artery insonation and is located below the external occipital protuberance. The temporal window is located above the zygomatic arch and permits insonating the Circle of Willis including the terminal internal carotid artery, the middle cerebral artery (MCA), the anterior cerebral artery (ACA), and the posterior cerebral artery (PCA). The temporal window is most commonly used due to its access to primary segments of the MCA (M1), ACA (A1) and PCA (P1), as well as secondary segments of the ACA (A2) and PCA (P2). Additionally, the temporal window provides the ability to use a headset for securing the probes in this position, allowing for

handsfree insonation and assessment. Within each acoustic window, insonation depth, flow direction, and flow velocity are used to help identify each vessel and optimize signal.

Transcranial Doppler is unable to measure absolute cerebral blood flow volume due to the unknown diameter of individual vessels. Previous literature shows MCA vessel diameter remains constant during hypercapnic, hypocapnic, and normocapnic protocols.[63,64] By assuming vessel diameters remain constant, we are able to compute CBFv from TCD raw data. During a cerebrovascular assessment, TCD spectral display will present mean cerebral blood flow velocity and pulsatility index. The mean velocity describes a time-averaged mean of the maximum velocity tracing.[65] Normative values for mean velocities are vessel-specific and depend on the age of the patient (**Table 2.1**).[39] Pulsatility index is an indication of distal flow resistance and is calculated by taking the difference of the peak systolic velocity and end diastolic velocity and dividing it by the mean velocity. A higher pulsatility index indicates greater distal resistance and correlates with absolute intracranial pressure.[65]

Table 2.1. Normative values for transcranial Doppler outcomes in patients younger than 30 years old.

Vessel	Mean velocity	Pulsatility Index
MCA	66.6 ± 14.4 cm/s	0.85 ± 0.13
ACA	53.6 ± 10.2 cm/s	0.85 ± 0.14
PCA	30.9 ± 5.7 cm/s	0.77 ± 0.11

Note: Normative values based on Tegeler et al.[66]

Historically, TCD has been used to monitor function in patients with acute and chronic cerebrovascular disease such as vasospasm, stenosis, emboli detection, intracranial occlusions, thrombosis, and brain death.[39] More recently, using TCD has expanded into concussed populations. As previously stated, absolute measures of cerebral blood flow volume are reduced immediately following concussion;[24] however, absolute cerebral blood flow velocity

does not seem to be sensitive to cerebrovascular changes following concussion.[9,20,67] Functional assessments of cerebrovascular responses including cerebral autoregulation, cerebrovascular reactivity and neurovascular coupling have recently been used in concussion literature to investigate the ability of the cerebrovascular system to adapt to different stimuli following injury.

Transcranial Doppler and Concussion

Assessing cerebrovascular function following concussion has recently gained popularity; however, most cerebrovascular studies use expensive, invasive imaging techniques. Transcranial Doppler allows for convenient, cost-effective cerebrovascular testing that can be transported to clinics and athletic training rooms.

Cerebral Autoregulation

Following moderate and severe TBI, decreases in cerebral perfusion pressure in the presence of impaired cerebral autoregulation can lead to decreased cerebral blood flow and increased brain tissue vulnerability to secondary ischemic injury.[36,68] Additionally, impaired cerebral autoregulation in moderate and severe TBI has been associated with poor outcomes and increased mortality.[68] Similar results have been found in patients following mild TBI, suggesting that if concussed patients experience decreased cerebral perfusion pressure following injury, they may be vulnerable to ischemic injury.[37] In sport-related concussions, impaired cerebral autoregulation is evident immediately following injury and may persist beyond clinical recovery.[42,69] This may be due to altered autonomic regulation of the cerebrovascular system, which can lead to delayed vascular response to changes in blood pressure.[42]

Cerebrovascular Reactivity

Cerebrovascular responses to changes in arterial CO_2 following head trauma are not completely understood. Following head injury, preserved cerebral autoregulation and impaired CVR is associated with severe brain damage, while impaired cerebral autoregulation and preserved CVR is associated with less severe deficits. This suggests that CVR integrity is more indicative of injury severity and may be more sensitive to cerebrovascular dysfunction relative to

cerebral autoregulation following head injury.[43] Following sport-related concussion, Len et al.[8,9] found that CVR is reduced immediately following injury and may remain impaired for up to 4 days. Similar results were found following moderate and severe TBI.[70] Bailey et al.[67] found impaired CVR in professional boxers with an implied history of subconcussive head impacts; however, there is a dearth of research with quantified cumulative subconcussive head impact load.

Neurovascular Coupling

Following head injury, NVC may be impaired due to neuronal death and/or astrocytic scar formation.[71] Additionally, autonomic dysfunction, which is known to occur following TBI,[26] is associated with impaired NVC[72] and has been shown to delay the response of cerebrovasculature to blood pressure changes.[73] There are currently few studies examining NVC in concussed patients. Wright et al.[10] studied the effect of acute concussion and concussion history on NVC response to a visual stimulation paradigm in contact-sport athletes. Concussed athletes had an elevated PCA response magnitude by 31.1% at 72 hours post-injury and that response was delayed by 56.6% relative to their preseason assessment. These results may be explained by the delay in cerebrovascular response due to autonomic dysfunction as well as compensatory recruitment of excessive neural resources to respond to a visual task.[10] Changes in NVC response dynamics were still evident at 2-weeks post-injury, despite symptom and cognitive performance having returned to baseline, suggesting cerebrovascular impairments may outlast clinical recovery. When comparing those with no concussion history to those with a history of 3 or more concussions, there were no differences in NVC responses suggesting that the effect of concussion may not be cumulative or persistent.[10] Further research is needed to determine how concussive and subconcussive impacts influence NVC response in athletes.

Head Impact Biomechanics

Concussion has most recently been defined as a TBI induced by biomechanical forces being transmitted directly or indirectly, typically resulting in neurological impairment.[18] The linear and rotational acceleration experienced at the time of impact initiates the aforementioned neurometabolic cascade and has shown to be the primary cause of concussive injuries.[74] The mechanical loading of linear and rotational acceleration on the brain are associated with pressure and shear forces, respectively. Linear acceleration has been correlated with peak pressures within the brain following head impact and has been associated with more focal injuries. Pressure gradients induced by linear acceleration can create strain on the brain stem[74] and craniocervical junction,[75] leading to possible neurologic dysfunction.[76] Though strain occurs with increased pressure, the brain is relatively resistant to slow or transient pressures. Therefore, tissue strains related to linear acceleration are generally smaller than those related to rotational acceleration.[74] Shear forces induced by rotational acceleration are responsible for up to 90% of tissue strain following head impact.[77] Brain tissue deforms more readily in response to shear forces relative to pressure,[74] with impacts in the coronal plane leading to increased damage to internal brain structures. Additionally, increases in peak rotational acceleration has been correlated with increased injury severity.[78,79]

Technological advances in the last two decades have led to growing research quantifying the relative contribution of linear and rotational accelerations to concussion in sport. As it is currently impossible to directly measure *in vivo* tissue response, skull acceleration has been measured as a correlate to the pressure and strain response of brain tissue. Using primarily cadaver and primate research, indices for injury tolerance were first published in the 1970's and 1980's in regard to car crash safety.[76,80] These studies largely focused on linear acceleration, finding that peak linear accelerations greater than 90 *g* and shorter duration impacts were associated with more severe injuries.[76,80] Laboratory researchers attempted to establish a "concussion threshold" in the late 1990's and early 2000's. These studies found that

linear accelerations between 70-85 *g* were associated with sustaining a concussion[81,82] and impacts over 98 *g* were 75% specific to concussion.[82] Later field studies found no evidence for an acceleration threshold for concussion, finding that only a small percentage (<0.35%) of hits over 80 *g* resulted in concussion[30] and that a single head impact exceeding 90 *g* did not result in acute neurological deficits.[31] Importantly, Guskiewicz and Mihalik[32] reported in 2010 that a concussion threshold likely does not exist as concussions occur at lower magnitudes of linear acceleration than previously thought, linear and rotational acceleration are both important factors in the overall magnitude of the impact, and that head impact biomechanics should not be used to predict acute outcomes. The scientific evolution of the role of head impact biomechanics in concussion diagnosis and management has played a large role in policy and rule changes within sport, specifically football in the last decade; however, these studies and policy changes have primarily focused on professional and collegiate athletics.

Head Impact Exposure in Youth Football

The relatively frequent collisions sustained by its participants and the accessibility of helmeted accelerometry devices has resulted in football as the primary sport in which youth athlete head impact exposure has been studied. Concussion rates across youth athletics have been consistently higher in football relative to other sports, with most recent studies reporting high school football concussion rates of 0.92 per 1,000 AEs,[1] and middle school football concussions rates of 2.61 per 1,000 AEs.[83]

Though head impact monitoring is not recommended for acute concussion diagnosis, measuring head impact biomechanics can still provide important information regarding youth athlete safety. In high school football, athletes typical sustain up to 775 head impacts per season with linemen consistently experiencing the greatest cumulative impact frequency followed by tight ends, running backs, and linebackers.[12,84] Head impact frequency is lower in middle school-aged athletes, who can experience up to 275 impacts per season.[85–87] Additionally, impact frequency is higher in games relative to practices across middle school[85–87]

and high school football. Contact practices have higher impact frequency than non-contact practices in youth football, suggesting that fewer contact practice days may reduce cumulative head impact exposure.[84,88] Impact location may play a role in concussion risk as players are more likely to sustain high magnitude impacts to the top of the head.[89] Finally, open field tackles and tackles with longer closing distances result in more severe head impact magnitudes.[86,89]

There is growing concern regarding the effect of cumulative subconcussive head impacts or head impact exposure on short- and long-term neurological outcomes. Typical post-concussion clinical assessments (e.g., symptom reporting, neurocognitive assessment, and postural control assessment) are not affected following subconcussive head impact exposure in adults. There are insufficient data in youth populations to make such a determination. In studies assessing collegiate football athletes, short-term clinical outcomes were not associated with head impact biomechanics,[90] did not decline throughout the season,[91] and were not significantly different from noncontact sport athletes.[92] In youth athletics, subconcussive head impacts may have a greater effect. Breedlove et al.[93] found that 54.5% of asymptomatic high school football players experienced a substantial deviation from baseline cognitive functioning score throughout their regular season. Additionally, Koerte et al.[94] found that while youth soccer players did not experience significant cognitive changes over the course of a season, the noncontact sport control group experienced cognitive improvement, suggesting that repetitive head impact exposure may be associated with a lack of improvement in cognitive performance.

Typical clinical assessments may not be sensitive enough to detect physiological changes occurring following subconcussive impacts. Additionally, clinical assessments becoming increasingly difficult to interpret when applied to youth athletes who are undergoing rapid neurodevelopmental changes. Advanced imaging metrics may be more appropriate for assessing physiological changes in youth athletes following head impact exposure. Neurophysiological impairments have been found in asymptomatic high school football athletes,[9] and these impairments have been associated with the number and location of head

impacts over the course of a season.[21,93] Additionally, cumulative linear and rotational accelerations over a single season have been associated with white matter tract changes in youth football athletes.[95,96]

Methodological Considerations

Population

Studying the neurophysiology of head impact exposure youth athletes poses several methodological considerations. The rapid ongoing neurodevelopment occurring in this age-group needs to be considered when looking at standard concussion assessments. Methodologically, the use of a non-contact control group may help improve internal validity of a study and better attribute any observed changes in neurophysiological assessments and standard concussion assessments over time to head impact exposure rather than normal developmental changes.

Transcranial Doppler Methodology

Using TCD in concussion literature is relatively new, and even more novel in a youth athlete population. Previously published methodologies for assessing CVR with TCD include 5 trials of breath-holding and hyperventilation, each for 20 seconds with a 40 second recovery following.[8,9] The authors state that participants did not have issue completing these respiratory stressors; however, the participants in both studies ranged in age from 14 – 25 years. Special consideration may need to be made for younger participants during the breath-holding and hyperventilation tasks. Additionally, self-reported levels of physical activity have been shown to be associated with cerebrovascular outcomes,[97] and therefore consideration should be made regarding the physical activity level of participants at the time of testing. NVC response as measured by TCD has been published using 5 trials of 40 seconds eyes-open to a visual stimulus.[10,49] These visual stimuli vary in complexity and have included colored dot identification, reading an article, and a visual search task.[49] In using these visual stimuli in a youth population, consideration may need to be made regarding the reading level of the participant. Similarly, self-

reported symptoms have been correlated with cerebral blood flow in concussed collegiate athletes and should therefore be considered when assessing for confounding variables.[98] Cerebral autoregulation as measured by TCD has been assessed using several different protocols, some of which pose patients comfort concerns.[37] For the present study, we will be limiting our protocol to include CVR and NVC only. Lastly, ADHD in children and adolescents has been associated with regional changes in cerebral blood flow,[99,100] which may also be effected by ADHD medication.[99,101] Medical history concerning ADHD and medications will be recorded to determine if these influence our cerebrovascular outcomes.

Conclusion

Advanced technologies allow for implementing cerebrovascular assessments using transcranial Doppler (TCD) in high school athletes. The neurophysiology of repetitive subconcussive head impacts is not clearly understood. While TCD has been used to measure meaningful change in cerebrovascular function following acute concussion in adult cohorts,[8,9] it is unknown if less severe neurophysiological deficits occur following repetitive subconcussive hits, which may accumulate to create a physiologically disadvantaged environment promoting risk for subsequent injury. Using TCD, the proposed study will non-invasively and cost-effectively measure cerebrovascular function following exposure to repetitive subconcussive impacts. This study will generate foundational knowledge about head impact exposure in youth athletes and contribute to our knowledge of pediatric neurophysiology.

CHAPTER 3

RESEARCH STRATEGY

Significance

Approximately 45 million youth athletes participate in organized sports each year.[16] Despite having greater athlete participation than collegiate athletics and widespread legislative progress regarding concussion education and management, medical coverage is not guaranteed at every athletic event. Additionally, concussion incidence rates are reported higher among collision sports such as football and ice-hockey.[1,2] This is concerning given recent associative evidence linking sport-related concussion to late-life cognitive and psychological dysfunction in retired athletes.[13,102] Following concussion, athletes typically experience increased symptom burden/endorsement, cognitive dysfunction, and balance deficits. Traditional care models permit athletes to return to unrestricted physical activity when post-injury deficits resolve to pre-injury levels on standardized clinical testing. Recent studies have shown physiological deficits can remain even after traditional clinical recovery[21] and that cumulative subconcussive impacts, or impacts not resulting in diagnosed concussions, may influence clinical and neurophysiological outcomes.[13] Unfortunately, these published findings focus almost exclusively on college[103,104] and retired football athletes.

Because subconcussive head impacts do not elicit symptoms and are therefore not evaluated, it is unknown if cumulative subconcussive head impacts result in measurable neurophysiological deficits (**Figure 3.1**). It is important to characterize neurophysiology early in the athletic career to begin understanding the true effects early head impact exposure may have on the late-life neurodegenerative changes recently associated with high school and collegiate football participation.[13] For many, high school marks the initiation to American football, and is a

population that needs further study. For example, the neurological response to repetitive subconcussive head impacts may differ considerably from more mature athletes. We know athletes under the age of 18 may be more vulnerable to the effects of brain injury[105] and take longer to recover.[5] The adolescent brain is undergoing functional alterations in cerebral blood flow perfusion,[106] rapid executive function development, and psychosocial changes.[5] It is unknown how repetitive head impact exposure influences the neurophysiologic function in this age group. The proposed study's short-term objective is to study the effect of a competitive sport season on high school athletes' cerebrovascular function.

Approach

Our overall objective is to determine the effect of a competitive sport season on the neurophysiology of high school collision sport athletes and compare these effects to those of non-collision sport athletes (**Figure 3.2**). Our central hypothesis is that athletes who participate in collision sports experience repetitive head impact exposure and will demonstrate significantly worse cerebrovascular assessment changes following one competitive season relative to non-collision sports athletes.

Specific Aim 1

To investigate baseline differences in cerebrovascular function between high school collision and non-collision athletes.

Rationale

Recent literature has shown that physiological deficits may outlast clinical recovery following concussion,[21–23] creating a potential area of added value for including objective, physiological measures to assess recovery in concussed athletes. Due to ongoing rapid neurodevelopmental changes, it is critical to be able to describe baseline neurophysiological function in adolescent athletes. Additionally, previous neurophysiological function studies have focused on expensive, non-portable, and inconvenient techniques including functional magnetic

resonance imaging[22,23,95,107] and diffusor tension imaging.[96] Thus, the proposed study will help to establish a valid and reliable representation of cerebrovascular function using more clinically applicable techniques.

Study Design & Participant Recruitment

For Aim 1, we employed a cross-sectional study design in a sample of high school collision sport (n=30) and non-collision athletes (n=23) which included athletes from the following teams: cross country, indoor track and field, and swimming.[108] Participants were included if they were between the ages of 13-19 years old and are a member of the school collision sport team or a non-collision sport team. Participants were not excluded from pre-season testing on the basis of prior concussion, but were excluded from post-season testing (Aim 2) if they sustain a concussion in the interim. Additionally, participants were excluded from the study if they have been diagnosed with sickle cell disease. Patients with sickle cell disease have higher resting cerebral blood flow velocities in all vessels directly stemming from the Circle of Willis, with children with sickle cell disease demonstrating higher velocities than adults with sickle cell disease.[109–112] The research team approached the athletes and parents about the project and screened the participants for inclusion. Participants who met this criterion and either consented (if ≥18 years old) or assented to participate with parental consent were enrolled in the study.

Procedures

Prior to beginning their respective athletic seasons, all participants completed a brief demographic questionnaire. Typical data collection sessions including the following assessments completed in this order: 1) Graded Symptom Checklist (GSC), 2) cerebrovascular TCD assessments to include cerebral blood flow velocity (CBFv), cerebral vasoreactivity (CVR), and neurovascular coupling response (NVC), 3) International Physical Activity Questionnaire – Short Form, and 4) the Test of Word Reading Efficiency – Second Edition (TOWRE-2).

Data Collection

Demographic Questionnaire

The Demographic Questionnaire included questions about the participant's medical history, academic history, and athletic history (**Appendix 1**). As a part of the medical history, participants were asked to self-report presence of attention deficit/hyperactivity disorder (ADHD) and what medications, if any, they were currently taking. Cerebral blood flow perfusion is influenced by ADHD and by common medications for ADHD.[99–101] Additionally, participants were asked if they have sickle cell disease or sickle cell trait. As a part of the academic history, participants were asked to report their school name and current grade level. As a part of the concussion history, participants were asked to report the number of previous concussions they've been diagnosed with, the approximate date of those previous concussions, and approximately how long they were symptomatic. All outcomes from the Demographic Questionnaire were assessed for their influence on our primary cerebrovascular outcomes to determine covariates.

Graded Symptom Checklist

The GSC is an objective measure of symptoms following sport-related concussion (**Appendix 2**). It has been found to be sensitive, reliable, and valid.[113] The checklist includes 22 symptoms that are graded on a Likert scale from 0 (not present) to 6 (severe). The total scores represent the total number of symptoms endorsed and the total severity score. The symptoms include: headache, "pressure in head," neck pain, nausea or vomiting, dizziness, blurred vision, balance problems, sensitivity to light, sensitivity to noise, feeling slowed down, feeling like "in a fog," "don't feel right," difficulty concentrating, difficulty remembering, fatigue or low energy, confusion, drowsiness, more emotional, irritability, sadness, nervous or anxious, and trouble falling asleep (if applicable).

Cerebrovascular function

Cerebrovascular function was measured using transcranial Doppler (TCD) which is sensitive to changes in mean CBFv,[114] cerebrovascular reactivity (CVR),[8] and neurovascular coupling (NVC) response[10] following concussion. Cerebrovascular reactivity (CVR) describes the brain's ability to adapt to varying arteriole partial pressures of carbon dioxide ($PaCO_2$) and is typically assessed by monitoring changes in MCA velocity (MCAv) during hypercapnia or hyponapnia.[8] Cerebrovascular responses to changes in $PaCO_2$ are typically greater than autoregulatory mechanisms indicating that CVR is a significant mediator of cerebral blood flow regulation.[35] Following sport-related concussion, overall CVR response is reduced immediately following injury[9] and may remain impaired for 4 days.[8] CVR deficits have also been found in those with an implied history of subconcussive head impacts, however there is a dearth of research with quantified cumulative subconcussive head impact load.[67] NVC describes the cerebrovascular response to neural activation. During neural activation, there is a demand for both nutrient delivery and by-product removal. The release of the neurotransmitter glutamate activates vasodilators and inhibits vasoconstrictors in both neurons and astrocytes. In a healthy brain, this combined stimulation of neurons and astrocytes creates a four-fold greater increase in cerebral blood flow relative to the energy being consumed to perform the neural task.[47] Following concussion, NVC response has been shown to be elevated, suggesting a period of overcompensation which can last up to two weeks post injury.[10,115] Due to the functional anatomy of the cerebrovascular system, NVC can be easily and reliably measured via TCD by monitoring changes in PCA velocity (PCAv) following a visual task.[48] Transcranial Doppler provides excellent temporal resolution.[39] Using a portable Neural Analytics (Los Angeles, CA) LUCID M1 TCD system, a Doppler probe was placed over the right and left temporal windows and adjusted until an optimal signal is found for the right middle cerebral artery (MCA) and the left posterior cerebral artery (PCA). Once optimal signal is found, transducers were held in place

with an adjustable headset (**Figure 3.3**). Cerebrovascular outcomes are summarized in **Table 3.1**.

Mean CBFv.

While seated at rest, mean CBF velocities were recorded for the right MCA and the left PCA for 2 minutes (eyes-open).

Cerebrovascular Reactivity (CVR).

The CVR protocol assessed the function of the cerebrovascular system in response to hypocapnia (hyperventilation) and hypercapnia (breath-holding). Each participant completed the following protocol (**Figure 3.4**):[8] The participants alternated 20 seconds of breath-holding and 40 seconds of normal breathing 5 times. Following a 2-min recovery period, the participants were instructed to breathe at a rate of 36 breaths per minute with cadence maintained with a metronome for 20 seconds, followed by normal breathing for 40 seconds. They repeated these procedures 5 times. End-tidal carbon dioxide ($P_{ET}CO_2$) and protocol compliance was monitored using a portable Nonin (Plymouth, MN) RespSense I capnograph. Main outcomes from CVR assessment are averaged response curves, breath-holding index (BHI), and vasomotor reactivity response (VMRr). Breath-holding index measures vasodilatory function at the end-range of hypercapnia and is calculated as follows:[116–118]

$$BHI = \frac{MCAv_{peak} - MCAv_{baseline}}{MCAv_{baseline}} \Big/ 20 \text{ seconds of breath-holding}$$

Vasomotor reactivity researve (VMRr) represents the full range of vasodilation and is calculated as follows:[116,118]

$$VMRr = 100 \times \frac{MCAv_{peak} - MCAv_{min}}{MCAv_{baseline}}$$

Graphical representations of these outcomes are shown **Figure 3.5.** Reductions in both BHI and VMRr have been associated with cerebrovascular diseases including carotid stenosis,[116] stroke,[117] and small-vessel disease.[118]

Neurovascular Coupling (NVC).

The NVC protocol assessed the function of the cerebrovascular system in response to two types of visual stimuli. Changes in PCAv was monitored during two visual tasks (**Figure 3.6**):[20] A visual screen (13" Apple Mac with 28.5cm x 18cm visual field) was placed 50-60 cm from the participant. The participants alternated five trials including 20 seconds of eyes-closed and 40 seconds of a reading task. Following a 2-minute recovery period, the participants completed five trials including 20 seconds of eyes-closed and 40 seconds of a search task. The search task included five Where's Waldo challenges. The participant was instructed to search for the full 40 seconds. Main outcome from the NVC assessment include averaged response curves and NVC response magnitude, defined by the area under the first 30 seconds of response curve relative to PCAv 3-5 prior to eyes open for each trial (**Figure 3.7**).

International Physical Activity Questionnaire – Short Form

The International Physical Activity Questionnaire – Short Form (IPAQ-SF) **(Appendix 3)** is a 9-item questionnaire that asks participants to estimate the time spent performing vigorous-intensity activity, moderate-intensity activity, walking, and sitting in the last seven days. . Frequency of activity is measured in days per week and duration in hours/minutes per day. From these frequencies, IPAQ-SF outcomes are recoded into "low," "moderate," or "high" physical activity as defined by the IPAQ working group (**Table 3.2**).[119] The IPAQ-SF demonstrates low criterion validty relative to objective measures, typically overestimating physical activity,[120] but similar validity relative to other self-report questionnaires. [121,122]

Test of Word Reading Efficiency – Second Edition

The Test of Word Reading Efficiency – Second Edition (TOWRE-2) is a quick assessment designed to measure the ability to pronounce words accurately and fluently

(Appendix 4). The TOWRE-2 is split into two subtests, Site Word Efficiency and Phonetic Decoding Efficiency. The Site Word Efficiency subtest includes real words and the Phonetic Decoding Efficiency test includes pseudowords. Participants were instructed to read a list of words from top to bottom as quickly as they can. The raw scores from these assessments are the total number of words the patient is able to read in 45 seconds. The test yields scaled scores and percentile rank which are normalized from raw scores. The TOWRE-2 can be used in patients aged 6-24 years and demonstrates strong criterion validity with average correlation coefficients ranging from 0.89 – 0.96 across subtest and index scores.[123]

Preliminary Data

In order to investigate how cerebrovascular function changes in response to breathing manipulations and visual stimuli using TCD, we conducted pilot testing on a group of 71 uninjured military soldiers(n=42 with concussion history (64.4%)). Cerebrovascular reactivity was measured in the right middle cerebral artery and neurovascular coupling response was measured in the left posterior cerebral artery. The data presented below used the protocol described above (**Figure 3.4 and Figure 3.5**), with the exception that the military soldiers performed breath-holding for 30 seconds rather than the proposed 20 seconds. The results from our preliminary investigation are shown below in **Figures 3.8 and 3.9**. When assessing cerebrovascular reactivity, we found no differences between those with and without a concussion history in averaged response profiles to breath-holding and hyperventilation (**Figure 3.8**). When assessing neurovascular coupling response to visual stimuli, we found that the differential stimulus response depended significantly on concussion history (**Figure 3.9**). Our preliminary findings may indicate that deficits in CVR may recovery over time, while deficits in NVC may still be apparent following injury recovery.

Specific Aim 2

To compare single-season neurophysiological function changes between collision sport high school aged athletes exposed to head impacts and non-collision sport control athletes.

Rationale

By measuring neurophysiological function in high school football athletes, the current study will investigate if neurophysiological and cerebrovascular function differs between those experiencing higher head impact exposure relative to low head impact exposure. Head impact exposure has recently surpassed actual concussive impacts as the factor most likely associated with late-life degenerative disease.[13] Previous head impact exposure studies have focused primarily on college,[103,124] professional.[125,126] Therefore, the present study will provide novel and impactful insight as to neurophysiological function of high school athletes in an understudied at-risk age group.

Study Design and Participant Recruitment

For Aim 2, we employed a prospective cohort study design to assess single-season changes in cerebrovascular function. Following the completion of their respective athletic seasons, all participants from Aim 1 repeated cerebrovascular assessments using the same protocols outlined in Aim 1. Participation in this study terminated after post-season data collection (**Figure 3.2**).

Preliminary Results

The neurophysiological effect of head impact exposure was measured by comparing high school football athletes, who sustained repetitive head impacts to non-collision sport athletes. Previous research has identified that sustaining a single severe head impact does not result in any measurable declines in neurocognition among college football players who otherwise don't endorse any symptoms to the sideline medical team.[127] Over the course of a 3-year study which employed the HIT System into the same high schools that will be used in the proposed study, head impact biomechanics variables remained consistent. Football athletes experienced an average of 474.35, 349.66, and 348.3 impacts per player in 2015, 2016, and 2017, respectively. This is slightly lower than previously published literature for this age group; however, was consistent over time in proposed sample.[12,84] Additionally, average linear

acceleration per impact remained consistent across seasons with 25.7g, 25.4g, and 25.2g in 2015, 2016, and 2017 respectively and was consistent with previously published literature.[12,84]

Data Reduction

Cerebrovascular Function **(Figure 3.10)**

Mean CBF velocity.

Baseline MCA and PCA velocities were derived by averaging velocities across the 2-minute eyes-open baseline period.

Cerebrovascular Reactivity.

All raw TCD data were measured at 125 Hz and filtered using a dual-pass 4th order Butterworth filter (2 Hz cutoff) using custom Matlab scripts (v2018b; Mathworks; Natwick, MA). For both breath-holding and hyperventilation tasks, filtered data were converted to time-series curves representing 60 consecutive 1-second averages for each trial. Time-series curves, calculated relative to the average velocity during the 2-minute eyes open baseline, were averaged across the 5 trials and time-aligned to task onset to generate two separate ensemble-averaged 60-second curves representing CVR response to breath-holding and hyperventilation, respectively, for each participant. To calculate BHI, the percent increase in MCA velocity from baseline to peak velocity during breath-holding was divided by the duration of breath-holding (20 seconds) for each trial[116–118]. The BHI was then averaged across trials. The VMRr was calculated as the percent change in MCA velocity from hyperventilation to breath-holding for each trial [100 × (maximum MCA velocity during breath-holding – minimum MCA velocity during hyperventilation / baseline MCA velocity)][118]. The VMRr was then averaged across trials. For BHI and VMRr, the trial-averaged value for both outcomes were used for analysis.

Neurovascular Coupling.

Filtered task data were converted to time-series profiles representing 40 consecutive 1-second averages for each trial. Profiles were then averaged across the 5 trials and time-aligned to stimulus onset (eyes-open) to generate a single ensemble-averaged 40-second profile

representing NVC response for each participant. To account for unknown insonation angles, PCAv changes were calculated relative to the average baseline PCAv. NVC response magnitude was defined as the area under the PCAv response curve in the first 30 seconds following visual stimulus onset, which was calculated using the trapezoidal rule, representing relative NVC response for each participant. Custom Matlab scripts were used to filter and reduce all data.

Data Analysis and Statistical Plan

Descriptive characteristics of the variables of interest were examined using means (±SDs) for resting MCA and PCA velocity and frequencies for categorical variables. For continuous dependent variables (resting MCA and PCA velocity, resting $P_{ET}CO_2$, BHI, VMRr, and NVC response magnitudes), distributions were assessed for normality using Shapiro-Wilks tests. Outliers were identified as values outside two standard deviations of the mean and removed from the dataset. Based on this criterion, one outlier was removed from the reading task NVC response magnitude analysis. For descriptive analysis, group differences in continuous demographic outcomes (age, height, mass, number of previous concussions) and resting-state MCA and PCA velocity and $P_{ET}CO_2$, total symptom endorsement, total symptom severity, and TWRE indices were calculated using independent samples t-tests. Fisher's exact tests were used to analyze group differences in categorical data with small cell counts (ADHD, IEP, concussion history, and IPAQ-SF category).

Separate analyses were run to determine the effect of potential confounding variables including age, mass, height, concussion history, attention deficit hyperactive disorder (ADHD), Individualized Education Plan (IEP), IPAQ-SF category and TWRE index. All outcomes of interest were assessed for group differences while controlling for potential confounding variables. Additionally, all models were ran removing participants with the presence of potential confounding variables (presence of concussion history, presence of ADHD, presence of an IEP, IPAQ-SF score below high, or a TWRE index below average (TWRE < 90)). Following analysis

of potential confounding variables, no results changed and therefore no covariates were included in final models **(Appendix 5).**

A summary of the analysis plan is provided in **Table 3.3**. For Aim 1 we used independent sample t-tests to assess group differences in BHI, VMRr, and NVC response magnitude to reading and visual search tasks between collision and non-collision sport athletes. For CVR response curves, linear mixed effects models were employed using fixed slopes and random intercepts with cubic mean structures to assess group differences in MCA velocity during the breath-holding and hyperventilation tasks. Relative change in MCA velocity during each task was modeled as a function of repeated time. For NVC response curves, linear mixed effect models were employed with fixed slopes and random intercepts using splines with one knot located at time=5. Relative change in PCA velocity during reading and visual search tasks was modeled as a function of repeated time. All linear mixed models were fit with compound symmetry covariance patterns, and empirical Wald tests based on the robust sandwich covariance estimator were used to evaluate statistical significance.

For Aim 2 we employed separate general linear models to determine group differences in change scores for our continuous dependent variables (breath-holding index, vasomotor reactivity response, and NVC response magnitude). To assess pre- to post- season differences in CVR and NVC response curves, we employed separate general linear mixed models. Pre- to post-season change in ensemble-averaged relative MCAv change curves during each task were modeled as a function of repeated time. For NVC response curves, linear mixed effect models were employed with fixed slopes and random intercepts using splines with one knot located at time=5. Differences in pre- to post-season relative PCAv changes during reading and visual search tasks were modeled using an interaction between group (collision vs. non-collision) and time point (pre- vs. post-season). All linear mixed models were fit with compound symmetry covariance patterns, and empirical Wald tests based on the robust sandwich covariance

estimator were used to evaluate statistical significance. For both aims, alpha was set to 0.05 a priori.

Power Analysis

Power analyses are calculated for analyses used for non-time series outcomes (BHI, VMRr, NVC response magnitude) for Aim 1 and Aim 2 samples sizes (**Table 3.4**).

Expected results, interpretation, possible pitfalls

We have strong and historical working relationship with the school system from which we will recruit our study sample. We do not anticipate issues with compliance in our study. We expect athletes exposed to quantifiably greater head impact exposure will demonstrate declines in neurophysiological function in addition to decreased performance in standardized clinical measures. These changes would point to the biological plausibility that these *repetitive subconcussive head impacts alter the participants' brain structure and function*. If positive associations are established, it would imply repetitive subconcussive head impacts may induce neurological changes similar to concussive impacts. In contrast, a null effect is possible. This would still offer meaningful clinical relevance to distinguish that these advanced neurophysiological assessments are most specific to the effects of concussive head impacts, and do not appear to be impacted by subconcussive—non-injurious—head impacts. Regardless, the presence of these changes by themselves is not a diagnosis of a negative health endpoint, and long-term prospective study would be needed to definitively test the hypothesis that repetitive subconcussive head impacts affects neurobehavioral outcomes. While diverse neurophysiological equipment has been posited as problematic to these study designs, all neurophysiological measurements was completed on the same equipment pre- and post-season.

Timetable for Project

	2019				2020	
	Q1	Q2	Q3	Q4	Q1	Q2
Institutional Review Board approval	+	+				
Recruit high school athletes		+	+			
Aim 1 – Preseason cerebrovascular assessments		+	+			
Aim 2 – Post-season cerebrovascular assessments			+	+	+	
Data Analysis				+	+	+
Prepare and submit Manuscript 1					+	+
Prepare and submit Manuscript 2					+	+

Table 3.1. Cerebrovascular variables measured via transcranial Doppler

Cerebrovascular Variable	Description	Data Reduction	Normal Ranges
Baseline Mean CBFv (cm/s)			
Middle Cerebral Artery	Mean CBFv in the middle cerebral artery	MCAv averaged across a 2-minute resting period	66.6 ± 14.4 cm/s
Posterior Cerebral Artery	Mean CBFv in the posterior cerebral artery	PCAv averaged across a 2-minute resting period	30.9 ± 5.7 cm/s
Cerebrovascular Reactivity			
Breath-holding Index (s^{-1})	Vasodilatory capacity during breath-holding	$\frac{\left(\frac{MCAv_{peak}-MCAv_{baseline}}{MCAv_{baseline}}\right)}{20\text{ s holding breath}}$	≥ 0.69 s^{-1}
Vasomotor Reactivity Response (%)	Vasodilatory capacity during breath-holding and hyperventilation	$100\times\frac{MCAv_{peak}-MCAv_{min}}{MCAv_{baseline}}$	86% ± 16%
Overall Response Curve	Sixty-second average response trajectory following each physiological stressor	Raw data from the 5 trials are averaged every second, then ensemble-averaged resulting in on 60-second response curve	N/A
Neurovascular Coupling (NVC)			
NVC Response Magnitude (cm)	Area under the PCAv response curve in the first 30 seconds following both visual stimuli normalized to baseline PCAv	$\frac{\text{Area under PCAv response curve}_{\ 30\text{ seconds}}}{PCAv_{baseline}}$	N/A
Overall Response Curve	Sixty-second average response trajectory following each visual stressor	Raw data from the 5 trials are averaged every second, then ensemble-averaged resulting in on 60-second response curve	N/A

Values are means ± standard deviation. Cerebral blood flow velocity (CBFv); Middle cerebral artery velocity (MCAv); Posterior cerebral artery velocity (PCAv); Peak middle cerebral artery velocity during breath-hold ($MCAv_{peak}$); Baseline middle cerebral artery velocity ($MCAv_{baseline}$); Minimum middle cerebral artery velocity during hyperventilation ($MCAv_{min}$); Baseline posterior cerebral artery velocity ($PCAv_{baseline}$). N/A indicates values for which normative ranges have not been previous published.

Table 3.2. Physical activity outcomes as measured by the International Physical Activity Questionnaire – Short Form

Level of Physical Activity	***Description***	***Data Reduction***
High	Those who move at least 12,500 steps per day, or the equivalent in moderate and vigorous activities.	a) Vigorous-intensity activity on at least 3 days achieving a minimum total physical activity of at least 1500 MET-min/week **OR** b) 7 or more days of any combination of walking, moderate-intensity or vigorous-intensity activities achieving a minimum total physical activity of at least 3000 MET-min/week
Moderate	Those who do some activity, equivalent to half an hour of at least moderate-intensity physical activity on most days	a) 3 or more days of vigorous-intensity activity of at least 20 min/day **OR** b) 5 or more days of moderate-intensity activity and/or walking of at least 30 min/day **OR** c) 5 or more days of any combination of walking, moderate-intensity or vigorous-intensity activities achieving a minimum total physical activity of at least 600 MET-min/week.
Low	Those who do not meet any of the criteria for either of the previous categories	Those who do not meet criteria for moderate or high physical activity are categorized as 'low'

Total physical activity MET-min/week: sum of Walking MET-min/week + moderate activity MET-min/week + vigorous activity MET-min/week scores; MET-min/week: metabolic rate of an activity multiplied by the minutes the activity is performed each week.

Table 3.3. Statistical analysis plan

Aim	Objective	Variables		Statistical Method
		Dependent	**Independent**	
1	Investigate baseline differences in cerebrovascular function between high school collision and non-collision athletes	**Continuous Variables:** Mean CBF velocity: average MCAv and PCAv during rest CVR: BHI, VMRr NVC: magnitude of response	**Head Impact Exposure:** Collision sport athletes Non-collision sport athletes	Separate general linear models
		Time-Series Variables: CVR response curve NVC response curve		Separate general linear mixed models
2	Compare single-season cerebrovascular function changes between collision and non-collision sport athletes	**Pre- to Post-Season Change Scores:** Mean CBFv CVR: BHI, VMRr NVC: magnitude of response	**Head Impact Exposure:** Collision sport athletes Non-collision sport athletes **Testing Time Point:** Pre-season Post-season	Separate general linear models
		Pre- to Post-Season Change: CVR response curve NVC response curve		Separate general linear mixed models

Table 3.4 Power analysis and effect sizes (Cohen's d) for two-tailed independent samples t-tests (alpha = 0.05).

Aim 1 (n=53)		Aim 2 (n=48)	
Power	Effect Size	Power	Effect Size
0.80	0.80	0.80	0.83

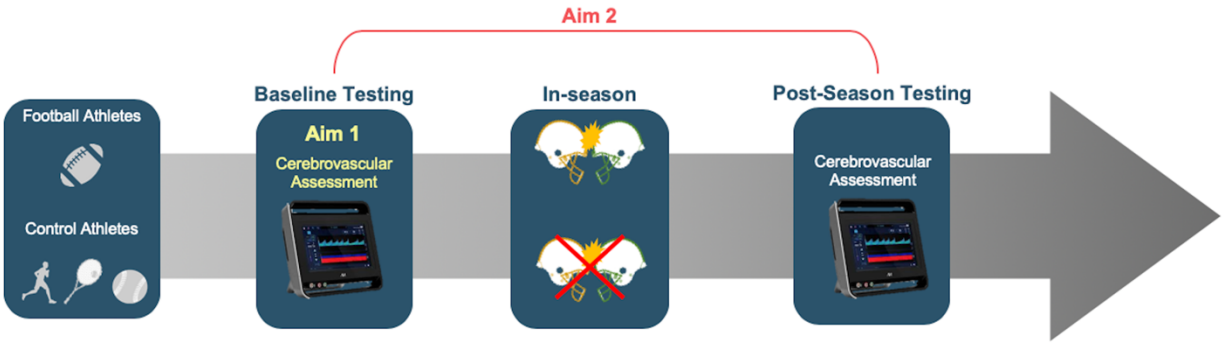

Figure 3.1. Study conceptual model.

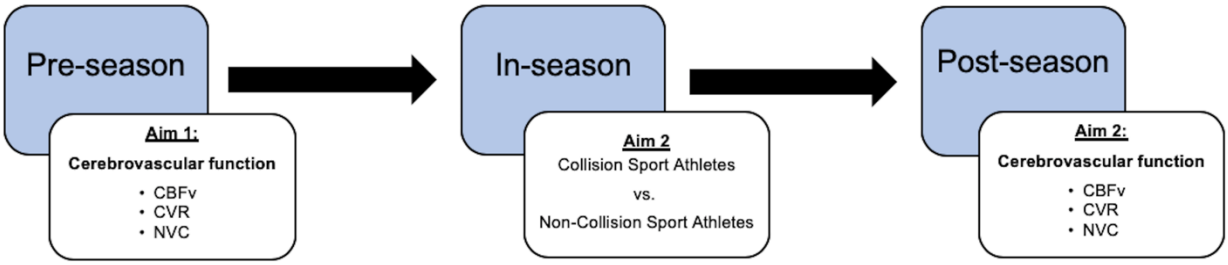

Figure 3.2. Schematic study design

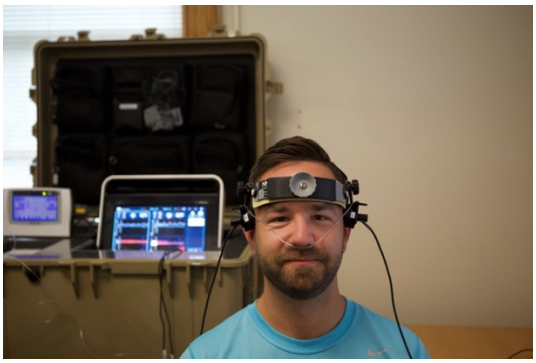

Figure 3.3. Typical participant set-up for transcranial Doppler protocol.

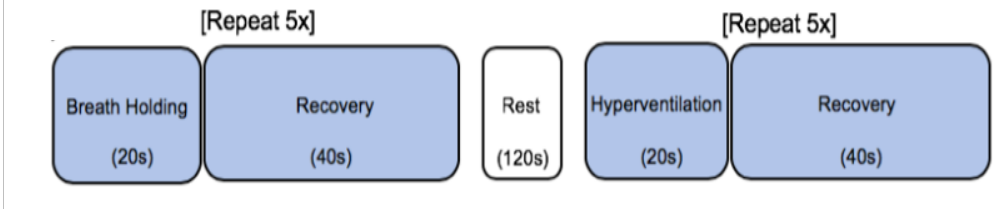

Figure 3.4. Functional transcranial Doppler protocol for cerebrovascular reactivity (CVR). Each participant completed five trials of CVR testing protocol.

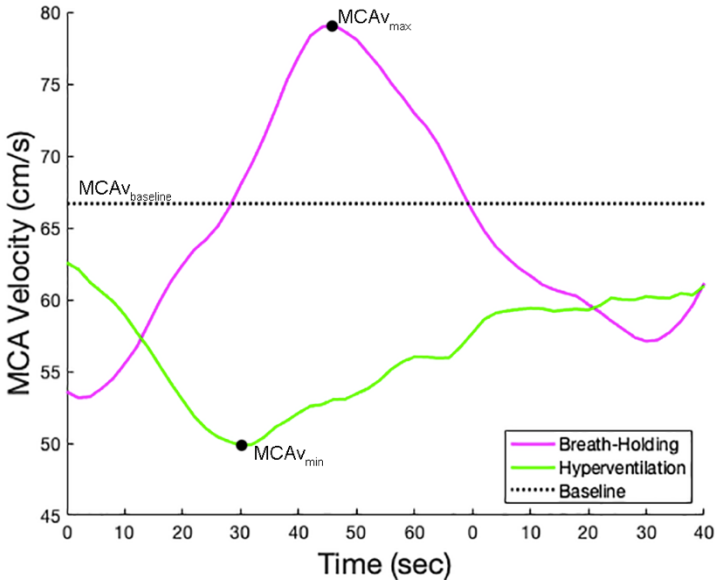

Figure 3.5. Cerebrovascular reactivity assessment outcomes. Representative traces of ensemble-averaged MCAv during breath-holding and hyperventilation. Average response curves are shown in red for breath-holding and orange for hyperventilation. BHI is defined as $[(MCAv_{max} – MCAv_{baseline}) / MCAv_{baseline}]$/ 20 seconds of breath-holding. VMRr is defined as 100 x $[(MCAv_{max} – MCAv_{min}) / MCAv_{baseline}]$.

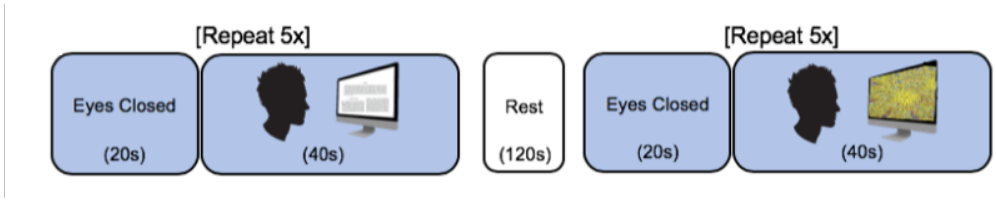

Figure 3.6. Functional transcranial Doppler protocol for neurovascular coupling (NVC).Each participant completed five trials of NVC testing protocol.

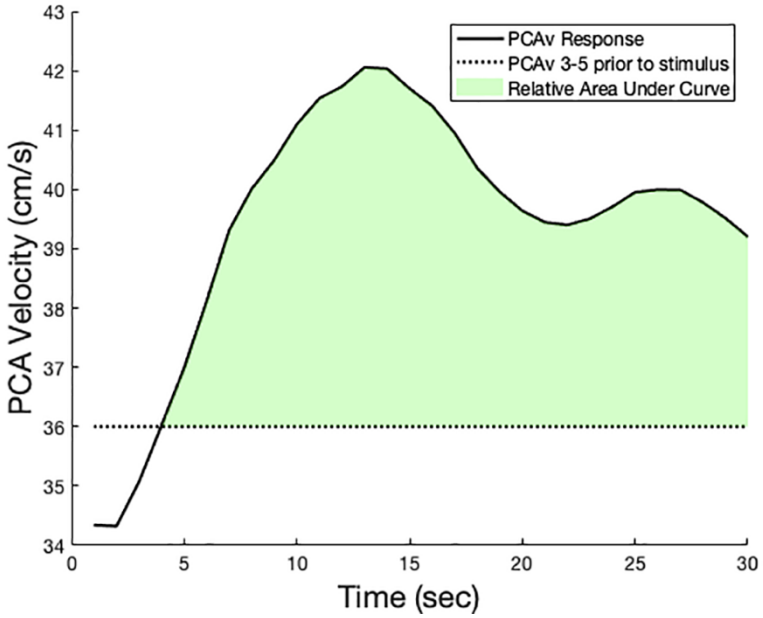

Figure 3.7. Neurovascular coupling (NVC) assessment outcome. Representative trace of ensemble averaged NVC response. Averaged response curve shown in black. NVC response magnitude, defined as area under the first 30 seconds of response curve relative to posterior cerebral artery velocity 3-5 seconds prior to each trial, shown in green.

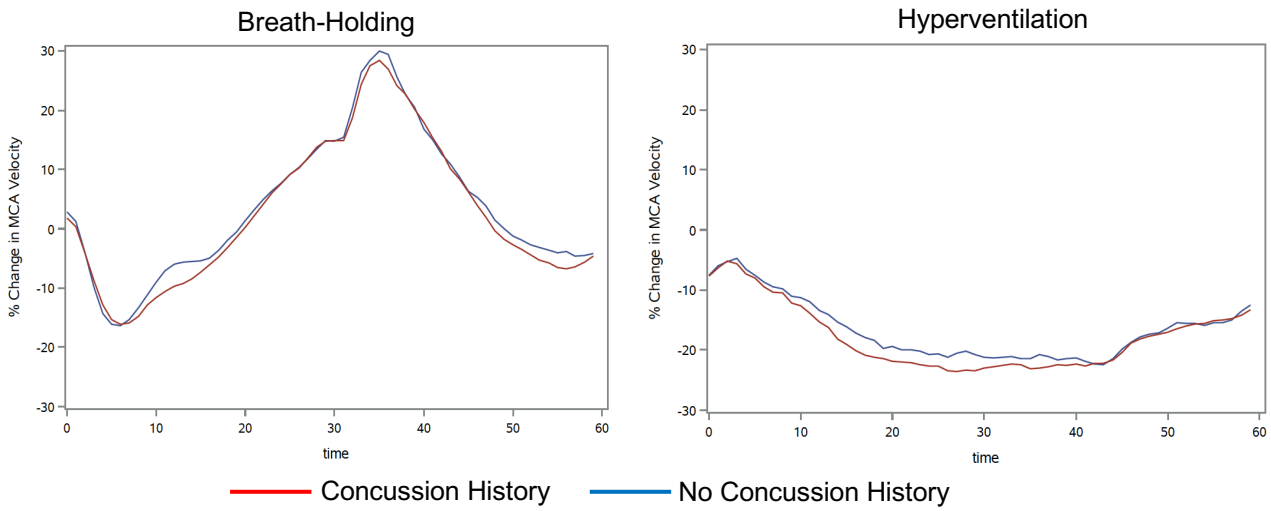

Figure 3.8. Pilot cerebrovascular reactivity data. Averaged response profiles of breath-holding and hyperventilation tasks stratified by concussion history.

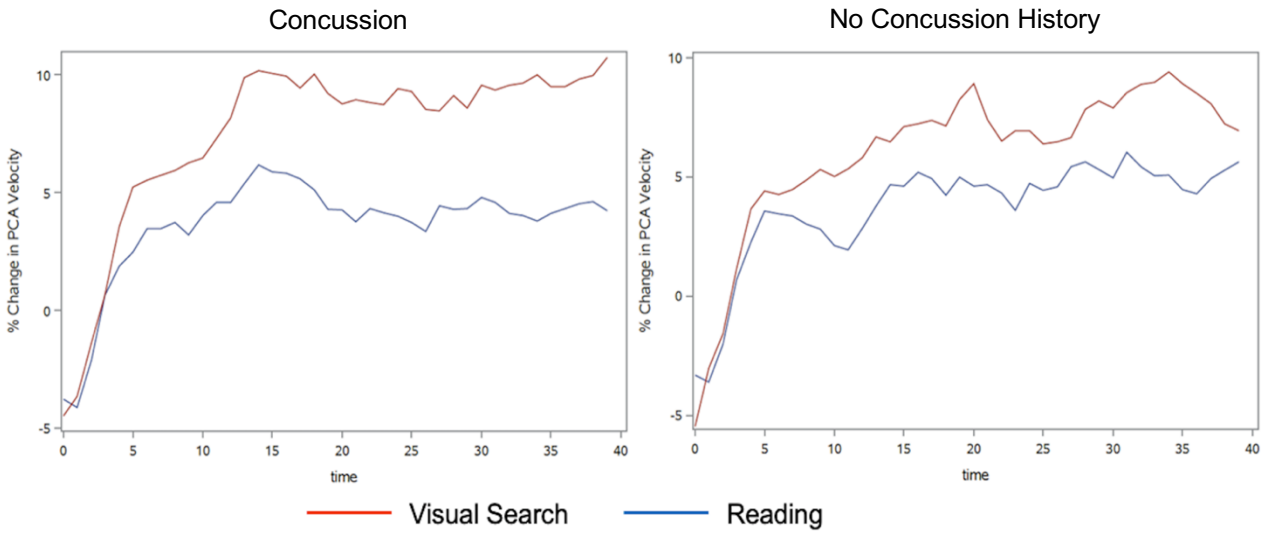

Figure 3.9. Pilot neurovascular coupling (NVC) data. Averaged response profiles of NVC response to a visual stimulus between those with and without concussion history.

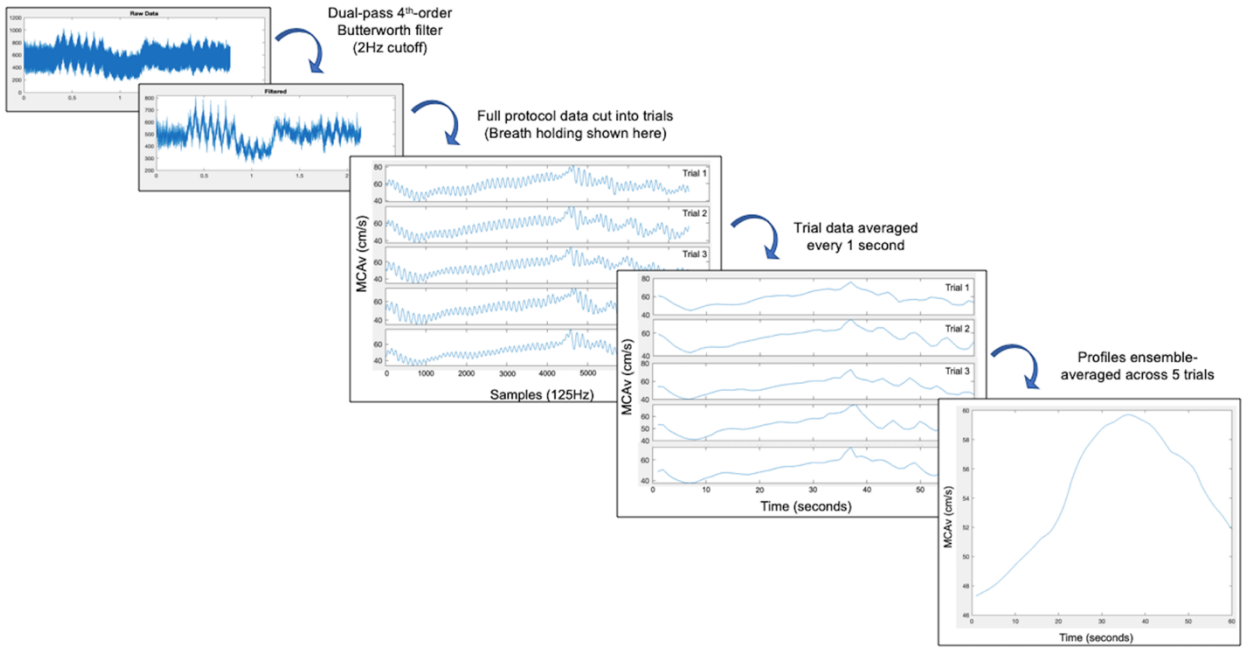

Figure 3.10. Data reduction for all TCD data (breath-holding data shown here). Raw data was filtered using a dual-pass 4th order Butterworth filter (2Hz cutoff). Filtered data was cut to each trial. Trial data was averaged every 1-second and then averaged over the five trials, creating a response curve representing cerebrovascular response to a respiratory or a visual stimulus.

CHAPTER 4

MANUSCRIPT 1 (AIM 1)

Introduction

There is growing concern surrounding the long-term effects of concussion history and cumulative subconcussive head impact exposure, particularly within collision-sport athletes (e.g., American football).[14] Late-life deficits in neurophysiological and psychosocial function have been associated with cumulative subconcussive head impact exposure.[13] Additionally, age of first exposure to repetitive head impacts has been linked to late-life cognitive impairments in former National Football League players,[128] suggesting tackle football participation during neurodevelopment may increase the risk for long-term impairment. However, these studies are limited by self-report information. Additionally, these studies primarily focus on former collegiate[13] and professional athletes,[129] which is concerning as the majority of football athletes do not participate beyond high school.[130] It is important to objectively characterize neurophysiology early in the athletic career to begin understanding the true effects early head impact exposure may have on the late-life neurodegenerative changes.

The brain is undergoing rapid neurodevelopmental changes throughout adolescence. Broadly, the adolescent brain is working to become more efficient. Synaptic pruning begins to remove synapses based on experiences[5] while white matter increases in volume, increasing myelination and neural conduction speed.[5,131] Functionally, this experience-based reorganization leads to immense cognitive and emotional changes.[5] The development of executive function, including thought, behavior, and the ability to adapt to new situational tasks, and social affective abilities including facial recognition, and empathy occurs during this stage.[50] Concurrently, cerebral blood flow steadily increases during childhood and gradually slows

during adolescence[132,133] eventually reaching adult levels around 19 years of age.[134] This active maturation of cerebral blood flow is thought to support the rapid structural changes occurring at a neural level.[133] Due to the dynamic nature of neurodevelopment occurring in this age group, it is critical to establish neurophysiological baseline prior to investigating the effect of head impact exposure.

Proper cerebrovascular function is critical for healthy cerebral metabolism and is a physiological system that is actively changing throughout adolescence making it an important neurophysiological biomarker to investigate in this age group. Cerebral regulation is primarily accomplished through three mechanisms: cerebral autoregulation, cerebrovascular reactivity, and neurovascular coupling.[39] Cerebrovascular reactivity (CVR) describes the brain's ability to adapt to variations in partial pressure of carbon dioxide ($PaCO_2$). Cerebrovascular responses to changes in $PaCO_2$ are typically greater than autoregulatory mechanisms indicating that CVR is a significant mediator of cerebral blood flow regulation.[35] Because $PaCO_2$ is a significant moderator of vasoconstriction and dilation, CVR is typically assessed by measuring middle cerebral artery (MCA) blood flow velocity differences from resting normal $PaCO_2$ (35-45 mmHg) to either hypocapnia ($PaCO_2$ < 35 mmHg) or hypercapnia ($PaCO_2$ > 45 mmHg).[44] Hypocapnia will lead to vasodilation and should cause decreases in cerebral blood flow velocity, while hypercapnia will lead to vasoconstriction and should cause increases in cerebral blood flow velocity. Cerebrovascular reactivity has been commonly quantified using the breath-holding index (BHI), which measures vasodilatory function at the hypercapnic end-range, and the vasomotor reactivity reserve (VMRr), which represents the full vasodilatory range. Reductions in both BHI and VMRr have been associated with cerebrovascular diseases including carotid stenosis,[116] stroke,[117] and small-vessel disease.[118] Neurovascular coupling (NVC) describes the cerebrovascular response to neural activation. During neural activation, there is a demand for both nutrient delivery and by-product removal. The release of the neurotransmitter glutamate activates vasodilators and inhibits vasoconstrictors in both neurons and astrocytes.[47] In a

healthy brain, this combined stimulation of neurons and astrocytes creates a four-fold greater increase in cerebral blood flow relative to the energy being consumed to perform the neural task.[47] Due to the functional anatomy of the cerebrovascular system, NVC can be easily and reliably measured through a visual, cognitive, or motor task.[39] Assessing NVC in response to a visual stimulus was first introduced by Aaslid et al.[48] in 1987, who showed a rapid response to light stimulation in the posterior cerebral artery (PCA). Since then, several different visual stimuli have been used to provoke NVC response ranging in difficulty from turning on lights[48] to a *Where's Waldo?* visual search task.[49]

Advanced neuroimaging techniques such as Transcranial Doppler (TCD) have been used to measure changes in cerebral blood flow velocity (CBFv) in the MCA and PCA to vasoactive and visual stimuli, respectively. In previous TCD studies, deficits have been found in both CVR and NVC acutely following sport-related concussion. [8–10] The studies either did not use report pre-season values or did not use a control group. In order to better interpret post-injury outcomes, it is imperative to describe baseline cerebrovascular function in adolescent athletes. Therefore, the primary purpose of this study was to investigate baseline differences in cerebrovascular function as measured by CVR and NVC between adolescent collision sport and non-collision sport athletes. We hypothesized that there would be no significant differences in CVR or NVC outcomes between these otherwise healthy groups of athletes in the absence of acute head impact exposure.

Methods

Study Design

Fifty-three high school-aged athletes (age=15.8 ± 1.2 yrs, height= 175.8 ± 8.1 cm, mass= 69.4 ± 13.5 kg) were recruited into two groups based on current sport participation to participate in this cross-sectional study. Collision sport athletes (n=30, 56.7%) included football players. To be eligible for participation, athletes had to be a high school-aged (13-19 years old), rostered, and able to participate in sport. Athletes were excluded if they had sickle cell disease

or a concussion in the last 6 months. Patients with sickle cell disease have higher resting cerebral blood flow velocities in all vessels directly stemming from the Circle of Willis, with children with sickle cell disease demonstrating higher velocities than adults with sickle cell disease.[109–112] No participants were excluded on these grounds. The research team approached the athletes and parents about the project and screened the participants for inclusion. Participants who met this criterion and either consented (if ≥18 years old) or assented to participate with parental consent were enrolled in the study. This study was approved by the University of North Carolina at Chapel Hill institutional review board prior to study initiation.

Procedure

All participants completed testing either in their high school athletic training room or a laboratory setting. Prior to testing, all participants completed a demographic questionnaire which included questions about the participant's medical history, academic history, and athletic history. As a part of the medical history, participants were asked to self-report presence of attention deficit/hyperactivity disorder (ADHD), sickle cell disease, concussion history, and if they currently had an Individualized Education Plan (IEP) **(Table 4.1).** All participants completed testing prior to the beginning of the athletic season in the following order: 1) Graded Symptom Checklist, 2) Cerebrovascular function, 3) International Physical Activity Questionnaire – Short Form, and 4) the Test of Word Reading Efficiency – 2nd Edition.

Instrumentation

Graded Symptom Checklist (GSC).

The GSC is an objective measure of symptoms following sport-related concussion (**Appendix 2**). It has been found to be sensitive, reliable, and valid.[113] The checklist includes 22 symptoms that are graded on a Likert scale from 0 (not present) to 6 (severe). The total scores represent the total number of symptoms endorsed and the total severity score. The symptoms include: headache, "pressure in head," neck pain, nausea or vomiting, dizziness, blurred vision, balance problems, sensitivity to light, sensitivity to noise, feeling slowed down, feeling like "in a

fog," "don't feel right," difficulty concentrating, difficulty remembering, fatigue or low energy, confusion, drowsiness, more emotional, irritability, sadness, nervous or anxious, and trouble falling asleep (if applicable).

Cerebrovascular Function.

Transcranial Doppler ultrasonography was used to monitor cerebral blood velocity. Ultrasound gel was applied to a 1.2 MHz Doppler probe (Lucid M1; NeuralAnalytics, Los Angeles, CA) which was then placed over the right and left temporal windows. The right MCA blood flow velocity was identified at approximately 50-65mm and the left PCA blood flow velocity was identified at approximately 60-70mm. If vessel signals could not be optimized on their respective sides, we attempted to identify the left MCA and right PCA. This occurred in only 2 (3.8%) participants. Previous literature shows no side-to-side differences in either of these vessels.[66,135] Additionally, if either signal could not be found and/or optimized, participant was removed from respective analyses. Of the fifty-three participants we successfully found and optimized 52 MCAs [n=29 collision (96.7%), n=23 non-collision (100%)] and 50 PCAs [n=28 collision (93.3%), n=22 non-collision (95.7%)]. Both signals were adjusted and optimized according to previously published criteria.[39] After vessels were identified and signals optimized, the ultrasound probe was locked in place with a fitted head frame. Partial pressure of end tidal carbon dioxide ($P_{ET}CO_2$) was monitored using a capnograph (RespSense I; Nonin Medical, Plymouth, MA) to ensure protocol compliance.

Once TCD and capnograph were in place, the participant was seated upright in a chair and instructed to move minimally throughout the protocol. While seated at rest with eyes open, mean CBF velocities were recorded for the right MCA, and the left PCA for 2 minutes. Cerebrovascular reactivity was measured using previously published breath-holding and hyperventilation protocols.[8] At the end of baseline collection, participants were instructed to hold their breath for 20 seconds then breathe normally for 40 seconds. Participants were instructed to take a normal breath in prior to holding their breath in order to avoid a Valsalva effect, which

can cause initial MCA velocity to decrease and underestimate reactivity.[116] This breath-holding protocol was repeated five times. Following a 2-min recovery period, participants were instructed to breathe at a rate of 36 breaths per minute with cadence maintained with a metronome for 20 seconds, followed by normal breathing for 40 seconds. They repeated these procedures 5 times. Neurovascular coupling response was measured in the PCA using previously published visual protocols. [20] A visual screen (13" Apple Mac with 28.5cm x 18cm visual field) was be placed 50-60 cm from the participant. The participants alternated five trials including 20 seconds of eyes-closed and 40 seconds of a reading task. Following a 2-minute recovery period, the participants completed five trials including 20 seconds of eyes-closed and 40 seconds of a search task. The search task included five *Where's Waldo?* challenges. Participants were instructed to search for the full 40 seconds.

International Physical Activity Questionnaire – Short Form (IPAQ-SF).

The IPAQ-SF **(Appendix 3)** is a 9-item questionnaire that asks participants to estimate the time spent performing vigorous-intensity activity, moderate-intensity activity, walking, and sitting in the last seven days. Frequency of activity is measured in days per week and duration in hours/minutes per day. From these frequencies, IPAQ-SF outcomes are recoded into "low," "moderate," or "high" physical activity as defined by the IPAQ working group (**Table 3.2**).[119] The IPAQ-SF demonstrates low criterion validity relative to objective measures, typically overestimating physical activity,[120] but similar validity relative to other self-report questionnaires.[121,122]

Test of Word Reading Efficiency – Second Edition (TOWRE-2).

The TOWRE-2 is a quick assessment designed to measure the ability to pronounce words accurately and fluently **(Appendix 4)**. The TOWRE-2 is split into two subtests, Site Word Efficiency and Phonetic Decoding Efficiency. The Site Word Efficiency subtest includes real words and the Phonetic Decoding Efficiency test includes pseudowords. Participants were instructed to read a list of words from top to bottom as quickly as they can. The raw scores from

these assessments are the total number of words the patient is able to read in 45 seconds. The test yields scaled scores and percentile rank which are normalized from raw scores. The TOWRE-2 can be used in patients aged 6-24 years and demonstrates strong criterion validity with average correlation coefficients ranging from 0.89 – 0.96 across subtest and index scores.[123]

Data Reduction

For the GSC, total symptom endorsement was calculated as the total number of symptoms self-reported by the athlete and total symptom severity was calculated as the sum of the self-reported symptom severity.

All raw TCD data were measured at 125 Hz and filtered using a dual-pass 4th order Butterworth filter (2 Hz cutoff) using custom Matlab scripts (v2018b; Mathworks; Natwick, MA). Baseline MCA and PCA velocity data were derived by averaging MCA and PCA velocity across the 2-minute baseline period. For CVR trials, filtered data were converted to time-series curves representing 60 consecutive 1-second averages for both breath-holding and hyperventilation tasks. Time-series curves were calculated relative to the average velocity during the 2-minute eyes open baseline to account for unknown insonation angles. Relative curves were then averaged across the 5 trials and time-aligned to task onset to generate two separate ensemble-averaged 60-second curves representing CVR response to breath-holding and hyperventilation, respectively, for each participant. To calculate BHI, the percent increase in MCA velocity from baseline to peak velocity during breath-holding was divided by the duration of breath-holding (*2*0 seconds) for each trial[116–118]. The BHI was then averaged across trials. The VMRr was calculated as the percent change in MCA velocity from hyperventilation to breath-holding for each trial [100 × (maximum MCA velocity during breath-holding – minimum MCA velocity during hyperventilation / baseline MCA velocity)][118]. The VMRr was then averaged across trials. For BHI and VMRr, the trial-averaged value for both outcomes were used for analysis **(Figure 4.1)**. For NVC trials, filtered task data were converted to time-series profiles representing 40

consecutive 1-second averages for stimulus response during each trial. To account for unknown insonation angles, time-series profiles were calculated relative to the average PCA velocity 3-5 seconds prior to stimuli onset. Profiles were then averaged across the 5 trials and time-aligned to stimulus onset to generate a single ensemble-averaged 40-second profile representing relative NVC response for each participant. The NVC response magnitude was defined as the area under the relative PCA velocity response curve during the first 30 seconds after stimulus onset, which was calculated using the trapezoidal rule **(Figure 4.2)**.[49]

For the IPAQ-SF, the total number of minutes and days of self-report physical activity were calculated for each athlete and categories were determined using established IPAQ guidelines.[119] For the TOWRE-2, the total number of correctly pronounced words from the Site Word Efficiency and Phonetic Decoding Efficiency subtests were scaled using age-based normative values, resulting in two scaled subtest scores. The two scaled subtest scores were totaled and indexed to create a Total Word Reading Efficiency Index (TWRE), which corresponds to the following descriptive terms: Very Poor = < 70, Poor = 70-79, Below Average = 80-89, Average = 90-110, Above Average = 111-120, Superior = 121-130, Very Superior > 130. [136]

Statistical Analysis

Descriptive characteristics of the variables of interest were examined using means (±SDs) for resting MCA and PCA velocity and frequencies for categorical variables. For continuous dependent variables (resting MCA and PCA velocity, resting $P_{ET}CO_2$, BHI, VMRr, and NVC response magnitudes), distributions were assessed for normality using Shapiro-Wilks tests. Outliers were identified as values outside two standard deviations of the mean and removed from the dataset. Based on this criteria, one outlier was removed from the reading task NVC response magnitude analysis. For descriptive analysis, group differences in continuous demographic outcomes (age, height, mass, number of previous concussions) and resting-state MCA and PCA velocity and $P_{ET}CO_2$, total symptom endorsement, total symptom severity, and

TWRE indices were calculated using independent samples t-tests. Fisher's exact tests were used to analyze group differences in categorical data with small cell counts (ADHD, IEP, concussion history, and IPAQ-SF category).

Separate analyses were run to determine the effect of potential confounding variables including age, mass, height, concussion history, attention deficit hyperactive disorder (ADHD), Individualized Education Plan (IEP), IPAQ-SF category and TWRE index. All outcomes of interest were assessed for group differences while controlling for potential confounding variables. Additionally, all models were ran removing participants with the presence of potential confounding variables (presence of concussion history, presence of ADHD, presence of an IEP, IPAQ-SF score below high, or a TWRE index below average (TWRE < 90)). Following analysis of potential confounding variables, no results changed and therefore no covariates were included in final models **(Appendix 5).**

Independent sample t-tests were employed to assess group differences in BHI, VMRr, and NVC response magnitude to reading and visual search tasks between collision and non-collision sport athletes. For CVR and NVC response curves, ensemble-averaged responses were plotted to inform the mean structures of the statistical models. For CVR response curves, linear mixed effects models were employed using fixed slopes and random intercepts with cubic mean structures to assess group differences in MCA velocity during the breath-holding and hyperventilation tasks. Relative change in MCA velocity during each task was modeled as a function of repeated time. For NVC response curves, linear mixed effect models were employed with fixed slopes and random intercepts using splines with one knot located at time=5. Relative change in PCA velocity during reading and visual search tasks was modeled as a function of repeated time. All linear mixed models were fit with compound symmetry covariance patterns, and empirical Wald tests based on the robust sandwich covariance estimator were used to evaluate statistical significance.

Results

Non-collision sport athletes (n=23, 44.3%) included cross-country, indoor track, and swimming athletes. Within collision sport athletes, football players began playing football at a median age of 13 years (IQR = 4 years). Within non-collision sport athletes, 6 (26.1%) of athletes had contact-sport[108] participation history including basketball, soccer, lacrosse, and wrestling, though no athletes were actively participating in a contact sport at the time of this study. Further demographic information can be found in **Table 4.1.** At rest, there was a significant relationship between group and self-reported levels of physical activity as assessed by the International Physical Activity Questionnaire – Short Form (IPAQ-SF) (p=0.004) such that more collision sport athletes reported high levels of physical activity relative to non-collision sport athletes. Additionally, there was a significant difference in TWRE indices (t_{50}=3.43, p=0.001) such that non-collision sport athletes demonstrated better reading efficiency relative to collision sport athletes. Graded symptom checklist scores did not differ between groups (P>0.05). Graded symptom checklist scores, IPAQ-SF categories, and TWRE indices are outlined in **Table 4.2**. At rest, there were no significant differences between collision and non-collision sport athletes in MCA velocity (t_{50}=1.70, p=0.10), PCA velocity (t_{50}=1.70, p=0.10), or $P_{ET}CO_2$ (t_{49}=-1.10, p=0.28).

Cerebrovascular Reactivity

Following data reduction and signal inspection, 52 participants had valid MCA signals (n=29 collision, n=23 non-collision). There were no significant group differences in BHI (t_{50}=0.68, p=0.50) or VMRr (t_{50}=1.70, p=0.10) between collision and non-collision sport athletes **(Table 4.3)**. Additionally, there were no group differences in relative MCA response curves during the breath-holding ($F_{1,2594}$=0.20, p=0.66) or hyperventilation ($F_{1,2594}$<0.001, p=0.99) **(Figure 4.3).**

Neurovascular Coupling

Following data reduction and signal inspection, 50 participants had valid PCA signals (n=28 collision, n=22 non-collision). There were no sig nificant group differences in NVC

response magnitude during the reading task (t_{48}=-0.91, p=0.37) or the visual search task (t_{49}=0.50, p=0.62) between collision sport and non-collision sport athletes **(Table 4.3).** When comparing relative NVC response curves, there were no group differences during the reading task ($F_{1,1448}$=0.82, p=0.36) or the visual search task ($F_{1,1448}$=0.08, p=0.78) **(Figure 4.4)**. There was a significant interaction between group (collision vs. non-collision) and task (reading vs. writing) such that the differential response to task was significantly larger in non-collision athletes relative to collision athletes ($F_{1,2946}$=38.69, p<0.0001) **(Figure 4.5).** On average, the relative response to the visual search task was 3.18% higher than the response to the reading task in non-collision athletes and 1.95% higher in collision athletes.

Discussion

The findings from the current study suggest that otherwise healthy adolescent collision sport athletes do not differ in cerebrovascular function as measured by CVR and NVC when compared to non-collision sport athletes. To our knowledge, this is the first study to investigate baseline cerebrovascular reactivity and neurovascular coupling in adolescent football athletes compared to their non-collision sport peers.

Cerebrovascular Reactivity

Cerebrovascular reactivity plays a critical role in the brain's ability to autoregulate cerebral blood flow and maintain stable cerebral perfusion pressure.[116] This is particularly critical in childhood and adolescence where cerebral blood flow perfusion rates are steadily changing.[54] We assessed CVR through three primary outcomes: BHI, VMRr, and overall response curves.

At baseline, we found no differences between collision sport and non-collision sport athletes in BHI or VMRr. Introduced over 25 years ago by Markus et al.,[116] BHI and VMRr have been widely published as descriptors of vasodilatory capacity under varying physiological stressors. Both outcomes have been associated with vascular disease[118] as well as the risk for ischemic events.[117] These studies have primarily investigated these outcomes in older populations with cerebrovascular disease, which likely accounts for the lack of differences in our

otherwise healthy groups. More recent studies have found significant increases in BHI up to 7 days post-concussion, suggesting that BHI may be sensitive to physiological changes in the acute stage following injury.[137,138] These studies investigated BHI in concussed adolescent athletes that were activity-matched with non-injured controls. Our study adds formative knowledge to this body of literature regarding pre-injury cerebrovascular reactivity of adolescent athletes and may better inform clinical interpretation of post-injury or post-exposure data in future research.

When assessing overall response curves, we found no significant differences between collision and non-collision sport athletes at baseline. Participants in both collision and non-collision sport groups reported a history of concussion (collision n=7 (23.3%); non-collision n=3 (13.0%)) and of contact sport participation (non-collision n=6 (26.1%)), yet groups demonstrated similar MCA velocity responses to hypercapnic and hypocapnic tasks. Research studying adolescent and young adult student-athletes have identified deficits in CVR immediately following injury[8] and as long as 3 months following injury.[139] Diminished CVR has also been reported following subconcussive head impact exposure following normal participation in high school soccer, high school football, and professional boxing.[61,62,67] Of these studies, few investigate the full response curve[9] and those that have only compare differences in relative MCA velocity at specific times within the task (i.e., 0 seconds, 20 seconds, 40 seconds). To our knowledge, our study is the first to look at global changes in the full response curve. In doing so, we are able to provide a more comprehensive look at the MCA response to hypercapnia and hypocapnia.

Neurovascular Coupling

We found no significant differences in NVC response magnitudes or overall response curve to either reading or visual search tasks between collision and non-collision sport athletes at baseline. For both groups, we saw the expected result that NVC response magnitude to the search task was greater than to the reading task. Considered a more complex visual task, a

Where's Waldo? search task results in more saccades and longer visual fixation times,[140,141] requiring greater processing within cortical regions supplied by the PCA.[49] Our results mirror previous research investigating the effect of single-season subconcussive head impact exposure on NVC response magnitude, which found no differences at baseline between contact sport (football and hockey) and non-contact sport controls.[20] Impairments in NVC response magnitude have been found acutely post-concussion in adult collision sport athletes,[10] though NVC changes are not evident following subconcussive head impact exposure.[20]

When assessing overall response curves separately by task, we found no difference between groups, though the differential response to task was significantly greater in non-collision sport athletes relative to collision. This effect seems to be driven by a greater response to the search task and a smaller response to the reading task relative to collision athletes. To our knowledge, analyzing global changes in relative PCA response curves is novel, and the clinical meaningfulness of our findings is unclear. Future research should continue pursuing these outcomes in order to determine how NVC function may be applied clinically.

Limitations

There are limitations to the current study. Our sample was restricted to male athletes, limiting our generalizability of our findings beyond this group. Throughout childhood, cerebral blood flow perfusion is steadily increasing until puberty where trajectories tend to diverge with females experiencing higher perfusion rates than males.[54] Additionally, high school females are known to sustain higher rates of concussions compared to males.[1] Future studies should expand cerebrovascular research to female athletes in order to capture this potentially unique population. We acknowledge limitations regarding the use of TCD to measure cerebrovascular function. Transcranial Doppler is unable to measure absolute cerebral blood flow volume due to the unknown diameter of individual vessels, and therefore must assume vessel diameter does not change, which is an inherent limitation of the equipment. Relative to advanced neuroimaging techniques, TCD lacks spatial resolution but has excellent temporal resolution[39] and is an

inexpensive, non-invasive, portable alternative to advanced imaging. Lastly, we acknowledge that BHI can be calculated with[142] and without[117,118,137] factoring $P_{ET}CO_2$. Measuring $P_{ET}CO_2$ during a complete breath-holding task is not possible. Our methods align with the majority of studies employing breath-holding methodologies.[137]

Conclusions

There is growing concern regarding the long-term effects of youth and adolescent collision sport participation. In order to appropriately interpret life-time neurological changes, is critical to first describe these outcomes in adolescent athletes. Our study indicates that baseline cerebrovascular function does not differ between current collision and non-collision sport athletes and provides formative data that may better inform the clinical meaningfulness of short- and long-term changes in physiological function.

Table 4.1. Demographic and medical history information for participants for total sample and by group.

	Total n=53	**Collision n=30**	**Non-Collision n=23**	**P**	**Effect Size**
Age, *yrs*	15.76 ± 1.17	15.77 ± 1.06	15.75 ± 1.31	0.94	0.02
Height, *cm*	178.83 ± 8.09	175.90 ± 7.93	175.73 ± 8.47	0.94	0.02
Mass, *kg*	69.37 ± 13.52	74.68 ± 12.61	62.43 ± 11.56	<0.001	1.01
ADHD,*+ *n (%)*	13 (25.00%)	5 (17.24%)	8 (34.78%)	0.21	0.27^
IEP, *+ *n (%)*	9 (16.98%)	7 (6.67%)	2 (22.22%)	0.34	0.21^
Concussion History,* *n (%)*	10 (18.87%)	7 (23.33%)	3 (13.04%)	0.48	0.13^
# Previous Concussions+	1.00 IQR = 0 (1-1)	1.00 IQR = 0 (1-1)	1.00 IQR = 0 (1-1)	0.17	0.81
Recency,* *n (%)*				0.03	0.80^
past year	5 (50.00%)	5 (71.43%)	-		
past 2 years	1 (10.00%)	1 (14.29%)	-		
> 2 years	4 (40.00%)	1 (14.29%)	3 (100.00%)		

ADHD: attention deficit hyperactive disorder; IEP: individualized education plan
All p-values represent independent samples t-tests unless otherwise noted
All effect sizes represent Cohen's d unless otherwise noted
*Fisher's Exact tests
^Phi Coefficient
+Median (IQR)

Table 4.2. Means (standard deviations) for resting middle cerebral artery velocity (MCAv), posterior cerebral artery velocity (PCAv), end tidal carbon dioxide ($P_{ET}CO_2$), and graded symptom checklist (GSC) for collision and non-collision sport athletes.

	Collision n=30	**Non-Collision n=23**	**P**	**Effect Size**
Resting MCAv *(cm/s)*	56.23 ± 10.89	60.61 ± 10.56	0.15	0.41
Resting PCAv *(cm/s)*	36.44 ± 6.32	38.31 ± 5.67	0.28	0.31
Resting $P_{ET}CO_2$ *(mmHg)*	41.09 (3.78)	39.85 (4.80)	0.28	0.32
Graded Symptom Checklist				
Total Endorsement	1.87 ± 2.93	2.57 ± 2.63	0.43	-0.25
Total Severity	2.97 ± 5.18	3.22 ± 3.57	0.84	-0.06
TWRE Index[+]	108.30 ± 9.44	96.67 ± 13.69	0.001	0.99
IPAQ-SF[+]**,** *n (%)*	2	1	0.004	0.42[^]
Low	0	0		
Moderate	0	6 (27.27%)		
High	30 (100.00%)	16 (72.73%)		

All p-values represent independent samples t-tests unless otherwise noted
All effect sizes represent Cohen's d unless otherwise noted
[^]Phi Coefficient
[+]1 missing

Table 4.3. Cerebrovascular outcomes by group reported as means ± standard deviation.

	Collision n=30	**Non-Collision n=23**	**P**	**Effect Size**
BHI (s^{-1})	1.37 ± 0.53	1.49 ± 0.70	0.50	0.19
VMRr (%)	45.62 ± 9.92	49.30 ± 15.24	0.10	0.29
NVC Response Magnitude (cm/s)				
Reading Task	32.54 ± 2.14	32.01 ± 1.80	0.37	0.27
Visual Search Task	33.22 ± 3.11	33.71 ± 3.32	0.59	0.15

BHI: Breath-holding index; VMRr: vasomotor reactivity response; NVC: neurovascular coupling
All p-values represent independent samples t-tests, all effect sizes represent Cohen's d

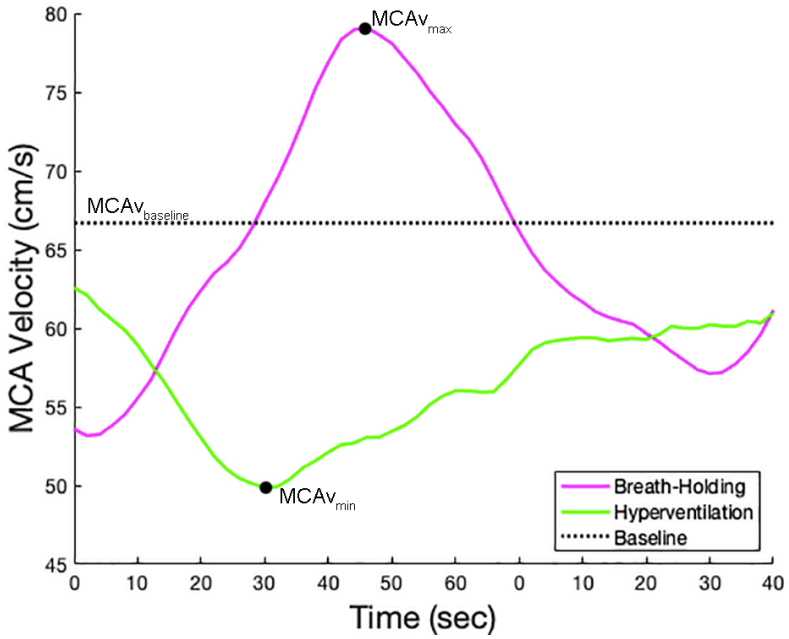

Figure 4.1. Cerebrovascular reactivity assessment outcomes. Representative traces of ensemble-averaged MCAv during breath-holding and hyperventilation. Average response curves are shown in red for breath-holding and orange for hyperventilation. BHI is defined as [($MCAv_{max}$ – $MCAv_{baseline}$) / $MCAv_{baseline}$]/ 20 seconds of breath-holding. VMRr is defined as 100 x [($MCAv_{max}$ – $MCAv_{min}$) / $MCAv_{baseline}$].

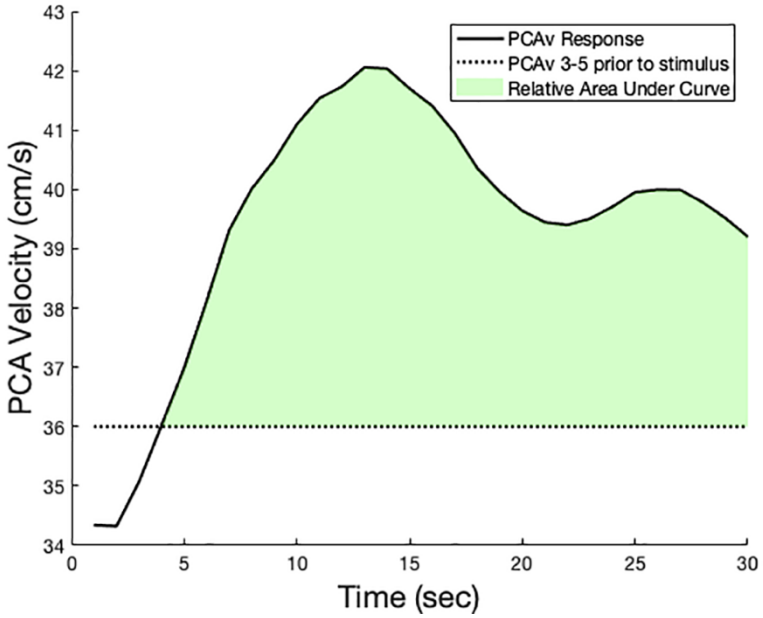

Figure 4.2. Neurovascular coupling assessment outcome. Representative trace of ensemble averaged NVC response. Averaged response curve shown in black. NVC response magnitude, defined as area under the first 30 seconds of response curve relative to PCAv 3-5 seconds prior to each trial, shown in green.

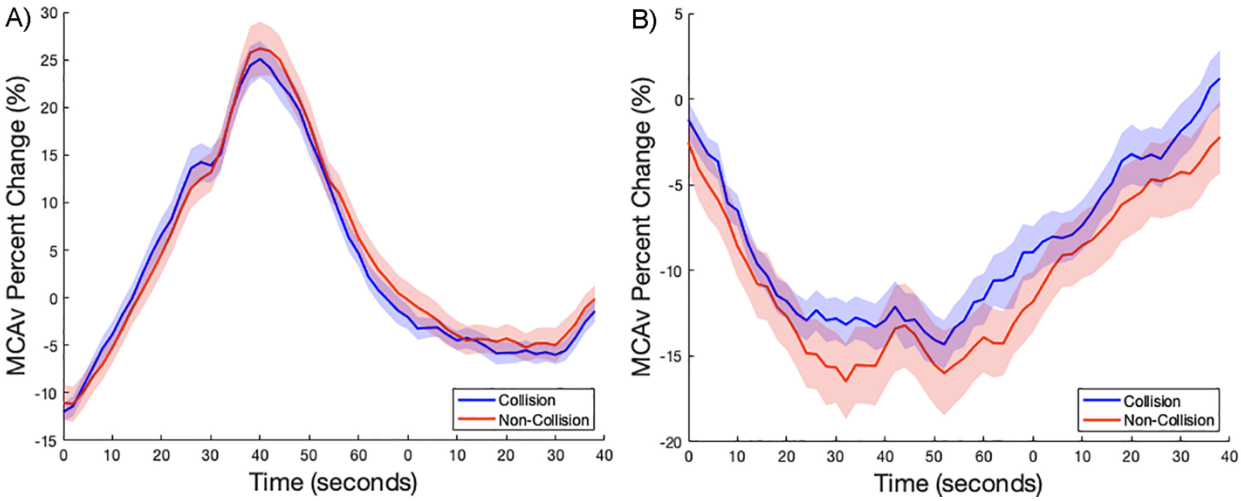

Figure 4.3. Relative MCA velocity ensemble-averaged across 5 trials in response to A) breath-holding task and B) hyperventilation task between collision and non-collision sport athletes. We found no group differences in relative MCA response curves during the breath-holding ($F_{1,2594}$=0.20, p=0.66) or hyperventilation ($F_{1,2594}$<0.01, p=0.99).

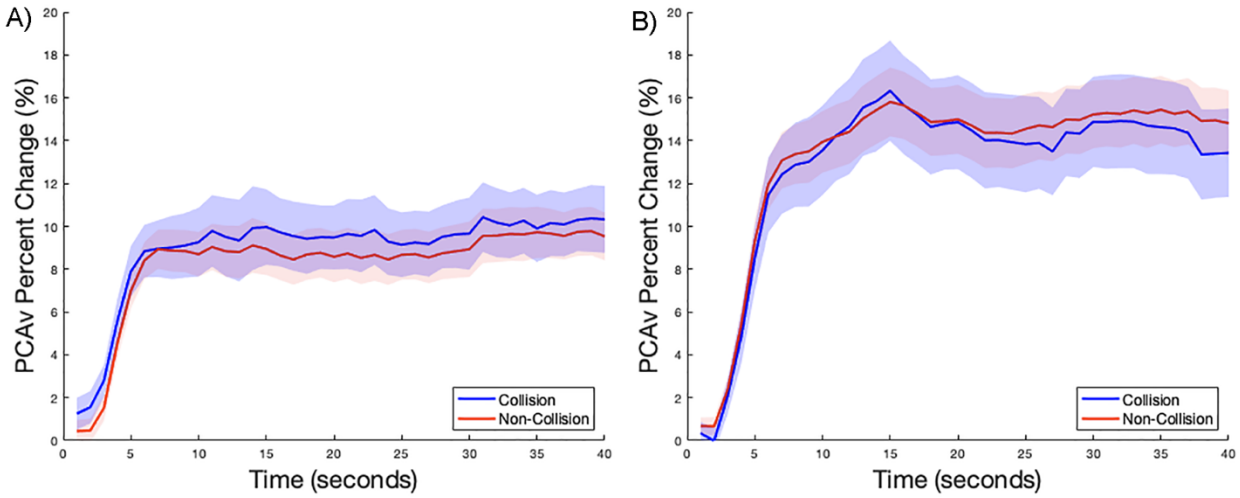

Figure 4.4. Relative PCA velocity ensemble-averaged across 5 trials in response to the A) reading task and the B) search task between collision and non-collision sport athletes. We found no group differences during the reading task ($F_{1,1448}$=0.82, p=0.36) or the search task ($F_{1,1448}$=0.08, p=0.78).

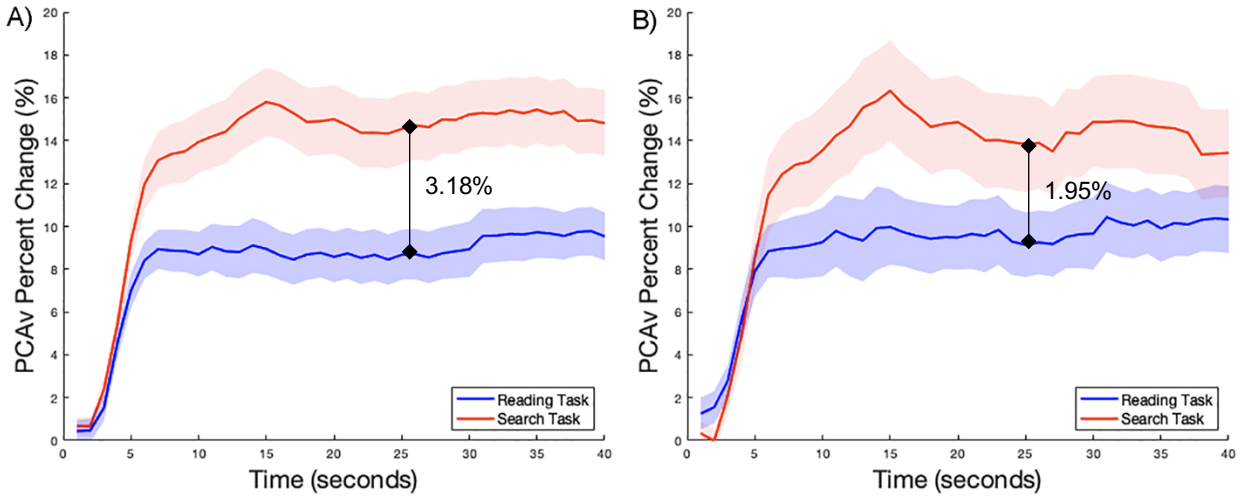

Figure 4.5. Relative PCA velocity ensemble-averaged across 5 trials in response to both tasks in A) non-collision sport athletes and B) collision sport athletes.The differential response to task was significantly greater in non-collision sport athletes compared to collision sport athletes ($F_{1,2946}$=38.69, p<0.0001).

CHAPTER 5

MANUSCRIPT 2 (AIM 2)

Introduction

Recent associative evidence has linked both cumulative subconcussive head impact exposure and younger exposure to repetitive head impacts with late-life cognitive and psychological dysfunction in retired athletes.[13,128] Additionally, white matter tract changes have been found following a single season in youth football athletes aged 13 and younger.[95] This is concerning as youth organized sport participation makes up the largest sport participation demographic[16] and most football players do not play past the high school level.[130] Additionally, subconcussive head impact exposures do not elicit symptoms and are therefore not evaluated. It is currently unclear if cumulative subconcussive head impacts result in measurable neurophysiological deficits in youth and adolescent athletes.

Previous studies assessing the effects of subconcussive head impact exposure have largely focused on older athletes. In college football athletes, short-term clinical outcomes were not associated with head impact biomechanics,[90] did not decline throughout the season,[91] and were not significantly different from noncontact sport athletes.[92] In youth athletics, subconcussive head impacts may have a greater effect on clinical outcomes. Breedlove et al.[93] found that 54.5% of asymptomatic high school football players experienced a substantial deviation from baseline cognitive functioning score throughout their regular season. Additionally, Koerte et al.[94] found that while youth soccer players did not experience significant cognitive changes over the course of a season, the noncontact sport control group experienced cognitive improvement. This suggests repetitive head impact exposure may be associated with a lack of improvement in cognitive performance.

The adolescent brain is undergoing rapid neurodevelopmental changes including functional alterations in cerebral blood flow perfusion,[106] rapid executive function development, and psychosocial changes.[5] The neurological response to repetitive subconcussive head impacts may differ considerably from more mature athletes due to these changes. Athletes younger than 18 years may be more susceptible to the effects of brain injury[105] and take longer to recover.[5] Following a single season of head impact exposure in high school football, deficits have been demonstrated in white matter integrity[143,144] and blood biomarkers,[145] suggesting that physiological impairments may be present without diagnosed injury.

Cerebrovascular regulation to head injury is a key element in concussion pathophysiology and may play a critical role in the neurophysiological response to head impact exposure. Cerebral blood flow is known to decrease following concussion and may remain low depending on severity.[24] Cerebrovascular impairments have also been shown following a single-season of repetitive head impact exposure[61,146,147] suggesting that cerebrovascular assessments may be sensitive to subtle neurophysiological changes following subconcussive head impacts. Cerebrovascular regulation is primarily accomplished through three mechanisms: 1) cerebral autoregulation, 2) cerebrovascular reactivity, and 3) neurovascular coupling.[39] Cerebrovascular reactivity (CVR) describes the brain's ability to adapt to variations in partial pressure of carbon dioxide ($PaCO_2$) and is typically assessed by monitoring changes in middle cerebral artery (MCA) velocity in response to either hypocapnia or hypercapnia.[44] Hypocapnia will lead to vasodilation and should cause decreases in cerebral blood flow velocity, while hypercapnia will lead to vasoconstriction and should cause increases in cerebral blood flow velocity. Cerebrovascular reactivity has been commonly quantified using the breath-holding index (BHI) and vasomotor reactivity reserve (VMRr). The BHI measures vasodilatory function at the hypercapnic end-range and VMRr represents the full vasodilatory range. Reductions in both BHI and VMRr have been associated with cerebrovascular diseases including carotid stenosis,[116] stroke,[117] and small-vessel disease.[118] Neurovascular coupling (NVC) describes the

cerebrovascular response to neural activation and is typically assessed by monitoring changes in posterior cerebral artery (PCA) velocity in response to visual stimuli.[47]

Advanced neuroimaging techniques such as Transcranial Doppler (TCD) have been used to measure changes in cerebral blood flow velocity (CBFv) in the MCA and PCA to vasoactive and visual stimuli, respectively. As functional cerebrovascular outcomes appear to be sensitive to subtle physiological changes following head impact exposure in collegiate and professional athletes, further investigation into cerebrovascular function in developing adolescent athletes is warranted. Therefore, the primary purpose of this study was to investigate the effect of repetitive head impact exposure on cerebrovascular function by assessing single season changes in CVR and NVC in adolescent collision sport and non-collision sport athletes. We hypothesized that collision sport athletes will demonstrate greater single-season changes in cerebrovascular function when compared to non-collision sport athletes. Specifically, collision sport athletes will demonstrate significantly reduced cerebrovascular reactivity outcomes and significantly elevated neurovascular coupling outcomes relative to non-collision sport athletes.

Methods

Fifty-three high school-aged athletes were recruited for participation in this prospective cohort study. Forty-eight collision and non-collision sport athletes (age=16.0 ± 1.2yrs, height=175.5 ± 8.1cm, mass= 68.6 ± 14.0kg) completed both pre- and post-season testing and were included in the final analysis. Collision sport athletes (n=25, 52.1%) included football players and non-collision sport athletes (n=23, 47.9%) included cross-country, indoor track, and swimming athletes. Within collision sport athletes, football players began playing football at a median age of 13 years (IQR = 4 years). Within non-collision sport athletes, 6 (26.1%) of athletes had contact-sport[108] participation history including basketball, soccer, lacrosse, and wrestling, though no athletes were actively participating in a contact sport at the time of this study. Further demographic information can be found in **Table 5.1.** To be eligible for participation, athletes had to be a high school-aged (13-19 years old), rostered, and able to

participate in sport. Athletes were excluded if they had sickle cell disease, a concussion in the last 6 months, or sustained a concussion during the study period. All participants completed testing either in their high school athletic training room or a laboratory setting prior to the start of their respective athletic seasons. Post-season was repeated 4-5 months following preseason testing (median = 113 days, IQR = 23 days). The research team approached the athletes and parents about the project and screened the participants for inclusion. Participants who met these criteria were informed of the study and its potential risks. All participants provided consent (if ≥18 years old) or assent (<18 years old) prior to completing any of the study procedures. Parental consent was also obtained for minors. The study was reviewed and approved by our institution's Office of Human Research Ethics.

Procedure

All participants completed testing either in their high school athletic training room or a laboratory setting. Prior to testing, all participants completed a demographic questionnaire which included questions about the participant's medical history, academic history, and athletic history. As a part of the medical history, participants were asked to self-report presence of attention deficit/hyperactivity disorder (ADHD), sickle cell disease, concussion history, and if they currently had an Individualized Education Plan (IEP) **(Table 5.1).** All participants completed testing prior to the beginning of the athletic season in the following order: 1) Graded Symptom Checklist, 2) Cerebrovascular function, 3) International Physical Activity Questionnaire – Short Form, and 4) the Test of Word Reading Efficiency – 2nd Edition.

Instrumentation

Graded Symptom Checklist

The Graded Symptom Checklist (GSC) is a sensitive, reliable, and valid[113] measure of symptoms following sport-related concussion (**Appendix 2**). The checklist includes 22 symptoms that are graded on a Likert scale from 0 (not present) to 6 (severe). Two measures are usually collected from the GSC: total number of symptoms endorsed (maximum score: 22)

and the total severity score, which is obtained by adding all the individual symptom severity scores (maximum score: 132). The symptoms include: headache, “pressure in head,” neck pain, nausea or vomiting, dizziness, blurred vision, balance problems, sensitivity to light, sensitivity to noise, feeling slowed down, feeling like “in a fog,” “don’t feel right,” difficulty concentrating, difficulty remembering, fatigue or low energy, confusion, drowsiness, more emotional, irritability, sadness, nervous or anxious, and trouble falling asleep (if applicable).

Cerebrovascular Function

Transcranial Doppler (TCD) ultrasonography was used to monitor cerebral blood velocity. Ultrasound gel was applied to a 1.2 MHz Doppler probe (Lucid M1; NeuralAnalytics, Los Angeles, CA) which was then placed over the right and left temporal windows. The right MCA blood flow velocity was identified at approximately 50-65mm and the left PCA blood flow velocity was identified at approximately 60-70mm. If vessel signals could not be optimized on their respective sides, the left MCA and right PCA were attempted. This occurred in 1 (2.1%) participant. Previous literature shows no side-to-side differences in either of these vessels.[66,135] Additionally, if either signal could not be found and/or optimized, the participant was removed from those respective analyses. Both signals were adjusted and optimized according to previously published criteria.[39] After vessels were identified and signals optimized, the ultrasound probe was locked in place with a fitted head frame. Partial pressure of expired carbon dioxide ($P_{ET}CO_2$) was monitored using a capnograph (RespSense I; Nonin Medical, Plymouth, MA) to ensure protocol compliance. Once TCD and capnograph were in place, the participant was seated upright in a chair and instructed to move minimally throughout the protocol. While seated at rest with eyes open, mean CBF velocities were recorded for the right MCA, and the left PCA for 2 minutes.

Cerebrovascular reactivity was measured using previously published breath-holding and hyperventilation protocols.[8] At the end of baseline collection, participants were instructed to hold their breath for 20 seconds then breathe normally for 40 seconds. Participants were instructed

to take a normal breath in prior to holding their breath in order to avoid a Valsalva effect, which can cause initial MCAv to decrease and underestimate reactivity.[116] This breath-holding protocol was repeated five times. Following a 2-min recovery period, participants were instructed to breathe at a rate of 36 breaths per minute with cadence maintained with a metronome for 20 seconds, followed by normal breathing for 40 seconds. They repeated these procedures 5 times.

Neurovascular coupling response was measured in the PCA using previously published visual protocols. [20] A visual screen (13" Apple Mac with 28.5cm x 18cm visual field) was be placed 50-60 cm from the participant. The participants alternated five trials including 20 seconds of eyes-closed and 40 seconds of a reading task. Following a 2-minute recovery period, the participants completed five trials including 20 seconds of eyes-closed and 40 seconds of a search task. The search task included five *Where's Waldo?* Challenges. Participants were instructed to search for the full 40 seconds. Our research team digitally removed Waldo from all presentations to increase search task compliance for the 40-second duration.

International Physical Activity Questionnaire – Short Form

The International Physical Activity Questionnaire – Short Form (IPAQ-SF) **(Appendix 3)** is a 9-item questionnaire that asks participants to estimate the time spent performing vigorous-intensity activity, moderate-intensity activity, walking, and sitting in the last seven days. Frequency of activity is measured in days per week and duration in hours/minutes per day. From these frequencies, IPAQ-SF outcomes are recoded into "low," "moderate," or "high" physical activity as defined by the IPAQ working group (Table 3.2).[119] The IPAQ-SF demonstrates low criterion validity relative to objective measures, typically overestimating physical activity,[120] but similar validity relative to other self-report questionnaires. [121,122]

Test of Word Reading Efficiency – Second Edition

The Test of Word Reading Efficiency – Second Edition (TOWRE-2) is a quick assessment designed to measure the ability to pronounce words accurately and fluently

(Appendix 4). The TOWRE-2 is split into two subtests, Site Word Efficiency and Phonetic Decoding Efficiency. The Site Word Efficiency subtest includes real words and the Phonetic Decoding Efficiency test includes pseudowords. Participants were instructed to read a list of words from top to bottom as quickly as they can. The total number of correctly pronounced words in 45 seconds from the Site Word Efficiency and Phonetic Decoding Efficiency subtests were scaled using age-based normative values, resulting in two scaled subtest scores. The two scaled subtest scores were totaled and indexed to create a Total Word Reading Efficiency Index (TWRE), which corresponds to the following descriptive terms: Very Poor = < 70, Poor = 70-79, Below Average = 80-89, Average = 90-110, Above Average = 111-120, Superior = 121-130, Very Superior > 130.[136] The TOWRE-2 can be used in patients aged 6-24 years and demonstrates strong criterion validity with average correlation coefficients ranging from 0.89 – 0.96 across subtest and index scores.[123]

Data Reduction

All raw TCD data were measured at 125 Hz and filtered using a dual-pass 4th order Butterworth filter (2 Hz cutoff) using custom Matlab scripts (v2018b; Mathworks; Natwick, MA). Baseline MCA and PCA velocity data were derived by averaging MCA and PCA velocity across the 2-minute baseline period. For CVR trials, filtered data were converted to time-series curves representing 60 consecutive 1-second averages for both breath-holding and hyperventilation tasks. Time-series curves were calculated relative to the average velocity during the 2-minute eyes open baseline to account for unknown insonation angles. Relative curves were then averaged across the 5 trials and time-aligned to task onset to generate two separate ensemble-averaged 60-second curves representing preseason CVR response to breath-holding and hyperventilation, respectively, for each participant. This process was repeated to generate two ensemble-average response curves for post-season testing. Preseason relative CVR response velocities were subtracted from post-season relative CVR response velocities for both tasks,

generating two 60-observation change curves for each participant to assess group differences in pre- to post-season change in CVR response to breath-holding and hyperventilation.

To calculate BHI, the percent increase in MCA velocity from baseline to peak velocity during breath-holding was divided by the duration of breath-holding (*2*0 seconds) for each trial[116–118]. The BHI was then averaged across trials. The VMRr was calculated as the percent change in MCA velocity from hyperventilation to breath-holding for each trial:[118]

$$VMRr = 100 \times \frac{MCAv_{peak} - MCAv_{min}}{MCAv_{baseline}}$$

The VMRr was then averaged across trials. For BHI and VMRr, pre- to post-season change scores were calculated from the trial-averaged values for both outcomes and used for analysis. For NVC trials, filtered task data were converted to time-series profiles representing 40 consecutive 1-second averages for stimulus response during each trial. To account for unknown insonation angles, time-series profiles were calculated relative to the average PCAv 3-5 seconds prior to stimuli onset. Profiles were then averaged across the 5 trials and time-aligned to stimulus onset to generate a single ensemble-averaged 40-second profile representing relative NVC response for each participant. NVC response magnitude was defined as the area under the relative PCAv response curve during the first 30 seconds after stimulus onset, which was calculated using the trapezoidal rule.[49] Pre- to post-season change scores in NVC response magnitude were calculated and used to analyze single-season change.

Statistical Analysis

Descriptive characteristics of the variables of interest were examined using means (±SDs) for resting MCA velocity, PCA velocity, and $P_{ET}CO_2$, total symptom endorsement, total symptom severity, and TWRE index and frequencies for categorical variables. For continuous dependent variables (pre- to post-season change scores for resting MCA and PCA velocity, resting $P_{ET}CO_2$, BHI, VMRr, and NVC response magnitudes), distributions were assessed for normality using Shapiro-Wilks tests. Outliers were identified as values outside two standard

deviations of the mean and removed from the dataset. Based on this criteria, one outlier was removed from the reading task NVC response magnitude analysis. Relative MCA velocity and PCA velocity response curves that visually inspected for any physiologically invalid data. Based on this criteria, one participant was removed from the relative PCA response to the reading task. For descriptive analysis, group differences in continuous demographic outcomes (age, height, mass, number of previous concussions) and pre- to post-season change scores for resting-state MCA velocity and PCA velocity were calculated using independent samples t-tests. Due to non-normal distribution, group differences in resting state $P_{ET}CO_2$ change scores were analyzed using Mann-Whitney U tests. Fisher's exact tests were used to analyze group differences in categorical data with small cell counts.

Separate analyses were run to determine the effect of potential confounding variables including age, mass, height, concussion history, attention deficit hyperactive disorder (ADHD), Individualized Education Plan (IEP), IPAQ-SF category and TWRE index. All outcomes of interest were assessed for group differences while controlling for potential confounding variables. Additionally, all models were ran removing participants with the presence of potential confounding variables (presence of concussion history, presence of ADHD, presence of an IEP, IPAQ-SF score below high, or a TWRE index below average (TWRE < 90)). Following analysis of potential confounding variables, no results changed and therefore no covariates were included in final models **(Appendix 5).**

Independent sample t-tests were employed to assess group differences in pre- to post-season change in BHI, VMRr, and NVC response magnitude to reading and visual search tasks between collision and non-collision sport athletes. To assess pre- to post- season differences in CVR response curves, we employed separate general linear mixed models. Pre- to post-season change in ensemble averaged relative MCAv change curves during each task were modeled as a function of repeated time. For NVC response curves, linear mixed effect models were employed with fixed slopes and random intercepts using splines with one knot located at

time=5. Differences in pre- to post-season relative PCAv changes during reading and visual search tasks were modeled using an interaction between group (collision vs. non-collision) and time point (pre- vs. post-season). All linear mixed models were fit with compound symmetry covariance patterns, and empirical Wald tests based on the robust sandwich covariance estimator were used to evaluate statistical significance. Alpha was set to 0.05 a priori.

Results

Time between pre-season and post-season testing was not significantly different ($t_{28.09}$=-1.73, p=0.09) between collision (mean=118.6 ± 12.2 days) and non-collision sport athletes (mean=106.6 ± 31.2 days). Of the 48 participants, we successfully found and optimized 46 MCAs [n=23 collision (92.0%), n=23 non-collision (100%)] and 47 PCA [n=24 collision (96.0%), n=23 non-collision (100%)] at pre- and post-season testing. From pre- to post-season, 11 (44.0%) collision sport athletes went from “high” to “moderate” self-reported physical activity on the IPAQ-SF while non-collision sport athletes did not change Both collision and non-collision sport athletes demonstrated improved TWRE indices from pre- to post-season. There were no significant differences between collision and non-collision sport athletes in single-season changes in resting MCA velocity (t_{44}=1.22, p=0.23), PCA velocity (t_{45}=1.74, p=0.09), or $P_{ET}CO_2$ (collision median = -1.7, IQR=7.8; non-collision median = 0.6, IQR=4.6; U=529.0, p=0.44, r=0.11)(**Table 5.2**).

Cerebrovascular Reactivity

Following data reduction and signal inspection, 46 participants had valid MCA signals (n=23 collision, n=23 non-collision). There was a significant group difference in pre- to post season BHI changes (t_{44}=-2.21, p=0.03) such that BHI in collision sport athletes increased by an average of $0.31s^{-1}$ and BHI in non-collision sport athletes decreased by an average of $0.11s^{-1}$ over the course of a single season. There were no significant group differences in VMRr (t_{44}=-0.72, p=0.48) between collision and non-collision sport athletes **(Table 5.2)**. Additionally, there were no group differences in single-season changes in relative MCA response curves during the

breath-holding ($F_{1,2294}$=0.27, p=0.60) **(Figure 5.1)** or hyperventilation ($F_{1,2294}$=0.35, p=0.55) **(Figure 5.2)**. Graphical representation of preseason and post-season relative MCA response curves by for both collision and non-collision sport athletes are shown in **Figure 5.3.** Graphical representation of mean group differences in pre- to post-season relative MCA curves for both breath-holding and hyperventilation tasks are shown in **Figure 5.4.**

Neurovascular Coupling

Following data reduction and signal inspection, 47 participants had valid PCA signals (n=24 collision, n=23 non-collision). There were no significant group differences in single-season NVC response magnitude change during the reading task (t_{44}=1.98, p=0.05) or the visual search task (t_{44}=-0.41, p=0.68) between collision sport and non-collision sport athletes **(Table 5.2).** When comparing relative NVC response curves, there was a significant interaction between group (collision vs. non-collision) and time point (pre- vs. post season) during the reading task ($F_{1,2710}$=101.54, p<0.001) **(Figure 5.5)**. Overall NVC response curves decreased by an average of 0.69% in collision sport athletes and increased by an average of 2.7% in non-collision sport athletes over the course of a single season. There were no significant interaction effects during the visual search task ($F_{1,2769}$=-0.24, p=0.80) **(Figure 5.6).** Graphical representation of preseason and post-season relative PCA response curves by for both collision and non-collision sport athletes are shown in **Figure 5.7.**

Discussion

This study investigated single-season changes in cerebrovascular function between high school-aged collision and non-collision sport athletes. We hypothesized that collision sport athletes would demonstrate significantly reduced cerebrovascular reactivity outcomes and significantly elevated neurovascular coupling outcomes relative to non-collision sport athletes. Our findings suggest that cerebrovascular function, specifically BHI and NVC response to reading, may be influenced by sport participation though clinical interpretations remain unclear.

Cerebrovascular Reactivity

Cerebrovascular reactivity is considered a significant mediator of cerebral blood flow regulation.[35] In assessing single-season changes in CVR, our study found that non-collision sport athletes demonstrated reduced BHI, while collision sport athletes demonstrated increased BHI. Breath-holding index is an outcome first coined by Markus et al.,[116] which describes vasodilatory function at the end-range of hypercapnia and has been noted as a predictor for ischemic events.[117] In more severe cerebrovascular pathologies, such as stroke and carotid artery occlusion,[117,148] higher indices are considered better with indices below 0.69 considered abnormal. Though we found significant group differences in single-season changes, the average BHI for both collision and non-collision sport athletes in the current study were above the previously describe index cut-off for more severe cerebrovascular pathology, indicating that otherwise healthy high school-aged athletes report normal BHI. Following concussion, BHI has been shown to be elevated immediately post-injury and may remain increased up to 7 days.[137] Our collision sport athletes demonstrated single-season increases in BHI, more closely resembling previous CVR literature in concussion, and may further support the uniqueness of the cerebrovascular sequalae following concussion and subconcussive head impact exposure.

When assessing single-season changes in overall CVR response curves, we found no differences between collision and non-collision sport athletes. Impairments in CVR following a single-season of repetitive head impact exposure have been described in collegiate[146] and high school[61] football athletes and high school female soccer athletes.[147] These studies all used advance imaging techniques (function magnetic resonance imaging) to assess changes, which are expensive, invasive, and not feasibly administered in an adolescent population. Studies utilizing TCD to assess CVR following concussion have shown significant impairments immediately following injury[8] and as long as 3 months following injury.[139] Of these studies, few investigate the full response curve[9] and those that have only compare differences in relative MCA velocity at specific times within the task (i.e., 0 seconds, 20 seconds, 40 seconds).

Analyzing global changes in relative MCA response curves is novel, and while the clinical meaningfulness of our findings is unclear, this analysis may more comprehensively describe single-season changes in MCA response to hypercapnia and hypocapnia.

Neurovascular Coupling

There were no significant group differences in single-season changes in NVC response magnitude for both reading and visual search task, though reading NVC response magnitude was trending toward significance. Our null findings are consistent with similar studies which describe no changes in this outcome following a single-season of collision sport participation (American football and ice hockey) relative to non-contact sport controls.[20] Additionally, our study demonstrates a greater increase in reading NVC response magnitude in non-collision sport athletes in relative to collision sport athletes, though non-significant, mirroring previous literature.[20] This indicates that single-season subconcussive head impact exposure elicits marginal effects on NVC response magnitude to both reading and visual search tasks.

We found a significant interaction between group (collision vs. non-collision) and time point (pre- vs. post-season) for the overall reading NVC response curve. On average, non-collision sport athletes demonstrated a 2.7% single-season increase in relative PCA velocity response while collision sport athletes demonstrated 0.7% single-season decrease in relative PCA velocity response. This results differs from previous literature which demonstrate no changes in NVC dynamics over the course of a single season.[20] Our results may be attributed to differences in participant age. Wright et al.[20] investigated these outcomes in young adults with a mean age of 19 years, which has been shown to be the age when regional cerebral blood flow reaches adult levels.[134] On average, our study participants were younger and still in the window of changing cerebral blood flow volume.[134] Similar to CVR, to our knowledge this study is the first to look at global changes in the full response curve. Our significant finding may indicate that assessing global changes in overall PCA response curve is more sensitive to single-season changes in cerebrovascular function.

Limitations

There are limitations to the current study. We did not collect head impact exposure data throughout the season and are therefore unable to precisely speak to the cumulative load experienced by our collision sport athletes. Our sample was restricted to male athletes, which limits the generalizability of our findings to both sexes. Throughout childhood, cerebral blood flow perfusion is steadily increasing until puberty where trajectories tend to diverge with females experiencing higher perfusion rates than males.[54] Additionally, high school females are known to sustain higher rates of concussions compared to males.[1] Future studies should expand cerebrovascular research to female athletes in order to capture this potentially unique population. We acknowledge limitations regarding the use of TCD to measure CVR and NVC. Transcranial Doppler is unable to measure absolute cerebral blood flow volume due to the unknown diameter of individual vessels, and therefore must assume vessel diameter does not change, which is an inherent limitation of the equipment. Relative to advanced neuroimaging techniques, TCD lacks spatial resolution but has excellent temporal resolution[39] and is an inexpensive, non-invasive, portable alternative to advanced imaging. Lastly, we acknowledge that BHI can be calculated with[142] and without[117,118,137] factoring $P_{ET}CO_2$. Measuring $P_{ET}CO_2$ during a complete breath-holding task is not possible. Our methods align with the majority of studies employing breath-holding methodologies.

Conclusions

There is growing concern regarding the long-term effects of youth and adolescent collision sport participation. Most athletes do not advance to collegiate or professional athletics and therefore it is critical that we investigate the effect of subconcussive head impact exposure at the high school level in order to appropriately interpret life-time neurological changes. Our study indicates that aspects of cerebrovascular function (BHI and reading task NVC response curve) are associated with single-season sport participation, though the clinical meaningfulness of these novel outcomes is still unclear. This study represents an important step toward

discovering innovative and feasible physiological assessments so that they may be refined and integrated into the current concussion assessment paradigm.

Table 5.1. Demographic and medical history information for participants by total sample and by group.

	Total n=48	**Collision n=25**	**Non-Collision n=23**	**P**	**Effect Size**
Age, *yrs*	16.0 ± 1.2	16.0 ± 1.1	16.0 ± 1.3	0.89	0.07
Height, *cm*	175.5 ± 8.1	175.4 ± 7.9	175.7 ± 8.5	0.92	0.03
Mass, *kg*	68.6 ± 14.0	74.9 ± 13.4	61.8 ± 11.5	<0.001	1.05
ADHD,*+ *n (%)*	12 (25.53%)	4 (16.67%)	8 (34.78%)	0.14	0.27^
IEP, *+ *n (%)*	8 (16.67%)	6 (24.00%)	2 (22.22%)	0.46	0.21^
Concussion History,* *n (%)*	9 (18.75%)	6 (24.00%)	3 (13.04%)	0.47	0.14^
# Previous Concussions	1.00 IQR = 0 (1-1)	1.00 IQR = 0 (1-1)	1.00 IQR = 0 (1-1)	0.55	0.81
Recency,* *n (%)*				0.10	0.79^
past year	4 (44.44%)	4 (66.67%)	-		
past 2 years	1 (11.11%)	1 (16.67%)	-		
> 2 years	4 (44.44%)	1 (16.67%)	3 (100.00%)		

ADHD: attention deficit hyperactive disorder; IEP: individualized education plan
All p-values represent independent samples t-tests unless otherwise noted
All effect sizes represent Cohen's d unless otherwise noted
*Fisher's Exact tests
^Phi Coefficient

Table 5.2. Pre-season and post-season means (standard deviations) for resting middle cerebral artery velocity (MCAv), posterior cerebral artery velocity (PCAv), end tidal carbon dioxide ($P_{ET}CO_2$), graded symptom checklist (GSC), breath-holding index (BHI), vasomotor reactivity response (VMRr) and neurovascular coupling (NVC) response magnitudes for collision (n=25) and non-collision (n=23) sport athletes.

	Pre-Season			**Post-Season**			**Pre- to Post-Season Change**		
	Collision	*Non-Collision*	*P*	*Collision*	*Non-Collision*	*P*	*Collision*	*Non-Collision*	*P*
Resting MCAv, *cm/s*	57.3 (11.6)	60.6 (10.6)	0.31	54.2 (8.5)	61.0 (8.7)	0.01	-3.3 (0.8)	0.4 (10.0)	0.23
Resting PCAv, *cm/s*	36.5 (6.7)	38.3 (5.3)	0.33	35.8 (5.7)	40.3 (5.3)	<0.001	-0.7 (6.3)	2.0 (4.2)	0.09
Resting $P_{ET}CO_2$, *mmHg*	41.1 (3.8)	39.9 (4.8)	0.28^	40.6 (6.7)	39.2 (5.5)	0.69^	-1.7 (7.8)	0.6 (4.6)	0.22^
GSC									
Total Endorsement	2.0 (3.1)	2.6 (2.6)	0.20^	2.4 (3.6)	2.7 (2.9)	0.28^	0.4 (2.0)	0.2 (2.8)	0.29^
Total Severity	3.1 (5.5)	3.2 (3.6)	0.31^	4.1 (6.4)	3.7 (4.6)	0.48^	1.0 (4.1)	0.5 (4.7)	0.42^
BHI (s^{-1})	1.4 (0.5)	1.5 (0.7)	0.47	1.7 (0.7)	1.4 (0.6)	0.17	0.3 (0.7)	-0.1 (0.6)	0.03
VMRr (%)	45.2 (10.1)	49.3 (15.2)	0.28	44.8 (14.5)	45.3 (13.3)	0.72	-0.3 (12.5)	-3.0 (13.1)	0.48
NVC Response Magnitude (cm)									
Reading Task	32.5 (2.1)	32.0 (1.8)	0.37	32.3 (1.9)	32.8 (1.6)	0.33	-0.2 (2.1)	0.9 (1.8)	0.05
Visual Search Task	33.2 (3.1)	33.7 (3.3)	0.59	33.6 (3.9)	33.8 (2.9)	0.80	0.5 (3.4)	0.02 (4.5)	0.68

All p-values represent independent samples t-tests unless otherwise noted
*Fisher's exact tests
^Mann-Whitney U Test

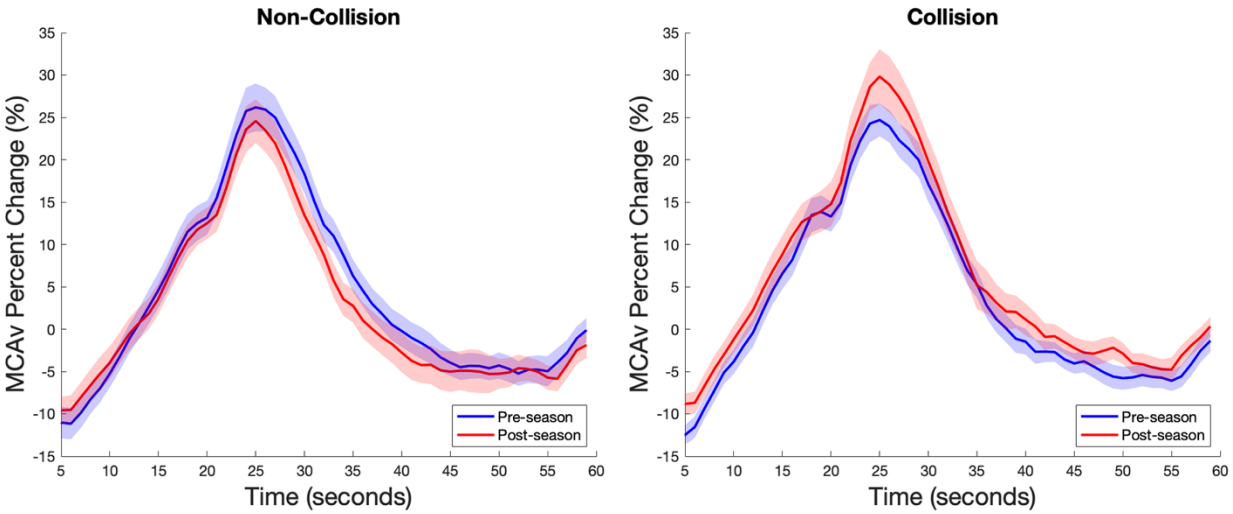

Figure 5.1. Relative MCA velocity ensemble-averaged across 5 trials in response to the breath-holding task for non-collision and collision sport athletes. Change in overall MCA velocity response curves from preseason to postseason did not differ between groups ($F_{1,2294}$=0.27, p=0.60).

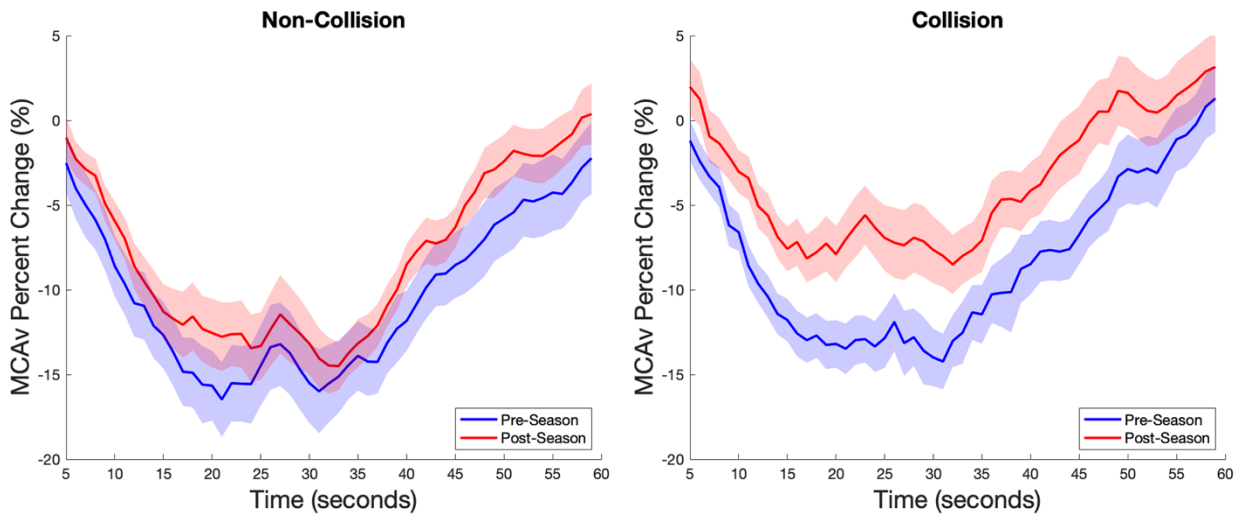

Figure 5.2. Relative MCA velocity ensemble-averaged across 5 trials in response to the hyperventilation task for non-collision and collision sport athletes. Change in overall MCA velocity response curves from preseason to postseason did not differ between groups ($F_{1,2294}$=0.35, p=0.55).

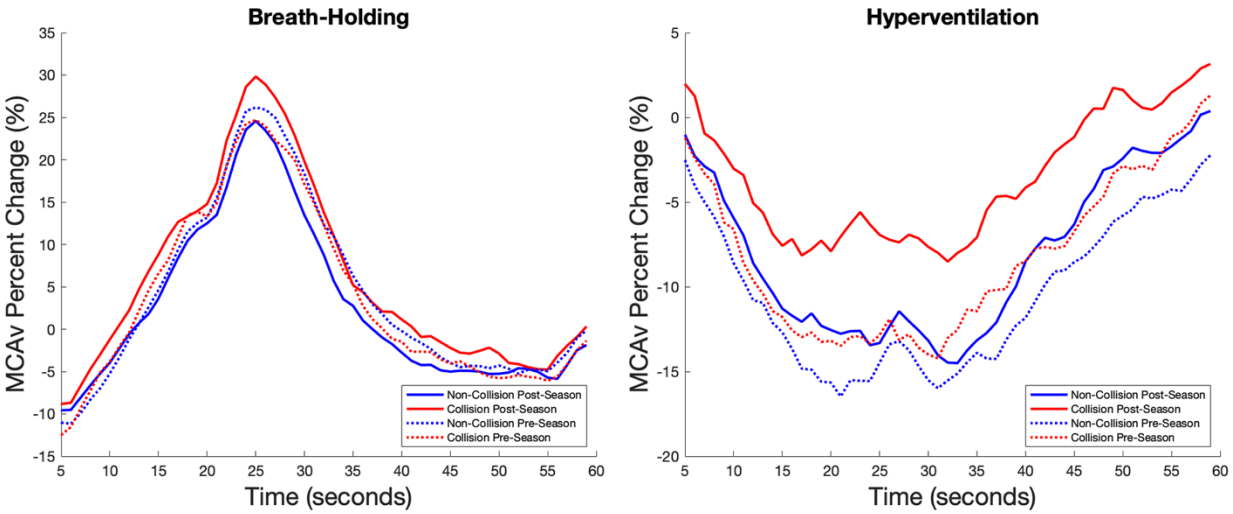

Figure 5.3. Relative MCA velocity ensemble-averaged across 5 trials in response to the breath-holding and hyperventilation tasks for non-collision and collision sport athletes at pre-season and post-season testing.

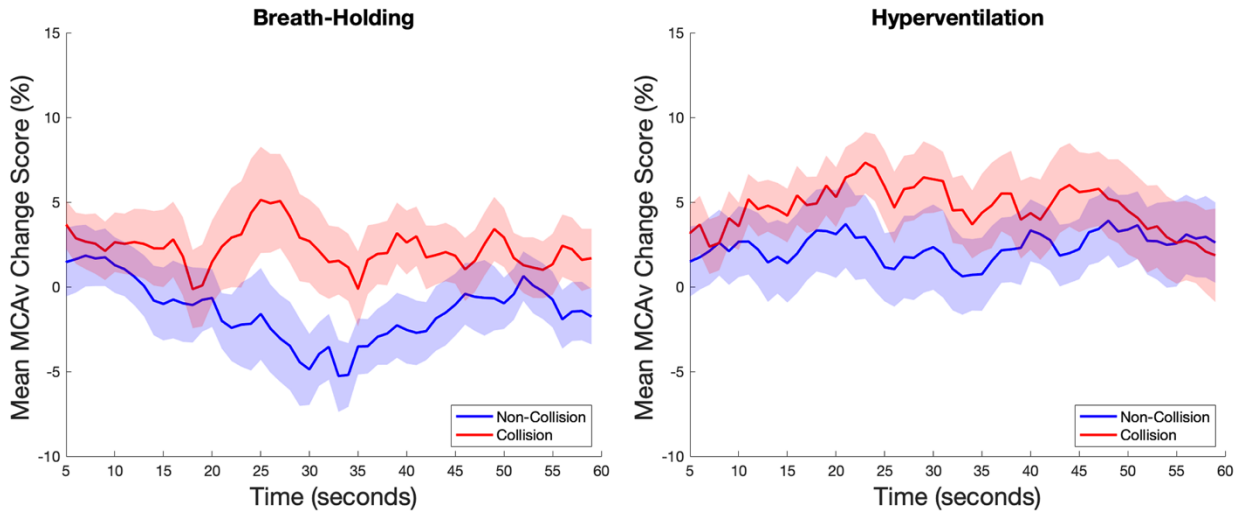

Figure 5.4. Pre- to post-season change curves in relative MCA velocity change to both breath-holding and hyperventilation tasks.

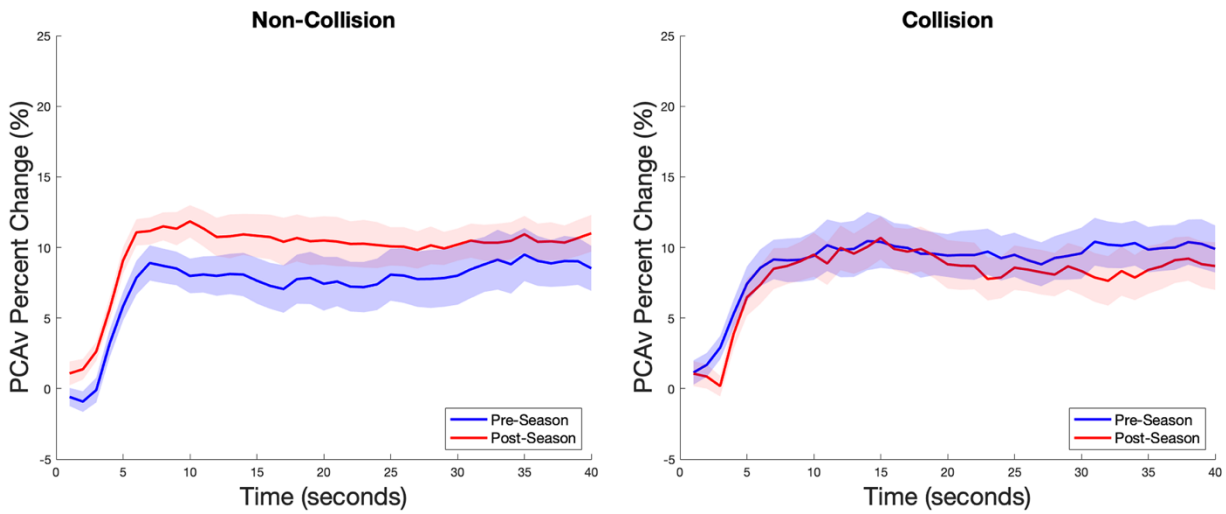

Figure 5.5. Relative PCA velocity ensemble-averaged across 5 trials in response to the reading task in non-collision and collision sport athletes at pre- and post-season time points. We found a significant interaction between group and time point ($F_{1,2710}$=101.54, p<0.001) such that overall NVC response curves decreased by an average of 0.69% in collision sport athletes and increased by an average of 2.7% in non-collision sport athletes over the course of a single season.

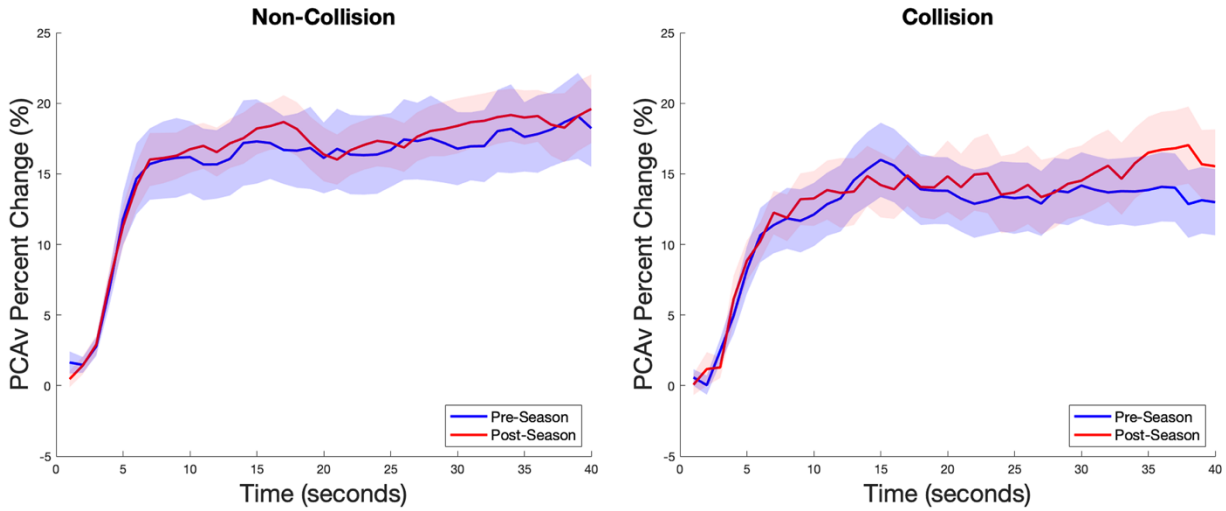

Figure 5.6. Relative PCA velocity ensemble-averaged across 5 trials in response to the visual search task in non-collision and collision sport athletes at pre- and post-season time points. We found no significant interaction effects during the visual search task ($F_{1,2769}$=-0.24, p=0.80).

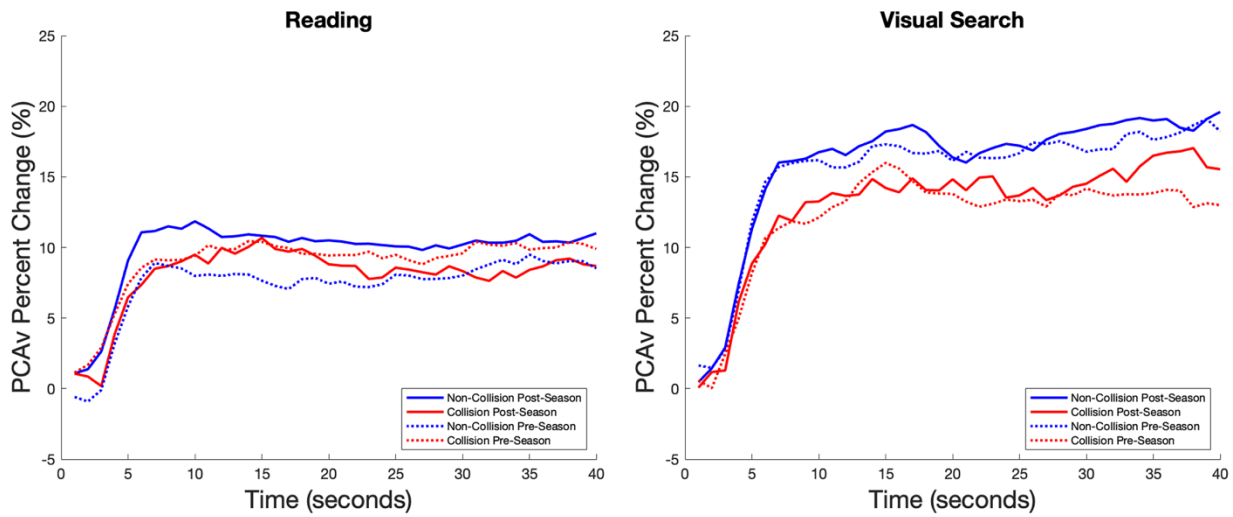

Figure 5.7. Relative PCA velocity ensemble-averaged across 5 trials in response to the reading and visual search tasks for non-collision and collision sport athletes at pre-season and post-season testing.

CHAPTER 6

EXECUTIVE SUMMARY

High school collision sports such as football, ice hockey, and lacrosse have consistently higher concussion incidence rates relative to other sports.[1,2] Due to the collision nature of these sports, athletes are more likely to be exposed to concussive and subconcussive head impacts. Following concussion, athletes typically experience an array of signs and symptoms including increased symptom endorsement, cognitive dysfunction, and postural control deficits. Recent studies have shown physiological deficits can remain even after traditional clinical recovery[21] and that cumulative subconcussive impacts, or impacts not resulting in diagnosed concussions, may influence clinical and neurophysiological outcomes.[13] This is concerning given recent evidence linking sport-related cumulative subconcussive head impacts to late-life cognitive and psychological dysfunction in retired athletes. Compounding this growing concern for youth sport safety are the previously published data demonstrating younger athletes may be more physiologically vulnerable to the effects of brain injury[3] and take longer to recover.[4] This is a critical need to further examine how repetitive head impact exposure may influence neurophysiological function in adolescent athletes.

Earlier age of first exposure to repetitive head impacts has been linked to late-life cognitive impairments in former National Football League players,[128] suggesting tackle football participation during neurodevelopment may increase the risk for long-term impairment. Neurophysiological deficits have also been found in youth athletes aged 13 and younger.[95] There is a need to describe these outcomes at the high school level as most football players do not play in college or professionally.[149] In order to appropriately interpret life-time neurological changes, is critical to first describe these outcomes in adolescent athletes. Cerebrovascular

function, as measured by cerebrovascular reactivity (CVR) and neurovascular coupling (NVC) has been shown to be sensitive acutely following sport-related concussion[8–10] and following subconcussive head impact exposure.[20] Additionally, both CVR and NVC can be non-invasively and feasibly assessed using transcranial Doppler (TCD). Our data show that high school-aged collision and non-collision sport athletes demonstrate similar cerebrovascular function prior to active repetitive head impact exposure (**Aim 1**). This is particularly interesting as our sample comprised of athletes in both groups with prior collision or contact sport experience. Within collision sport athletes, football players began playing football at a median age of 13 years (IQR = 10 -14 years). Within non-collision sport athletes, six (26.1%) of these athletes had contact-sport[108] participation history including basketball, soccer, lacrosse, and wrestling. Additionally, seven (23.3%) collision sport athletes and three (13.0%) non-collision sport athletes reported history of concussion. Despite collision sport participation, our football athletes did not present with significantly different cerebrovascular function relative to non-collision sport athletes prior to the start of their seasons. Importantly, our findings from Aim 1 provide formative data to better interpret cerebrovascular findings following head impact exposure (**Aim 2**).

Adolescent athletes experience ongoing neurodevelopmental changes and the neurological response to repetitive subconcussive head impacts may differ considerably from more mature athletes. Following a single season of high school football athletes have shown in white matter integrity[143,144] and blood biomarkers,[145] though it is currently unclear how single-season head impact exposure may effect cerebrovascular function. Our data indicate that single-season changes in cerebrovascular outcomes may differ between collision and non-collision sport athletes. Collision sport athletes demonstrated a greater change and a positive change in CVR, as measured by breath-holding index (BHI), relative to non-collision sport athletes. According to previously published normative values in more severe cerebrovascular pathology, positive change indicates improved BHI;[117,148] however, a recent study assessing BHI acutely post-concussion shows increased BHI for up to 7 days post injury.[137] Additionally, non-

collision sport athletes demonstrated a greater single-season change in NVC response to a reading task, as measure by overall PCA response curves. At post-season testing, non-collision sport athletes had an increased PCA response, which is thought to indicate increased neural resource recruitment and therefore an impairment.[10] While the clinical interpretation is still unclear, our study shows single-season changes in BHI and NVC response to a reading task differ between collision and non-collision sport athletes and warrants continued research in this age group.

Overall, our study demonstrates that CVR and NVC assessments may be sensitive to the dynamic cerebrovascular changes occurring in adolescent athletes. Additionally, this study represents an important step toward discovering innovative and feasible physiological assessments so that they may be refined and integrated into the current concussion assessment paradigm. Future research should continue to assess these outcomes following both subconcussive head impact exposure and throughout the recovery trajectory following concussion. Importantly, normative values of cerebrovascular function in adolescent athletes should be established and assessment should expand to broader pediatric populations including sex-comparable sports (e.g., soccer, basketball, etc.), and different age-groups/organizations (e.g., middle school, club sports, etc.). The development of these research ideas will be my early career focus, and the data observed in my dissertation will provide the scientific rational and formative physiological understanding for future study development

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APPENDIX 1: DEMOGRAPHIC INTAKE FORM

The neurophysiology of subconcussive head impacts in high school athletes

Entry Survey

Thank you for participating in our study. Please complete this questionnaire and return it with your consent forms in the envelope provided. The information you provide will not be shared with anyone at your school.

Today's Date: ____/_____/_____

Please complete the following information for both player and parent(s). Please print.

Player

First Name _____ Last Name: _____

Email:_____ Cell Phone: (_____) _____-_____

Best way to contact: ☐ Email ☐ Cell Phone ☐ Text

Parent 1

First Name _____ Last Name: _____

Email:_____ Cell Phone: (_____) _____-_____

Best way to contact: ☐ Email ☐ Cell Phone ☐ Text

Parent 2

First Name _____ Last Name: _____

Email:_____ Cell Phone: (_____) _____-_____

Best way to contact: ☐ Email ☐ Cell Phone ☐ Text

For Office Use **ID#:**_____

Data Entry **#1 Date:** **Initials:** **#2 Date:** **Initials:**

Section 1: Demographic Information

Q1. Date of Birth (mm/dd/yyyy) :_____ **Q1a. Sex/Gender** :_____

Q2. Height: ____ ft ____ in **Q3. Weight:** _____lbs

Q4. Primary Language: ☐ English(1) ☐ Spanish(2) ☐
Other(3):_____

Q4a.If English is NOT your primary language, are you fluent in English?

☐ Yes (1) ☐ No (0)

Q5. Race: ☐ African American/Black (1) ☐ American Indian/Alaskan Native (2)
☐ Asian(3) ☐ Native Hawaiian/Pacific Islander (4) ☐ White (5)
☐ Unknown (98) ☐ Prefer not to answer (97)

Q6. Ethnicity: ☐ Hispanic/Latino (1) ☐ Not Hispanic/Latino (0)
☐ Unknown (98) ☐ Prefer not to answer (97)

Section 2: Sport Participation

Q1.

Sport	Participation	Level		Age range in which sport was played
		High School	Club	
Football	☐	☐	☐	_____
Hockey	☐	☐	☐	_____
Soccer	☐	☐	☐	_____
Lacrosse	☐	☐	☐	_____
Baseball	☐	☐	☐	_____
Basketball	☐	☐	☐	_____
Other: _____	☐	☐	☐	_____

(Collision sport athletes only)

Football Only

Q2a. Primary Position on your football team for 2019 season: *Select only one*

☐ QB (1) ☐ Running Back (2) ☐ Receiver (3) ☐ Tight End (4) ☐ Offensive Lineman (5)

☐ Defensive Lineman (6) ☐ Linebacker (7) ☐ Defensive Back (8) ☐ Special Teams/Kicker (9) ☐ Uncertain (98)

Q2b. Secondary Position(s) on your football team for 2019 season: *Select **all** that apply*

☐ QB (1) ☐ Running Back (2) ☐ Receiver (3) ☐ Tight End (4) ☐ Offensive Lineman (5)

☐ Defensive Lineman (6) ☐ Linebacker (7) ☐ Defensive Back (8) ☐ Special Teams/Kicker (9) ☐ Uncertain (98)

Ice Hockey Only

Q3a. Primary Position on your ice hockey team for 2019 season: *Select only one*

☐ Forward (1) ☐ Defense (2) ☐ Goalie (3)

Q3b. Secondary Position on your ice hockey team for 2019 season:

☐ Forward (1) ☐ Defense (2) ☐ Goalie (3)

Section 3: Academic/Health History

Q1. High School: ☐ CHHS (01) ☐ CHS (02) ☐ ECHHS (03) ☐ OHS (04)
☐ Other (05): _____

Q2. What will be your academic year during the 2019-2020 school year?

☐ Freshman (9) ☐ Sophomore (10) ☐ Junior (11) ☐ Senior (12)

Do you currently have any of the following?

Q3. Individualized Education Plan (IEP) ☐ Yes (1) ☐ No (0) ☐ Don't Know (98)

Q4. 504 Plan ☐ Yes (1) ☐ No (0) ☐ Don't Know (98)

Have you ever been diagnosed by a doctor with:

Q5. Vision Disorder (corrective lenses, Lasik surgery) ☐ Yes ☐ No ☐ Don't Know (98)

Q6. Sickle Cell Disease ☐ Yes ☐ No ☐ Don't Know (98)

Q7. Sickle Cell Trait ☐ Yes ☐ No ☐ Don't Know (98)

Q8. Attention Deficit Hyperactive Disorder (ADHD) ☐ Yes ☐ No ☐ Don't Know (98)

Q8a. If you have been diagnosed with ADHD, please list any medications you are currently taking:

_____

Section 4 Concussion History Update **Please complete the table below for ANY concussion(s) you may have had.**

Definition of Concussion: A change in brain function following a force to the head, which may be accompanied by temporary loss of consciousness, but is identified in awake individuals with measures of neurologic and cognitive dysfunction. Common concussion symptoms include:

- Headache
- Feeling slowed down
- Difficulty concentrating or focusing
- Dizziness, balance problems, loss of balance
- Fatigue/lack of energy
- Feeling in a fog
- Irritable
- Drowsiness
- Nausea
- Forgetting things (before or after the injury)
- Sensitivity to light/noise
- Blurred vision

IMPORTANT: A) A concussion can occur without being "knocked out" or unconscious
B) getting your "bell rung", "clearing the cobwebs" is a concussion

Q1. Have you ever had a concussion related to sport or other activities?
☐ Yes (1) ☐ No (0)
Q1a. If YES, how many concussions have you had? _____
If you have had more than 10 concussions, please report only the 10 most recent

	Sport or Non-Sport Related Concussion	Approximate date of injury (mm/yyyy)	How many days did you experience symptoms related to the injury?
#1	☐ Sport (1) ☐Non-Sport(0)	_____/____	_____(days) ☐ Unknown (98)
#2	☐ Sport (1) ☐Non-Sport(0)	_____/____	_____(days) ☐ Unknown (98)
#3	☐ Sport (1) ☐Non-Sport(0)	_____/____	_____(days) ☐ Unknown (98)
#4	☐ Sport (1) ☐Non-Sport(0)	_____/____	_____(days) ☐ Unknown (98)
#5	☐ Sport (1) ☐Non-Sport(0)	_____/____	_____(days) ☐ Unknown (98)
#6	☐ Sport (1) ☐Non-Sport(0)	_____/____	_____(days) ☐ Unknown (98)
#7	☐ Sport (1) ☐Non-Sport(0)	_____/____	_____(days) ☐ Unknown (98)
#8	☐ Sport (1) ☐Non-Sport(0)	_____/____	_____(days) ☐ Unknown (98)
#9	☐ Sport (1) ☐Non-Sport(0)	_____/____	_____(days) ☐ Unknown (98)
#10	☐ Sport (1) ☐Non-Sport(0)	_____/____	_____(days) ☐ Unknown (98)

APPENDIX 2: GRADED SYMPTOM CHECKLIST

SCAT Symptom Evaluation

How do you feel?

You should score yourself on the following symptoms, based on how you feel now.

	None	Mild		Moderate		Severe	
Headache	0	1	2	3	4	5	6
"Pressure in Head"	0	1	2	3	4	5	6
Neck Pain	0	1	2	3	4	5	6
Nausea or Vomiting	0	1	2	3	4	5	6
Dizziness	0	1	2	3	4	5	6
Blurred Vision	0	1	2	3	4	5	6
Balance Problems	0	1	2	3	4	5	6
Sensitivity to Light	0	1	2	3	4	5	6
Sensitivity to Noise	0	1	2	3	4	5	6
Feeling Slowed Down	0	1	2	3	4	5	6
Feeling like "in a fog'	0	1	2	3	4	5	6
"Don't feel right"	0	1	2	3	4	5	6
Difficulty Concentrating	0	1	2	3	4	5	6
Difficulty Remembering	0	1	2	3	4	5	6
Fatigue or Low Energy	0	1	2	3	4	5	6
Confusion	0	1	2	3	4	5	6
Drowsiness	0	1	2	3	4	5	6
Trouble Falling Asleep	0	1	2	3	4	5	6
More Emotional	0	1	2	3	4	5	6
Irritability	0	1	2	3	4	5	6
Sadness	0	1	2	3	4	5	6
Nervous or Anxious	0	1	2	3	4	5	6

How many hours did you sleep last night? _____(hrs)

Do the symptoms get worse with physical activity? ☐Yes ☐No ☐N/A

Do the symptoms get worse with mental activity? ☐Yes ☐No

APPENDIX 3: INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE – SHORT FORM

I am going to ask you about the time you spent being physically active in the last 7 days. Please answer each question even if you do not consider yourself to be an active person. Think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

1. **Now, think about all the *vigorous* activities which take *hard physical effort* that you did in the last 7 days. Vigorous activities make you breathe much harder than normal and may include heavy lifting, digging, aerobics, or fast bicycling. Think only about those physical activities that you did for at least 10 minutes at a time.** During the **last 7 days**, on how many days did you do **vigorous** physical activities?
2. How much time did you usually spend doing **vigorous** physical activities on one of those days?
3. ***If clarification on #2 is needed:** An average time for one of the days on which you do vigorous activity is being sought. If the respondent can't answer because the pattern of time spent varies widely from day to day, ask: "How much time in total would you spend **over the last 7 days** doing vigorous physical activities?"
4. **Now think about activities which take *moderate physical effort* that you did in the last 7 days. Moderate physical activities make you breathe somewhat harder than normal and may include carrying light loads, bicycling at a regular pace, or doubles tennis. Do not include walking. Again, think about only those physical activities that you did for at least 10 minutes at a time.** During the **last 7 days**, on how many days did you do **moderate** physical activities?
5. How much time did you usually spend doing **moderate** physical activities on one of those days?
6. ***If clarification on #5 is needed:** An average time for one of the days on which you do moderate activity is being sought. If the respondent can't answer because the pattern of time spent varies widely from day to day, or includes time spent in multiple jobs, ask: "What is the total amount of time you spent over the **last 7 days** doing moderate physical activities?"
7. **Now think about the time you spent walking in the last 7 days. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.** During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?
8. How much time did you usually spend **walking** on one of those days?
9. ***If clarification on #8 is needed:** An average time for one of the days on which you walk is being sought. If the respondent can't answer because the pattern of time spent varies widely from day to day, ask: "What is the total amount of time you spent walking over **the last 7 days**?"
10. **Now think about the resistive activities that you did in the last 7 days. Again, think about only those physical activities that you did for at least 10 minutes at a time.** During the **last 7 days**, on how many days did you do **resistive** physical activities?
11. How much time did you usually spend doing **resistive** physical activities on one of those days?
12. ***If clarification on #11 is needed:** An average time for one of the days on which you do resistive activity is being sought. If the respondent can't answer because the pattern of time spent varies widely from day to day, or includes time spent in multiple jobs, ask: "What is the total amount of time you spent over the **last 7 days** doing resistive physical activities?"

Physical Activity	Days per week	Time per day	Time per week*
Vigorous	Days/week ☐ Don't know/Not sure ☐ Refused	Hours/day Minutes/day ☐ Don't know/Not sure ☐ Refused	Hours/day Minutes/day ☐ Don't know/Not sure ☐ Refused
Moderate	days ☐ Don't know/Not sure ☐ Refused	Hours/day Minutes/day ☐ Don't know/Not sure ☐ Refused	Hours/day Minutes/day ☐ Don't know/Not sure ☐ Refused
Walking	days ☐ Don't know/Not sure ☐ Refused	Hours/day Minutes/day ☐ Don't know/Not sure ☐ Refused	Hours/day Minutes/day ☐ Don't know/Not sure ☐ Refused
Resistive	days ☐ Don't know/Not sure ☐ Refused	Hours/day Minutes/day ☐ Don't know/Not sure ☐ Refused	Hours/day Minutes/day ☐ Don't know/Not sure ☐ Refused

APPENDIX 4: THE TEST OF WORD READING EFFICIANCY – 2ND EDITION

Test of Word Reading Efficiency–Second Edition

TOWRE-2

Examiner Record Booklet Form A

Joseph K. Torgesen, Richard K. Wagner, and Carol A. Rashotte

Section 1. Identifying Information

Name _____ Female ☐ Male ☐

	Year	Month	Day			
Date Tested	_____	_____	_____	School	_____	Grade _____
Date of Birth	_____	_____	_____	Examiner's Name	_____	Title _____
Age	_____	_____	_____			

Section 2. Test Performance

**Scaled score based on* ☐*age* ☐*grade*

	Raw Score	Age Equiv.	Grade Equiv.	%ile Rank	Scaled Score*	*SEM*	Descriptive Term
Sight Word Efficiency (SWE)	____	____	____	____	____	(5)	_____
Phonemic Decoding Efficiency (PDE)	____	____	____	____	+____	(4)	_____
				Sum of Scaled Scores =			
Total Word Reading Efficiency Index (TWRE)				____	____	(3)	_____

Section 3. Descriptive Terms

Descriptive Term	Very Poor	Poor	Below Average	Average	Above Average	Superior	Very Superior
Scaled Score	<70	70–79	80–89	90–110	111–120	121–130	>130

Section 4. Other Test Scores

	Test Name	Date	Standard Score	TOWRE-2 Equiv.
1.				
2.				
3.				
4.				

Section 5. Interpretation and Recommendations

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Subtest 1

Sight Word Efficiency

Materials: Stopwatch, Sight Word Efficiency reading card Form A

Ceiling: Administer all items until 45 seconds have elapsed.

Scoring: Record the total number of words the examinee reads correctly in 45 seconds. If the examinee finishes all the words before the time is up, note the time required to read all the words. Mark all the words the examinee reads correctly with a one (1), and mark all the words that are pronounced incorrectly with a zero (0). Draw a line after the examinee's last word. If the examinee skips a word, simply count the item as an error. If the examinee makes an error and then self-corrects, count the item as correct. If the examinee hesitates for more than 3 seconds on a word and is instructed to go to the next word, mark the word as incorrect.

Practice: Present the practice words on the Sight Word Efficiency card. Say, **I want you to read some lists of words as fast as you can. Let's start with this practice list. Begin at the top, and read down the list as fast as you can. If you come to a word you cannot read, just skip it and go to the next word. Use your finger to help you keep your place if you want to.** Have the examinee read the words aloud. If the examinee skips around, ask him or her to read the words from top to bottom, without jumping around. If the examinee cannot respond correctly to at least one item, discontinue testing and do not record a score.

Practice Words: *on, my, bee, old, warm, bone, most, spell*

Test: Give the following instructions while holding the Sight Word Efficiency card. Say, **OK, now you will read some longer lists of words. The words start out pretty easy, but they get harder as you go along. Read as many words as fast as you can until I tell you to stop. Begin here** (turn over the card to show the word list and point to the upper left corner of the list) **and read down the list** (draw finger down the list) **before you start on the next list** (point to top of second column). **Read the words in order, but if you come to one you can't read, skip it and go to the next one. Use your finger to keep your place if you want to, and if you skip more than one word, point to the word you are reading next.** (Turn the card back to the practice words.) **Do you understand? OK, you will begin as soon as I turn over the card.**

Quickly turn over the card to the list of words to be administered and start timing as soon as the examinee says the first word. **After 45 seconds, tell the examinee to stop**, and draw a line under the last word read. If, before the time is up, the examinee indicates that he or she cannot read any more words, ask the examinee to look over the whole list to see if there are any more words that he or she can read. If the examinee then indicates that he or she can read no more words, stop testing.

Form A

____ 1. is	____ 23. men	____ 45. space	____ 67. morning	____ 89. understand
____ 2. up	____ 24. baby	____ 46. short	____ 68. resolve	____ 90. emphasis
____ 3. cat	____ 25. new	____ 47. left	____ 69. describe	____ 91. confident
____ 4. red	____ 26. stop	____ 48. people	____ 70. garment	____ 92. intuition
____ 5. me	____ 27. work	____ 49. almost	____ 71. business	____ 93. boisterous
____ 6. to	____ 28. jump	____ 50. waves	____ 72. qualify	____ 94. plausible
____ 7. no	____ 29. part	____ 51. child	____ 73. potent	____ 95. courageous
____ 8. we	____ 30. fast	____ 52. strong	____ 74. collapse	____ 96. alienate
____ 9. he	____ 31. fine	____ 53. crowd	____ 75. elements	____ 97. extinguish
____ 10. the	____ 32. milk	____ 54. better	____ 76. pioneer	____ 98. prairie
____ 11. and	____ 33. back	____ 55. inside	____ 77. remember	____ 99. limousine
____ 12. yes	____ 34. lost	____ 56. plane	____ 78. dangerous	____ 100. valentine
____ 13. of	____ 35. find	____ 57. pretty	____ 79. uniform	____ 101. detective
____ 14. him	____ 36. paper	____ 58. famous	____ 80. necessary	____ 102. recently
____ 15. as	____ 37. open	____ 59. children	____ 81. problems	____ 103. instruction
____ 16. book	____ 38. kind	____ 60. without	____ 82. absentee	____ 104. transient
____ 17. was	____ 39. able	____ 61. finally	____ 83. advertise	____ 105. phenomenon
____ 18. help	____ 40. shoes	____ 62. strange	____ 84. pleasant	____ 106. calculated
____ 19. then	____ 41. money	____ 63. budget	____ 85. property	____ 107. alternative
____ 20. time	____ 42. great	____ 64. repress	____ 86. distress	____ 108. collective
____ 21. wood	____ 43. father	____ 65. contain	____ 87. information	
____ 22. let	____ 44. river	____ 66. justice	____ 88. recession	

Number of words read correctly: _____. If examinee finishes list before 45 seconds, note time to finish: _____

Subtest 2

Phonemic Decoding Efficiency

Materials: Stopwatch, Phonemic Decoding Efficiency reading card Form A

Ceiling: Administer all items until 45 seconds have elapsed.

Scoring: Record the total number of nonwords the examinee reads correctly in 45 seconds. If the examinee skips a nonword, simply count it as an error. If the examinee hesitates for more than 3 seconds on a nonword, mark it as incorrect and point to the next item and say, Go on. If the examinee initially pronounces the nonword incorrectly but then self-corrects to the correct pronunciation, count the item as correct. Some of the items have more than one correct pronunciation for the vowel. Score the item correct if the child gives any of the correct pronunciations. Alternative correct pronunciations are indicated with real-word examples, with the vowel in question underlined. For words with more than two syllables, alternative pronunciations are given separately for each syllable where needed for clarification. Put a slash through nonwords read incorrectly. Draw a line after the examinee's last word.

Practice: Present the practice items on the Phonemic Decoding Efficiency card. Say, I want you to read some made-up words that are not real words. Just tell me how they sound. Let's start with this practice list. Begin at the top, and read down the list as fast as you can. If you come to a made-up word you cannot read, just skip it and go to the next word. Use your finger to help you keep your place if you want to. Have the examinee read the nonwords. If the examinee skips around, ask him or her to read the words from top to bottom, without jumping around. If the examinee tries to substitute real words for the nonwords, remind him or her that these are made-up words, not real words, and the goal is to try to say how they sound. If the examinee simply pronounces each letter sound separately, say, You are giving me the sounds each letter makes. Try to blend the sounds together to make a made-up word.

Practice Words: *ba* (*b<u>a</u>t*, *f<u>a</u>te*, *pizz<u>a</u>*), *um* (*<u>u</u>mpire*), *fos* (*f<u>o</u>ssil*), *gan* (*g<u>a</u>nder*), *rup* (*r<u>u</u>pture*), *nasp* (*cl<u>a</u>sp*), *luddy* (*m<u>u</u>ddy*), *dord* (*f<u>o</u>rd*)

Test: Give the following instructions while holding the Phonemic Decoding Efficiency card. Say, OK, now you will read some longer lists of made-up words. The made-up words start out pretty easy, but they get harder as you go along. Read as many of them as you can until I tell you to stop. Begin here (turn over the card to show the nonword list and point to the upper left corner of the list) and read down the list (draw finger down the list) before you start on the next list (point to top of second column). Read the made-up words in order, but if you come to one you can't read, skip it and go to the next one. Use your finger to keep your place if you want to, and if you skip more than one word, point to the word you are reading next. (Turn the card back to the practice words.) Do you understand? OK, you will begin as soon as I turn over the card.

Quickly turn over the card to the list of nonwords to be administered and start timing as soon as the examinee says the first nonword. **After 45 seconds, tell the examinee to stop**, and draw a line under the last nonword read. If, before the time is up, the examinee indicates that he or she cannot read any more nonwords, ask the examinee to look over the whole list to see if there are any more nonwords that he or she can read. If the examinee then indicates that he or she can read no more nonwords, stop testing.

Form A

Stimulus	Pronunciation	Stimulus	Pronunciation
____ 1. ip	(tip)	____ 34. sline	(line)
____ 2. ga	(law, gap, gate)	____ 35. dreff	(dref)
____ 3. ko	(law, code, cot)	____ 36. prain	(pain)
____ 4. ta	(tack, tape)	____ 37. zint	(lint)
____ 5. om	(on)	____ 38. bloot	(loot, book)
____ 6. ig	(pig)	____ 39. trisk	(brisk)
____ 7. ni	(nip, nice)	____ 40. kelm	(helm)
____ 8. pim	(him)	____ 41. strone	(stone)
____ 9. wum	(sum)	____ 42. lunaf	lu (tune, bun) naf (after)
____ 10. lat	(fat)	____ 43. cratty	(fatty)
____ 11. baf	(bat)	____ 44. trober	(sober)
____ 12. din	(pin)	____ 45. depate	de (dee, deck) pate (fate)
____ 13. nup	(cup)	____ 46. glant	(plant)
____ 14. fet	(met)	____ 47. sploosh	(loose, book)
____ 15. bave	(save)	____ 48. dreker	dre (met, meet) ker (her)
____ 16. pate	(fate)	____ 49. ritlun	rit (sit) lun (bun)
____ 17. herm	(term)	____ 50. hedfert	hed (bed) fert (fern)
____ 18. dess	(mess)	____ 51. bremick	bre (tree, bed) mick (tick)
____ 19. chur	(blur)	____ 52. nifpate	nif (sniff) pate (late)
____ 20. knap	(nap)	____ 53. brinbert	brin (fin) bert (her)
____ 21. tive	(hive)	____ 54. clabom	cla (clay, clap) bom (bomb)
____ 22. barp	(tarp)	____ 55. drepnort	drep (pep) nort (fort)
____ 23. stip	(tip)	____ 56. shrattec	shrat (rat) tec (neck)
____ 24. plin	(fin)	____ 57. plofent	plo (toe, mop) fent (bent)
____ 25. frip	(trip)	____ 58. smucrit	smu (fun, few) crit (bit)
____ 26. poth	(moth)	____ 59. pelnador	pel (fell) na (nap, nut, hip) dor (for, fur)
____ 27. vasp	(clasp)	____ 60. fornalask	forn (born) a (at, it, up) lask (task)
____ 28. meest	(feast)	____ 61. fermabalt	ferm (firm) a (at, it, up) balt (salt)
____ 29. shlee	(flee)	____ 62. crenidmoke	cre (hen, see) nid (lid) moke (hope)
____ 30. guddy	(muddy)	____ 63. emulbatate	e (eel, egg) mul (hull) ba (bat, bit, but) tate (late)
____ 31. skree	(tree)	____ 64. strotalanted	stro (stroll, on) ta (fun, fin, fan) lant (plant), ed (ted)
____ 32. felly	(jelly)	____ 65. prilingdorfent	pri (pry, print) ling (sling) dor (for), fent (vent)
____ 33. clirt	(shirt)	____ 66. chunfendilt	chun (fun) fen (fence) dilt (tilt)

Number of words read correctly: _____. If examinee finishes list before 45 seconds, note time to finish: _____.

Test of Word Reading Efficiency–Second Edition

TOWRE-2

Sight Word Efficiency Form A

Joseph K. Torgesen, Richard K. Wagner, and Carol A. Rashotte

Practice Words

on

my

bee

old

warm

bone

most

spell

5 6 7 8 9 10 23 22 21 20 19 18

13912

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is	jump	inside	absentee
up	part	plane	advertise
cat	fast	pretty	pleasant
red	fine	famous	property
me	milk	children	distress
to	back	without	information
no	lost	finally	recession
we	find	strange	understand
he	paper	budget	emphasis
the	open	repress	confident
and	kind	contain	intuition
yes	able	justice	boisterous
of	shoes	morning	plausible
him	money	resolve	courageous
as	great	describe	alienate
book	father	garment	extinguish
was	river	business	prairie
help	space	qualify	limousine
then	short	potent	valentine
time	left	collapse	detective
wood	people	elements	recently
let	almost	pioneer	instruction
men	waves	remember	transient
baby	child	dangerous	phenomenon
new	strong	uniform	calculated
stop	crowd	necessary	alternative
work	better	problems	collective

Test of Word Reading Efficiency–Second Edition

TOWRE-2

Phonemic Decoding Efficiency Form A

Joseph K. Torgesen, Richard K. Wagner, and Carol A. Rashotte

Practice Words

ba

um

fos

gan

rup

nasp

luddy

dord

5 6 7 8 9 10 23 22 21 20 19 18

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ip	stip	depate
ga	plin	glant
ko	frip	sploosh
ta	poth	dreker
om	vasp	ritlun
ig	meest	hedfert
ni	shlee	bremick
pim	guddy	nifpate
wum	skree	brinbert
lat	felly	clabom
baf	clirt	drepnort
din	sline	shrattec
nup	dreff	plofent
fet	prain	smucrit
bave	zint	pelnador
pate	bloot	fornalask
herm	trisk	fermabalt
dess	kelm	crenidmoke
chur	strone	emulbatate
knap	lunaf	strotalanted
tive	cratty	prilingdorfent
barp	trober	chunfendilt

APPENDIX 5: SEPARATE COVARIATE ANALYSES

Group Differences

Independent samples t-tests unless otherwise noted

Demographics

	Collision n=30	Non-Collision n=23	p-value
Age, *yrs*	15.77 ± 1.06	15.75 ± 1.31	0.94
Height, *cm*	175.90 ± 7.93	175.73 ± 8.47	0.94
Mass, *kg*	74.68 ± 12.61	62.43 ± 11.56	<0.001
Sport, *n (%)*			
Football	30 (100.00%)		
Cross-Country	-	14 (60.87%)	
Indoor Track	-	3 (13.04%)	
Swimming	-	6 (26.01%)	
Race, [+] *n (%)*			<0.0001
African American	15 (50.00%)	0 (0.00%)	
White	10 (33.33%)	19 (82.61%)	
Asian	0 (0.00%)	2 (8.70%)	
Multiple	4 (13.33%)		
Unreported	1 (3.33%)	2 (8.70%)	
Ethnicity, [+] *n (%)*			1.00
Hispanic/Latino	3 (10.00%)	2 (8.70%)	
Not Hispanic/Latino	20 (66.67%)	19 (82.61%)	
Unreported	7 (23.33%)	2 (8.70%)	
Academic Year, [+] *n (%)*			0.30
8th grade	0 (0.00%)	1 (4.35%)	
Freshman	7 (23.33%)	8 (34.78%)	
Sophomore	12 (40.00%)	4 (17.39%)	
Junior	6 (20.00%)	7 (30.43%)	
Senior	5 (16.67%)	3 (13.04%)	
IEP, [+] *n (%)*			0.34
Yes	7 (23.33%)	2 (8.90%)	
No	21 (70.00%)	18 (78.26%)	
Unreported	2 (6.67%)	3 (13.04%)	
504, [+] *n (%)*			0.46
Yes	1 (3.33%)	1 (4.35%)	
No	26 (86.67%)	18 (78.26%)	
Unreported	3 (10.00%)	4 (17.39%)	

p-values represent independent samples t-tests unless otherwise noted
[+] Fisher's exact test

Medical History

	Collision n=30	Non-Collision n=23	p-value
Visual Disorder	4 (13.33%)	5 (21.74%)	0.48
Sickle Cell Trait	3 (10.00%)	0 (0.00%)*	0.16
Sickle Cell Disease	-	-	-
ADHD+	5 (17.24%)	8 (34.78%)*	0.15
Concussion History+	7 (23.33%)	3 (13.04%)	0.48
# Previous Concussions	1.29 ± 0.49	1.00 ± 0.00	0.36
Recency+			0.03
Within Year	5 (71.43%)	0 (0.00%)	
Within 2 Years	1 (14.29%)	0 (0.00%)	
> 2 years	1 (14.29%)	3 (100.00%)	

p-values represent independent samples t-tests unless otherwise noted
+ Fisher's exact test

Other Variables

	Collision n=30	Non-Collision n=23	p-value
Total Symptom Endorsement	1.87 ± 1.53	2.56 ± 2.63	0.37
Total Symptom Severity	2.97 ± 5.18	3.22 ± 3.57	0.84
TWRE	96.67 ± 13.69	108.30 ± 9.44	0.001
IPAQ - SF+			0.004
Low	0 (0.00%)	0 (0.00%)	
Moderate	0 (0.00%)	6 (27.27%)	
High	30 (100.00%)	16 (72.73%)	

p-values represent independent samples t-tests unless otherwise noted
+ Fisher's exact test

Effect of Variables on Outcomes

General Linear Model
E(outcome) = $ß_1$ + $ß_2$*Variable + e

Demographics

Variable	Outcome (p-value)					
	MCA mean	PCA mean	BHI	VMRr	Read AUC	WW AUC
Age	0.11	0.11	0.97	0.62	0.0498	0.0386
Height	0.27	0.70	0.73	0.94	0.77	0.98
Weight	0.0456	0.08	0.49	0.48	0.25	0.16
Race	0.08	0.39	0.21	0.59	0.55	0.51
Ethnicity	0.79	0.64	0.99	0.74	0.93	0.90
Academic Year	0.20	0.0508	0.93	0.64	0.06	0.0430
IEP	0.38	0.22	0.86	0.91	0.39	0.62
504	0.80	0.06	0.96	0.99	0.56	0.17
Visual Disorder	0.14	0.48	0.40	0.43	0.44	0.39
SC Trait (n=3)	0.19	0.72	0.97	0.44	0.14	0.01
ADHD	0.98	0.98	0.15	0.09	0.98	0.94
Conc Hx	0.46	0.96	0.23	0.72	0.62	0.86

Other Variables

Variable	Outcome (p-value)					
	MCA mean	PCA mean	BHI	VMRr	Read AUC	WW AUC
Total Symptom Endorsement	0.57	0.16	0.80	0.73	0.73	0.42
Total Symptom Severity	0.46	0.14	0.91	0.79	0.59	0.35
IPAQ	0.16	0.66	0.09	0.07	0.92	0.76
TWRE	0.36	0.92	0.30	0.56	0.47	0.39

Mixed Models with Cubic Mean Structure

$E(outcome) = ß_1 + ß_2*time + ß_3*time^2 + ß_4*time^3 + ß_5*variable + ß_6*variable*time + ß_7*variable*time^2 + ß_8*variable*time^3 + e$

Demographics

Variable	Outcome (p-value)							
	BH Curve			HV Curve			Read Curve	WW Curve
	Time*IV	Time^2*IV	Time^3*IV	Time*IV	Time^2*IV	Time^3*IV		
Age	0.81	0.70	0.65	0.86	0.94	0.89	0.0443	0.26
Height	0.59	0.94	0.94	0.91	0.84	0.74	0.77	0.67
Weight	0.65	0.54	0.49	0.46	0.30	0.21	0.25	0.26
Race	0.53	0.66	0.75	0.27	0.31	0.35	0.55	0.82
Ethnicity	0.41	0.72	0.99	0.39	0.48	0.61	0.93	0.86
Academic Year	0.83	0.87	0.88	0.68	0.72	0.86	0.530	0.25
IEP	0.33	0.37	0.37	0.88	0.87	0.81	0.39	0.27
504	0.23	0.31	0.35	0.99	0.90	0.91	0.56	0.31
Visual Disorder	0.04	0.09	0.19	0.56	0.40	0.36	0.44	0.28
SC Trait (n=3)	0.02	0.03	0.02	0.82	0.0003	0.001	0.13	0.01
ADHD	0.23	0.39	0.66	0.89	0.88	0.65	0.98	0.91
Conc Hx	0.33	0.32	0.29	0.90	0.77	0.73	0.61	0.69

Other Variables

Variable	Outcome (p-value)							
	BH Curve			HV Curve			Read Curve	WW Curve
	Time*IV	Time^2*IV	Time^3*IV	Time*IV	Time^2*IV	Time^3*IV		
Total Symptom Endorsement	0.77	0.85	0.94	0.59	0.62	0.66	0.73	0.39
Total Symptom Severity	0.62	0.70	0.81	0.84	0.84	0.83	0.59	0.36
IPAQ	0.33	0.42	0.56	0.61	0.94	0.60	0.92	0.65
TWRE	0.39	0.33	0.31	0.51	0.54	0.53	0.47	0.58

Controlling for Variable in Full Model

General Linear Model

E(outcome) = $ß_1$ + $ß_2$*Group(collision vs. non-collision) + $ß_3$*Variable e

Demographics

Variable	Outcome (p-value)					
	MCA mean	PCA mean	BHI	VMRr	Read AUC	WW AUC
Age	0.10	0.28	0.50	0.31	0.36	0.60
Height	0.09	0.29	0.50	0.30	0.37	0.63
Weight	0.37	0.73	0.68	0.42	0.12	0.89
Race	0.39	0.23	0.20	0.15	0.56	0.38
Ethnicity	0.06	0.51	0.58	0.28	0.51	0.56
Academic Year	0.11	0.33	0.50	0.32	0.29	0.71
IEP	0.14	0.14	0.43	0.45	0.64	0.55
504	0.41	0.34	0.24	0.44	0.58	0.92
Visual Disorder	0.12	0.32	0.55	0.34	0.39	0.59
SC Trait (n=3)	0.16	0.25	0.53	0.41	0.56	0.27
ADHD	0.10	0.24	0.34	0.17	0.35	0.50
Conc Hx	0.07	0.28	0.60	0.32	0.41	0.64

Other Variables

Variable	Outcome (p-value)					
	MCA mean	PCA mean	BHI	VMRr	Read AUC	WW AUC
Total Symptom Endorsement	0.11	0.38	0.48	0.32	0.34	0.70
Total Symptom Severity	0.10	0.31	0.50	0.31	0.36	0.65
IPAQ - SF	0.25	0.27	0.93	0.83	0.35	0.69
TWRE	0.18	0.17	0.85	0.44	0.17	0.96

Mixed Models with Cubic Mean Structure

E(outcome)= $ß_1 + ß_2$*time + $ß_3$*$time^2$ + $ß_4$*$time^3$ + $ß_5$*group + $ß_6$*group*time + $ß_7$*group*$time^2$ + $ß_8$*group*$time^3$ + $ß_9$*variable + e

Variable	Outcome (p-value)							
	BH Curve			HV Curve			Read Curve	WW Curve
	Time*IV	$Time^2$*IV	$Time^3$*IV	Time*IV	$Time^2$*IV	$Time^3$*IV		
Age	0.65	0.46	0.36	0.96	0.90	0.84	0.36	0.77
Height	0.65	0.46	0.36	0.96	0.90	0.84	0.37	0.77
Weight	0.65	0.46	0.36	0.96	0.90	0.84	0.11	0.82
Race	0.49	0.35	0.28	0.87	0.75	0.68	0.56	0.62
Ethnicity	0.96	0.78	0.68	0.65	0.68	0.69	0.50	0.88
Academic Year	0.65	0.46	0.36	0.96	0.90	0.84	0.29	0.83
IEP	0.58	0.40	0.32	0.80	0.67	0.58	0.63	0.56
504	0.64	0.42	0.32	0.99	0.85	0.76	0.58	0.84
Visual Disorder	0.65	0.46	0.36	0.96	0.89	0.84	0.39	0.73
SC Trait (n=3)	0.49	0.32	0.24	0.96	0.89	0.84	0.55	0.37
ADHD	0.49	0.32	0.24	0.96	0.89	0.84	0.35	0.75
Conc Hx	0.65	0.46	0.36	0.99	0.90	0.84	0.40	0.82

Variable	Outcome (p-value)							
	BH Curve			HV Curve			Read Curve	WW Curve
	Time*IV	$Time^2$*IV	$Time^3$*IV	Time*IV	$Time^2$*IV	$Time^3$*IV		
Total Symptom Endorsement	0.65	0.46	0.36	0.99	0.90	0.84	0.34	0.87
Total Symptom Severity	0.65	0.46	0.36	0.99	0.90	0.84	0.36	0.81
IPAQ - SF	0.14	0.07	0.05	0.46	0.32	0.24	0.35	0.60
TWRE	0.49	0.32	0.24	0.96	0.89	0.84	0.31	0.84

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