SEX-SPECIFIC DIFFERENCES IN THE ASSOCIATION BETWEEN HISTORY OF CONCUSSION AND CEREBRAL BLOOD FLOW VOLUME

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ABSTRACT

Concussion injuries are currently diagnosed using subjective symptom-reporting. The objective of this thesis was to use objective measurement of cervical arteries contributing to cerebral blood flow (Common Carotid Artery; CCA, Internal Carotid Artery; ICA and Vertebral artery; VA) between male and female athletes with and without a history of concussion (HOC). One hundred and two (102) asymptomatic university athletes were recruited; 41 athletes reported HOC and 61 did not (controls). Doppler ultrasound was used to measure blood flow volume (BFV) in CCA, ICA and VA, bilaterally. Female controls had significantly lower BFV compared to male controls in bilateral CCA and right ICA (p= 0.004; p= 0.048; p <0.001, respectively). Females with HOC had even lower BFV compared to male controls in bilateral CCA and ICA (p< 0.001; p <0.001; p< 0.001; p= 0.035, respectively). Data suggests sex-related differences, particularly in anterior cervical vessels contributing to CBF in athletes with HOC.

ACKNOWLEDGEMENTS

To Dr. Loriann Hynes, thank-you for taking a chance on me. I came into your lab unsure of my future. Three years later, research has taught me to be not uncertain, but excited, to explore and discover. Your guidance, patience, and compassion has made my journey as a graduate student a wonderful one. Thank-you for your continuous support- I could not have asked for a better supervisor.

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TERMINOLOGY

Abbreviation	Expanded Word
mTBI	Mild traumatic brain injury
TBI	Traumatic brain injury
WHO	World Health Organization
SCAT	Sport Concussion Assessment Tool
PCS	Post-concussion syndrome
PCD	Post-concussion disorder
CBF	Cerebral blood flow
BFV	Blood flow volume
CCA	Common carotid artery
ICA	Internal carotid artery
VA	Vertebral artery
DU	Doppler ultrasound
PC-MRI	Phase-contrast magnetic resonance imaging
HOC	History of concussion
ANS	Autonomic nervous system

CHAPTER ONE: INTRODUCTION

Concussion Terminology

Concussion, a form of mild traumatic brain injury (mTBI), is becoming an increasingly prominent health concern, and sport-related concussion is contributing to much of the epidemiological burden.[1] Although concussion injuries have gained popularity in recent decades, the term dates back to 460-370 BC when it was mentioned in the writings of Hippocrates.[2] It is considered probable that the term was used generically to include the entire spectrum of head injury. Concussion was first clearly described in the 10th century by the Persian physician Rhazes, who distinguished concussion as an abnormal physiologic state rather than a brain injury.[2] Despite being recognized many centuries ago, there is still confusion when it comes to terminology due to a lack of consensus on a clear definition and the terms concussion and mTBI being used interchangeably in contemporary literature.

The World Health Organization (WHO) collaborating task force on mild traumatic brain injury defines mTBI as an acute brain injury resulting from external physical forces. It must include i) one or more of the following: loss of consciousness for 30 minutes or less, post-traumatic amnesia for less than 24 hours, confusion or disorientation and/or other transient neurological irregularities; ii) Glasgow Coma Scale score of 13-15 post injury.[3,4] The latest Consensus Statement on Concussion in Sport defines concussion as a traumatic brain injury induced by biomechanical forces due to either a direct or indirect blow to the head, face, neck or body resulting in transient impairment of neurological function.[5] While both mTBI and concussion may incur adverse pathophysiological changes, the term concussion emphasizes the transient nature of the disturbance in function caused by head trauma.[6]

Epidemiology

The WHO task force on mTBI reported key findings of a systematic review of the literature, stating the incidence of hospital-treated mTBI is 100-300 per 100,000 people world-wide. However, due to significant under-recognition and underreporting in the literature,[7] the WHO estimates the true mTBI rate to be above 600 per 100,000 people.[4] In the United States, it is estimated that 1.6-3.8 million sports-related TBI's occur annually.[8] In 2009-2010, Statistics Canada reported an estimated 98,440 people (2.4% of the population) sustained a concussion or other brain injury.[9] The most recent data shows the overall incidence rate of reported sport-related concussions to be 221 per 100,000 Canadians; or 1 in 450 people.[10] There has been a significant rise in concussion incidence rate, particularly since 2010. One study observed a 4.4 fold increase in the number of emergency department visits for pediatric concussion, highlighting the potential burden concussion injuries can place on the healthcare system.[11] While the incidence rate of sport-related concussion is mounting annually, this rise may be due in part to a combination of increased awareness and improved protocols for concussion identification and management. Concussion education programs have shown to significantly improve knowledge and attitudes about concussion.[12] This improved awareness may enable coaches and athletes to better understand concussion injuries, influencing the culture of concussion reporting in sport. Depending on the sport, risk of concussion can range from quite low at 0.74% per athletic exposure (game or practice) in non-contact sports (e.g. baseball) to significantly higher at 7.92% in contact and collision sports (e.g. wrestling).[13] Another study conducted at a Canadian University observing concussions in athletes reported an incidence proportion of 10.24 concussions per 100 athletes.[14] This study also observed a significant

difference between days to physical symptom recovery and days to cognitive recovery. This highlights the need for objective tools to evaluate an athlete's true return to baseline function following a concussion injury.

Current Diagnostic Tools

Currently, there is no perfect diagnostic tool or marker clinicians can use for concussion diagnosis.[5] Instead, health care professionals rely on several tests to evaluate individuals with concussion, including symptom inventories, neurocognitive tests and postural assessments. Neurocognitive tests are found to be the most sensitive to concussion (up to 79.2%); followed by subjective symptom reporting (68%); postural assessments have the lowest sensitivity (61.9%).[15] When the complete battery is assessed, sensitivity exceeds 90% providing support for a multifaceted assessment of concussion.

The Sport Concussion Assessment Tool (SCAT) is a validated instrument that includes a combination of symptom reporting, neurocognitive and postural tests which allow for sideline evaluation in a sporting environment. The most recent version (SCAT-5) identified administering baseline testing increases the efficacy of this tool for follow up comparisons as it was found to be most useful when using normative baseline/post-injury comparisons.[16] The SCAT5 is broken down into two components: "immediate or on-field assessment" and "office or off-field assessment." The on-field assessment involves checking for red flags of potential severe injury (neck pain or tenderness, double vision, weakness or tingling in arms or legs, severe or increasing headache, seizure or convulsion, loss of consciousness, deteriorating conscious state, vomiting or increased restlessness, agitated or combative), other observable signs, memory assessment (Maddock's questions), examination of Glasgow Coma Scale and cervical spine

assessment. The off-field assessment involves a background report (details about concussion injury; medical history), symptom evaluation (22 symptoms are ranked on a scale from 0 to 6, with 0 being none and 6 being severe), cognitive screening known as Standardized Assessment of Concussion (includes tests of orientation, immediate memory and concentration), neurological screen (includes movement, coordination, balance and vison tests) and lastly, delayed recall assessment.

SCAT5 is the most well-established instrument for concussion diagnosis, however there are a variety of other tools clinicians can choose from. In non-sport settings, evaluation of symptoms can be done using the Post-Concussion Symptom Scale or the Rivermead Post Concussion Questionnaire. There is no universal protocol, however almost all protocols include assessment of symptoms and neurocognition.[5] Identifying objective biomarkers of concussion is critically important because objective measures in conjunction with subjective reporting will provide clinicians with a better, more wholesome understanding of concussion recovery. This may better inform clinicians in tailoring more individualized rehabilitation protocols.

Concussion Signs/Symptoms and Pathophysiology

The diagnosis of concussion includes one or more of the following domains: clinical symptoms, which can be somatic (ie. headache), cognitive (ie. feeling like in a fog) or emotional (ie. lability); physical signs (ie. loss of consciousness, amnesia, neurological deficit); cognitive impairments (ie. slowed reaction times); balance impairment (ie. gait unsteadiness); behavioural changes (ie. irritability) and sleep/wake disturbance (ie. somnolence, drowsiness).[5] For the majority, resolution of symptoms/signs will occur within 10-14 days[5] but 10–30 % of individuals will exhibit prolonged symptoms.[17] A systematic review revealed symptoms can

persist up to 6 months in 14% to 26% of individuals, and up to several years for approximately 23% of individuals.[18,19] In individuals for whom symptoms persist beyond the generally accepted time frame, it is commonly referred to as post-concussion syndrome (PCS).[20] The Diagnostic and Statistics Manual (DSM-IV) defines PCS as: (1) cognitive deficits in attention or memory and (2) at least three or more of the following symptoms: fatigue, sleep disturbance, headache, dizziness, irritability, affective disturbance, or apathy or personality change that persist for three months or greater.[21] The WHO's International Classification of Diseases (ICD-10) defines PCS as the persistence of three or more of the following symptoms: fatigue, sleep disturbance, headache, dizziness, irritability, insomnia, concentration and memory difficulties; this definition is more sensitive for identifying PCS however it does not specify a time frame.[22] A limitation of these PCS definitions is their non-specific criteria and their inclusion of symptoms that also reflect other neurological disorders, such as depression or migraines.[23] PCS was removed from the DSM-V; instead, clinicians use the term "prolonged symptoms" or "persistent symptoms" to refer to symptoms that do not resolve within the typical 10-14 day time frame. A framework has been proposed for categorizing the prolonged symptoms into post-concussion disorders (PCD). The three categories identified are: physiologic PCD, which is characterized by impairments in global cerebral metabolism; vestibulo-ocular PCD, which is characterised by dysfunction of the vestibular and oculomotor systems; and cervicogenic PCD which is characterised by dysfunction of the cervical spine and somatosensory system.[24]

Giza and Hovda[25] provided the original explanation of the neurometabolic cascade that leads to impairments in the brain metabolism as seen in physiologic PCD. Following biomechanical injury to the brain, there is an indiscriminate release of the neurotransmitter

glutamate, causing neuronal depolarization which results in an influx of calcium and efflux of potassium. High levels of intracellular calcium can result in accumulation of calcium in the mitochondria of nerve cells, which may cause impaired oxidative metabolism.[26] This ionic flux may also be correlated to clinical characteristics of concussion such as headaches, photophobia and phonophobia.[27] In an effort to restore ionic homeostasis, adenosine triphosphate (ATP)-dependent pumps are activated. This leads to an increase in glucose metabolism or "hypermetabolism." Usually, cerebral blood flow (CBF) is tightly coupled to cerebral metabolism, where an increase in metabolism results in an increase in CBF. However, this hypermetabolism occurs in a setting of normal or diminished CBF, leading to a mismatch between supply and demand and consequently resulting in a cerebral energy crisis (Figure 1).[25,27] After the initial period of hypermetabolism which may occur up to 24 hours, glucose metabolism diminishes. Positron Emission Tomography (PET) in humans shows a reduction in global cerebral glucose metabolism that can last for 2-4 weeks post-concussion.[25]

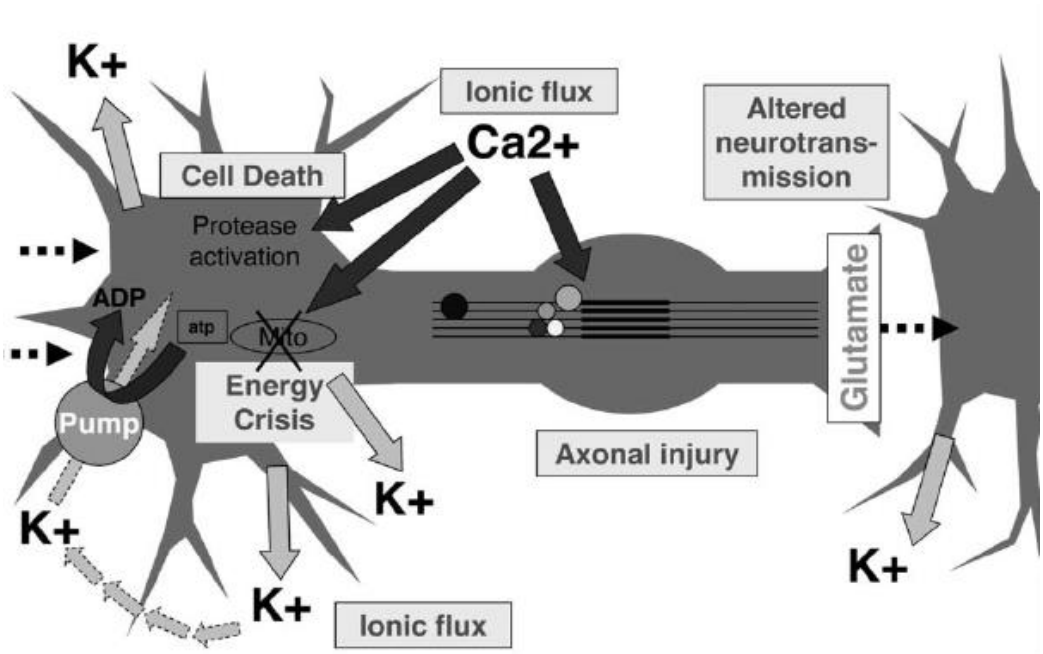

Figure 1. Neurometabolic cascade occurring after concussion.[27]

Cerebral Blood Flow

Current research demonstrates alterations in cerebral blood flow (CBF) as a result of head trauma, suggesting the potential for CBF to be used as a biomarker of concussion. Some studies report elevated CBF,[28,29] while others report reduced CBF post-injury.[30–33] The majority of these studies evaluated CBF in the acute stage (ranging from 24 hours to 10 days) and found a mixture of results as some reported elevated CBF and others reported reduced CBF; studies that assessed CBF in the subacute stage (ranging from 3 days to one month) all found a reduction in CBF. CBF is regulated by the autonomic nervous system (ANS) in response to changes in blood pressure to maintain stable perfusion; the ANS can control CBF via constriction or dilation of cerebral blood vessels. Studies have shown impairments to cerebral autoregulation following a brain injury,[34–36] however, it is not known whether this impairment is due to incongruency in supply and demand as witnessed in the neurometabolic cascade.

Constant CBF is vital to the human brain which receives approximately 15% of total cardiac output.[37] There are two ways by which to assess total CBF: total CBF can be calculated by measuring flow in the capillaries or by measuring flow in the supplying vessels of the brain.[38] The brain is supplied by four major arteries: the two internal carotid arteries (ICA) and the two vertebral arteries (VA). CBF can be calculated by adding the blood flow volumes (BFV) of the ICA and VA together, a method which has been validated.[39]

CBF supply begins at the aortic arch of the heart, which branches into the left common carotid artery (CCA) and the brachiocephalic artery which gives rise to the right CCA. At the level of the fourth cervical vertebrae, both left and right CCA bifurcate into the ICA and external carotid artery. The ICA supplies most of the ipsilateral cerebral hemisphere.[40] The subclavian arteries also branch out from the aorta of the heart and give rise to the bilateral vertebral arteries (VA), which ascend the neck by passing through the foramina in the transverse process of the cervical vertebrae (Figure 2). The bilateral VA merge together to form the basilar artery travelling to the brainstem, pons, and midbrain where it converges with the bilateral ICA to form the Circle of Willis. The circle of Willis is formed by the anastomosis between the two ICA and the two VA.[40] Within the circle of Willis, the ICA bifurcates into the anterior cerebral artery and the middle cerebral artery; the basilar artery bifurcates into the posterior cerebral artery which connects to the middle cerebral artery via the posterior communicating artery.[41] Branches from the bilateral anterior, middle, and posterior arteries perfuse different parts of the cortex (Figure 3).[37]

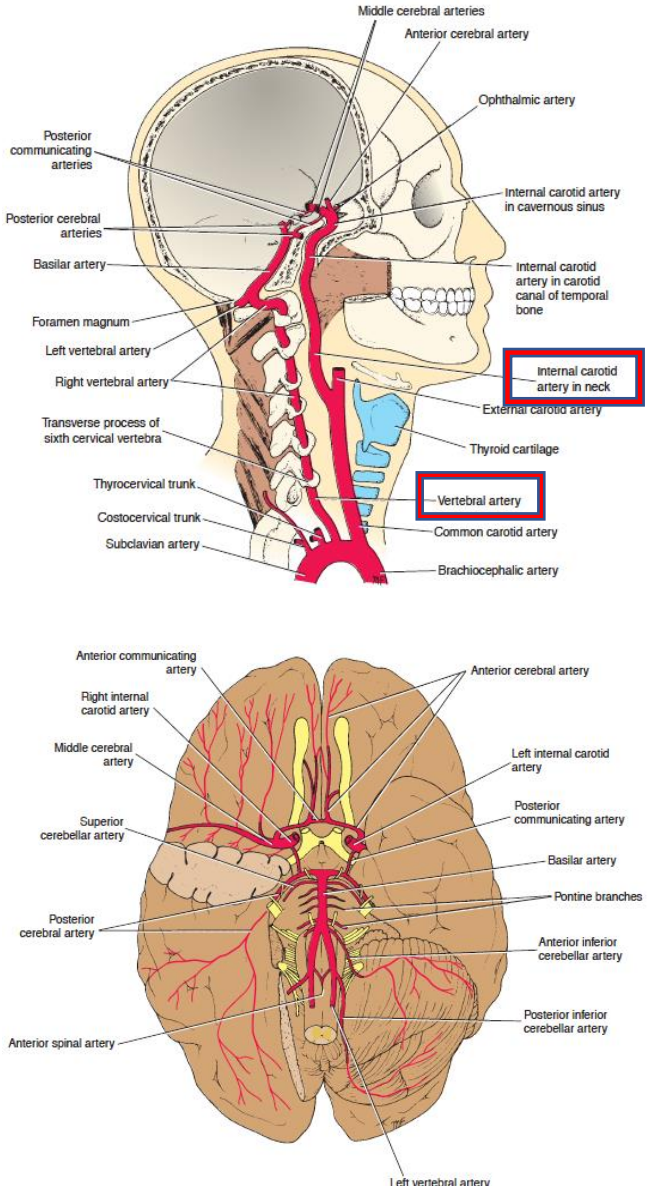

Figure 2. Blood supply of the brain: A) origin and courses of the internal carotid and vertebral carotid arteries as they ascend the neck to enter the skull. B) Internal carotid and basilar arteries converge to form circle of Willis.[41]

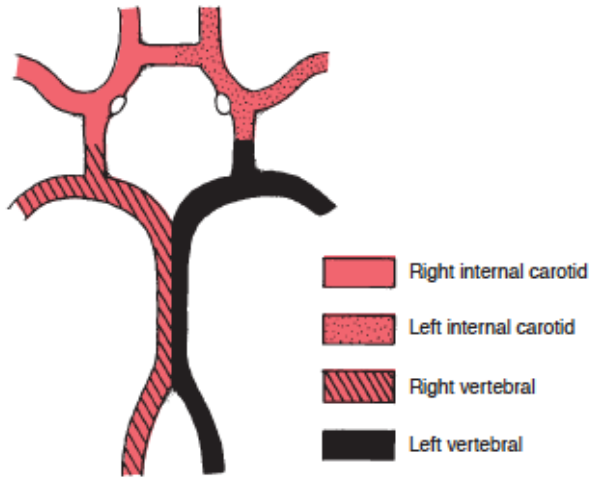

Figure 3. Circle of Willis distributes blood to different areas of the cortex.[41]

Sonography and phase-contrast magnetic resonance imaging (PC-MRI) are two non-invasive imaging techniques that allow assessment of individual blood vessels.[42] Blood flow of the capillaries of the brain can be measured using MRI whereas blood flow in the supplying vessels of the brain can be measured by both Doppler Ultrasound (DU) and MRI. Studies have validated both methods of blood flow measurement.[39,43–45] Although PC-MRI is considered the gold standard for blood flow volume quantification, DU is more readily available and has been shown to be a practical method to measure CBF.[39] Its benefits include low operational costs, accessibility to repeated measurements and its use for bedside monitoring. However, it has limitations; it is operator-dependent and it can overestimate BFV.[46] Despite this, studies have established a correlation between PC-MRI and sonography, with B-flow ultrasound providing the closest flow-volume values to PC-MRI flow quantification (correlation coefficient of 0.917 for ICA and 0.794 for VA); B-flow imaging portrays the blood echoes in a grayscale presentation, providing unobstructed views of the vessel lumen.[44] This thesis will use DU to measure blood flow volume in the supplying vessels of the brain to estimate cerebral blood flow.

Sex Differences in Concussion

Sex refers to a set of biological attributes primarily related to physical and physiological features such as chromosomes, gene expression, hormone expression and function, and reproductive/sexual anatomy.[47] Within the realm of concussion, sex is a contributing factor of incidence rate, symptom reporting, post-concussion impairments and recovery time.

In the context of incidence rates, a systematic review revealed evidence indicating females may be at greater risk of sustaining a concussion compared to males in sex-comparable sports (including soccer and basketball). Nine out of the ten studies included in this review of incidence rates observed higher absolute concussion injury rates for females.[48] Other studies show that females are 1.4 to almost 2 times more likely to sustain a sport-related concussion compared to their male counterparts.[49–51] Concussion incidence for females in para-sport is also reportedly higher, as a study observed females to be 2.5 times more likely to sustain a concussion in wheelchair basketball.[52] Higher female susceptibility to concussion may be explained by differences in anatomy and biomechanics. Females reportedly have less head mass and neck girth, as well as weaker neck flexor and extensor muscles.[53] As a result of weaker neck stabilization muscles, females experience greater acceleration of the head-neck complex during impact to the body, increasing their susceptibility to injury.[53,54]

Sex differences in symptom reporting are also observed in sex-comparable sports.[55] A meta-analysis conducted on acute symptom reporting disclosed that although there are no differences between males and females on the likelihood of reporting symptoms, there is a difference in the total number of symptoms reported; females generally report higher symptom scores, at both baseline and post-concussion.[55] Another meta-analysis found contradictory

results- males reported greater number of symptoms than females post-concussion.[56] It is worth noting that sex-comparable sports are underrepresented in this meta-analysis and majority of the athletes included are football players; therefore the results may be biased by athletes in this sport. A systematic review observing sex differences in prolonged symptoms indicates that female sex is also associated with poorer chronic outcome as females are more likely to develop PCS.[57] It is important to consider that concussion symptoms such as headaches, difficulty concentrating, energy/sleep disturbance and emotional symptoms are also associated with premenstrual syndrome (PMS),[58-59] suggesting that symptom presentation may be hormonally influenced in females. These sex differences in symptom reporting may also be attributed to differences in pain perception as females are generally more sensitive to pain compared to males.[60] It is also important to consider non-biological factors that may contribute to sex differences in symptom reporting. For example, due to societal norms and constructions of masculinity, males may be more likely to downplay symptoms to appear "strong."[61] Overall, subjective measures of concussion such as symptom reporting can be influenced by a range of factors including those mentioned above.

When it comes to post-concussion impairments, results are inconsistent. A meta-analysis evaluating acute neuropsychological outcomes following concussion reveals that females demonstrate a larger neuropsychological deficit compared to males.[56] However, other studies not included in this analysis found no significant differences between males and females[14,62] Another recent study looking at long-term effects of concussion observed that females with a history of concussion (HOC) performed significantly slower on memory tasks compared to males with HOC.[63] Although females may have greater (and perhaps chronic)

neuropsychological deficits following concussion injury, the data is insufficient and therefore it is difficult to make conclusions. There is variability in the neurocognitive tests administered and without a standardized protocol, it is challenging to consolidate study results.

Recovery time following concussion injury is also considered to differ by sex. The most recent systematic review observing predictors of clinical recovery established that females, on average, take longer to recover compared to males.[64] Females are also more likely to have symptoms persist for more than one-month post-concussion.[64] However, studies that corroborate these findings were predominantly youth population studies, and therefore these results may be confounded by differences in neurodevelopment; maturation of the cortex extends past adolescence and occurs at different rates for males and females.[65] Stereotypical conceptions of masculinity and femininity may also play a role in these sex differences. Physicians may have a gender bias against clearing females too early (to protect them), leading to longer clinical recovery times for females.[66] Overall, there appear to be sex-related differences across many domains of concussion injury. Other studies may demonstrate results that are inconsistent with those mentioned in this paper, however this paper focused on systematic reviews and meta-analyses to highlight the main findings in each domain.

CHAPTER TWO: MANUSCRIPT

Introduction

Concussion, a form of mild traumatic brain injury (mTBI), is becoming an increasingly prominent public health concern in the athletic world.[1] The term concussion was first clearly described in the 10th century by Persian physician Rhazes[2], but despite being acknowledged many centuries ago, there is still confusion with the terminology. The latest Consensus Statement on Concussion in Sport defines concussion as a traumatic brain injury caused by biomechanical forces due to either a direct or indirect blow to the head, face, neck or body resulting in transient impairment of neurological function.[5] Within literature the terms concussion and mTBI are often used interchangeably. While both may incur adverse pathophysiological changes, the term concussion emphasizes the *transient* disturbance in function caused by head trauma.[6]

From 2009-2010, Statistics Canada reported an estimated 98,440 people sustained a concussion or other brain injury.[9] There has been a significant rise in concussion incidence, particularly since 2010; one study observed a 4.4 fold increase in the number of paediatric concussion-related visits to the emergency department in Ontario.[11] The most recent Canadian Community Health Survey stated the overall incidence rate of reported sport-related concussions to be 221 per 100,000 Canadians; or 1 in 450 people.[10] A study conducted at a Canadian University observing concussions in athletes reported an incidence proportion of 10.24 concussions per 100 athletes.[14] This study also observed a significant difference between days to physical symptom recovery and days to cognitive recovery. This difference between

physical and cognitive recovery highlights the need for objective tools to help better evaluate an athlete's recovery following a concussion injury.

Giza and Hovda[25] provided the original explanation of the neurometabolic cascade that leads to impairments in brain metabolism. Following biomechanical injury to the brain, there is an indiscriminate release of the neurotransmitter glutamate, causing neuronal depolarization which results in an influx of calcium and an efflux of potassium. In an effort to restore homeostasis, adenosine triphosphate (ATP)-dependent pumps are activated. This leads to an increase in glucose metabolism or "hypermetabolism." Usually, cerebral blood flow (CBF) is tightly coupled to cerebral metabolism, where an increase in metabolism results in an increase in CBF. However, this hypermetabolism occurs in a setting of normal or diminished CBF, leading to a mismatch between supply and demand and consequently resulting in a cerebral energy crisis.[25,27]

Continual CBF is vital to the human brain which receives approximately 15% of total cardiac output.[37] The brain is supplied by four major arteries: the two internal carotid arteries (ICA) and the two vertebral arteries (VA); the ICA stem from the common carotid arteries (CCA) bilaterally, which bring blood up from the heart.[40] CBF can be estimated by measuring blood flow volume (BFV) of the supplying vessels in the neck, a method that has been previously validated.[39]

Studies report alterations in CBF as a result of head trauma, suggesting the potential for CBF to be used as a biomarker for concussion injury. Some studies report elevated CBF,[28-29] while others report reduced CBF.[30–33] However, majority of these studies document changes in CBF in the acute or subacute stages (ranging from 24 hours to one month post-concussion).

There remains a dearth of longitudinal data evaluating CBF beyond one-month post-concussion. The objective of the current study is twofold. First, to examine the effects of history of concussion (HOC) on blood flow volume in the cervical arteries which contribute to CBF within an athletic cohort; and second, to examine sex-specific differences in the effects of HOC on blood flow volume in the cervical arteries which contribute to CBF. It is hypothesized that both males and females with HOC will present lower BFV compared to athletes without HOC, as demonstrated in previous studies. It is also hypothesized that females with HOC will present lower BFV, as literature reports sex-related differences in blood flow.[42]

Methods

Participants

Participants were recruited from a variety of varsity sports teams during pre-season (soccer, hockey, basketball, volleyball and rugby). A total of 102 athletes were recruited for the study; participants were separated into two groups- those with HOC (41) and those without HOC, referred to as controls (61). Participants were reported to be healthy and were not diagnosed with a concussion at the time of testing; participants with HOC were “asymptomatic” at the time of testing. Participants were briefed on the study protocol and informed consent was obtained prior to their participation. The study protocol was approved by the Human Participants Review Committee (HPRC) at the Office of Research Ethics, York University, Toronto, ON.

Doppler Ultrasound Collection and Analysis

Doppler ultrasound (DU) is a non-invasive imaging technique that allows for assessment of individual blood vessels.[42] DU has been validated as a reliable measure of CBF.[39] DU is also comparable and correlates to phase-contrast magnetic resonance imaging.[44,67]

Blood flow velocity and diameter were collected on a Logiq-e Ultrasound System (GE Healthcare). Bilateral CCA, ICA, and VA velocities and diameters were measured using a 4-12MHz linear array transducer. The CCA was located in the transverse plane (Figure 4-A) and imaged in the longitudinal plane (Figure 4-B); the ICA was imaged at the level of CCA bifurcation or slightly above, approximately at the level of the C4 vertebrae (Figure 4-C); and the VA was imaged at the level of the intertransverse segments of the vertebrae (Figure 4-D). To ensure consistency and uniformity between vessel measurements, an angle-corrected measurement was taken across the entire vessel lumen with an insonation angle of 60° for all vessel measurements.

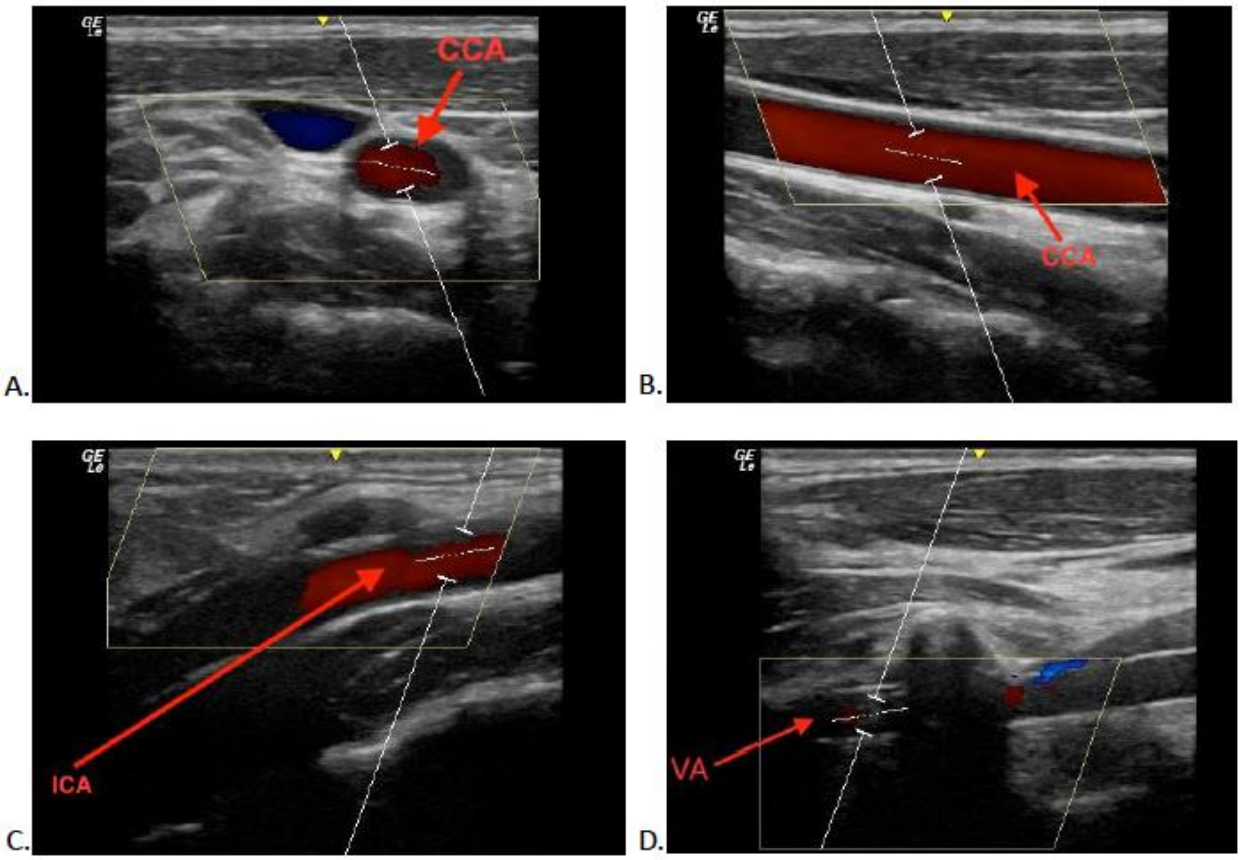

Figure 4. Doppler ultrasound imaging of the supplying vessels in the neck.
A) Common carotid artery (CCA) in the transverse plane. B) CCA in the longitudinal plane C) Internal carotid artery (ICA) at level of bifurcation. D) Vertebral artery (VA) at level of intertransverse segments of the vertebrae.

Blood Flow Volume (BFV) (mL/min) was calculated as: time-averaged velocity (TAMEAN)(cm/s) X cross sectional area of the vessel (cm^2) X 60s to adjust for a single cardiac cycle. Cross-sectional area was measured as $(d/2)^2 X \boldsymbol{\pi}$ where (d) is the vessel diameter. This is in line with methods adopted in the current literature.[68] Diameter was calculated three times per cardiac cycle; three cardiac cycles were utilized to provide a total of 9 measures to obtain the total average. Measurements were recorded during the end-diastolic phase when the velocity profile becomes parabolic and the mean velocity is lower than the maximum velocity; this was done to counter the overestimation of BFV by DU.[46,68]

Variables

Exposure variables were concussion history (HOC or control) and sex (male or female). Outcome variables were BFV in the six cervical arteries which contribute to CBF: CCA, ICA and VA, bilaterally.

Statistical Analysis

Assumptions of normality were first assessed using the Shapiro-Wilk tests and confirmed for all variables. Histograms were also produced to observe distribution in the outcome variables, and all were found to be normally distributed. Stem-and-leaf plots were used to look for outliers and parametric tests were subsequently run.

In the primary analysis, a Pearson correlation test was used to explore the association between the outcome variables. Independent t-tests were used to identify significant differences between athletes with HOC and without HOC across all outcome variables. Independent t-tests were also used to identify significant differences between males and females across all outcome variables. A multiple linear regression was conducted to observe associations between HOC, sex and BFV.

In a secondary analysis, participants were divided into the following four cohorts: male athletes without HOC, to be referred as male controls; male athletes with HOC; female athletes without HOC, to be referred as female controls; and female athletes with HOC. A one-way analysis of variance (ANOVA) was performed to look for differences between the four cohorts across all outcome measures. A Bonferroni post-hoc test was applied to observe where the differences were located. Another linear regression analysis was performed to observe the relationship between the four cohorts for each outcome variable, adjusting for both HOC and

sex. Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS), Version 26 (IBM Corp. Armonk, New York). All significance levels were set to $p < 0.05$.

Results

In total, 102 varsity athletes participated in the study; however, 9 athletes (8.82%) did not have conclusive DU recordings and were excluded from the data analysis. A total of 93 athletes were included in the final data analysis: 37 with HOC (39.8%) and 56 controls (60.2%). Of those included in the data analysis, 5 athletes were missing DU recordings for the right VA; 3 athletes were missing DU recordings for the left VA; and 14 athletes were missing age. Demographics and descriptive statistics are outlined in Table 1.

Table 1: Demographic characteristics and blood flow volume of the bilateral common carotid, internal carotid, and vertebral arteries in athletes both with and without history of concussion.

	Control	**HOC**	***p*-value**
N (%)	56 (60.2)	37 (39.8)	
Age [mean (SD)]*	20.36 (1.75)	20.59 (1.68)	0.558
Sex, N (%)			
Male	30 (32.3)	19 (20.4)	
Female	26 (27.9)	18 (19.4)	
Vessel BFV [mean (SD)]			
Right CCA	631.82 (123.03)	580.62 (99.91)	0.037
Left CCA	660.7 (108.48)	643.1 (134.97)	0.489
Right ICA	468.76 (112.18)	431.39 (100.33)	0.105
Left ICA	490.04 (106.75)	482.32 (115.39)	0.742
Right VA*	109.26 (50.89)	102.91 (35.44)	0.522
Left VA*	145.06 (56.82)	128.93 (42.88)	0.151

Abbreviations: HOC, history of concussion; BFV, blood flow volume; CCA, common carotid artery; ICA, internal carotid artery; VA, vertebral artery

*For this variable there were missing data

Primary Analysis

The Pearson correlation test showed significant correlation between the bilateral CCA and bilateral ICA. There was also significant correlation between the right CCA and ICA and between the left CCA and ICA. Lastly, there was correlation between the left CCA and right ICA. These results are summarized in Table 2. Independent t-tests indicated significantly lower BFV in the right CCA in athletes with HOC compared to controls, $t(91)= 2.112$, $p= 0.037$. Although there were no significant differences in the other vessels, there was a trend towards lower BFV in all vessels in athletes with HOC (Figure 5). There were also significant differences in BFV between males and females in the right CCA, $t(91)= 3.922$, $p= <0.001$; left CCA, $t(91)= 4.156$, $p <0.001$; right ICA, $t(91)= 4.326$, $p= <0.001$; left ICA, $t(91)= 2.269$, $p= 0.026$; and left VA, $t(88)= -2.264$, $p= 0.026$. Females had lower BFVs in all vessels except for bilateral VA where the opposite was true (Figure 6). Multiple linear regressions revealed that females had significantly lower BFV after adjustment for HOC in the following vessels: right CCA ($\beta= -87.26$; $p= <0.001$); left CCA ($\beta= -94.62$; $p< 0.001$); right ICA ($\beta= -88.61$; $p= <0.001$); and left ICA ($\beta= -50.43$; $p= 0.027$). They also had significantly higher BFV after adjustment for HOC in the left VA ($\beta= 24.92$; $p= 0.022$) (Table 3).

Table 2: Pearson correlation coefficients for the bilateral common carotid, internal carotid, and vertebral arteries.

	Right CCA		Left CCA		Right ICA		Left ICA		Right VA		Left VA	
	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*
Right CCA	1		0.38**	<0.001	0.58**	<0.001	0.18	0.084	0.02	0.831	0.02	0.873
Left CCA	0.38**	<0.001	1		0.44**	<0.001	0.54**	<0.001	0.13	0.245	0.02	0.865
Right ICA	0.58**	<0.001	0.44**	<0.001	1		0.45**	<0.001	0.16	0.143	0.01	0.964
Left ICA	0.18	0.084	0.54**	<0.001	0.45**	<0.001	1		0.13	0.247	0.12	0.279
Right VA	0.02	0.831	0.13	0.245	0.16	0.143	0.13	0.247	1		0.02	0.863
Left VA	0.02	0.873	0.02	0.865	0.01	0.964	0.12	0.279	0.02	0.863	1	

Abbreviations: CCA, common carotid artery; ICA, internal carotid artery; VA, vertebral artery
r= Pearson Correlation Coefficient
**= p< 0.01

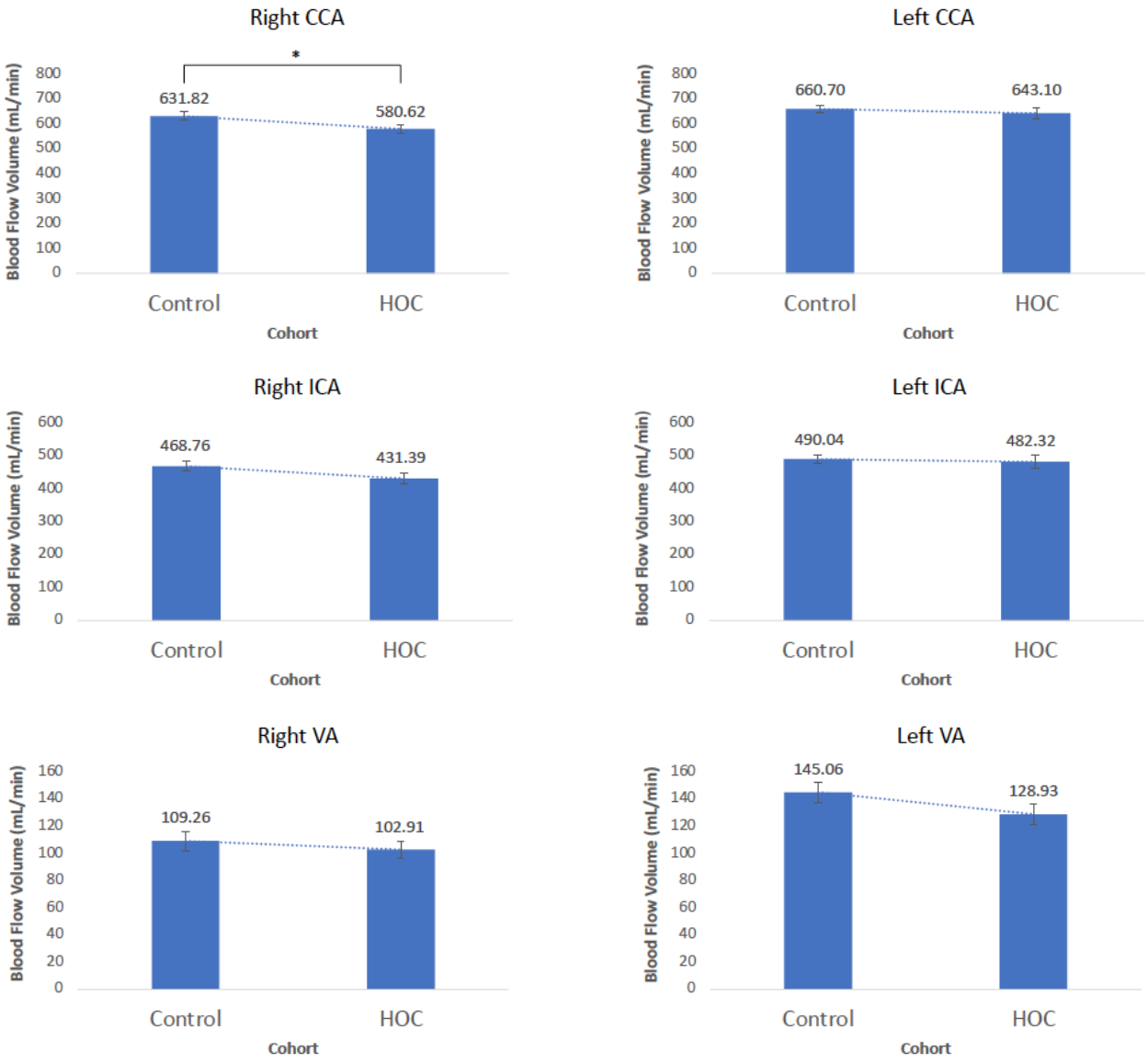

Figure 5. Independent t-tests comparing athletes without history of concussion (control) to athletes with history of concussion (HOC). Blood flow volumes were compared in bilateral common carotid arteries (CCA), bilateral internal carotid arteries (ICA) and bilateral vertebral arteries (VA). *= $p < 0.05$. Error bars represent standard error of the mean.

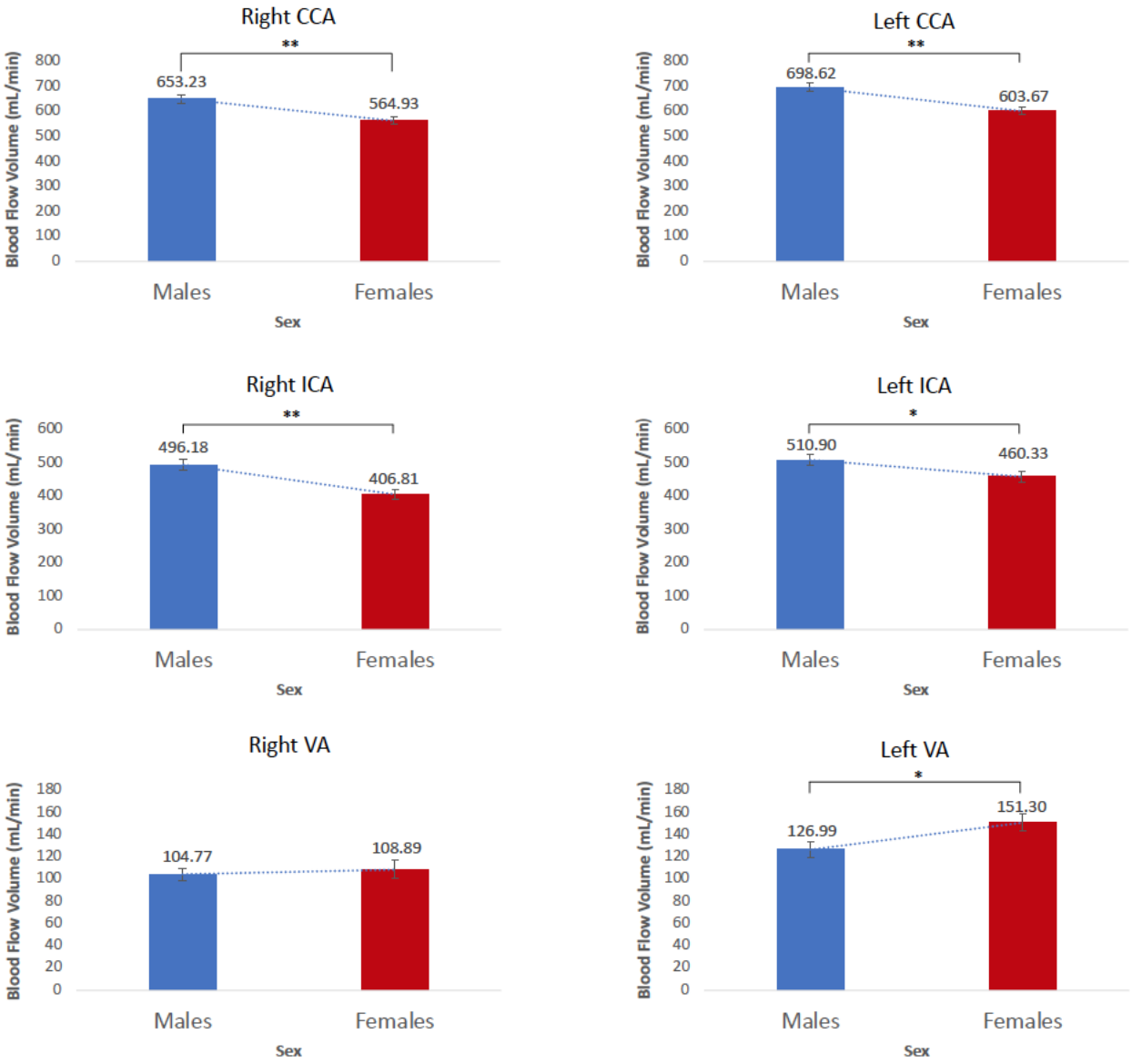

Figure 6. Independent t-tests comparing males to females. Blood flow volumes were compared in bilateral common carotid arteries (CCA), bilateral internal carotid arteries (ICA) and bilateral vertebral arteries (VA). *= $p< 0.05$, **= $p<0.01$. Error bars represent standard error of the mean.

Table 3: Linear regression analysis of blood flow volume in the bilateral common carotid, internal carotid, and vertebral arteries, after adjusting for HOC and sex. Controls are the reference group for the HOC analysis; males are the reference group for the sex analysis.

Vessel	HOC			Sex		
	β	95% CI	*p*	*β*	95% CI	*p*
Right CCA	-49.26	-93.98 to -4.55	0.031	-87.26	-131.09 to -43.42	<0.001
Left CCA	-15.51	-61.96 to 30.95	0.509	-94.62	-140.16 to -49.08	<0.001
Right ICA	-35.4	-76.86 to 6.05	0.093	-88.61	-129.25 to -47.98	<0.001
Left ICA	-6.6	-52.01 to 38.81	0.773	-50.43	-94.94 to -5.91	0.027
Right VA	-6.42	-26.18 to 13.35	0.520	4.2	-15.16 to 23.57	0.667
Left VA	-17.06	-38.65 to 4.54	0.120	24.92	3.74 to 46.1	0.022

Abbreviations: HOC, history of concussion; CCA, common carotid artery; ICA, internal carotid artery; VA, vertebral artery; β, beta coefficient; CI, confidence interval

Secondary Analysis

Secondary analysis assessed the data after dividing it into 4 cohorts (male controls, males with HOC, female controls, and females with HOC). A one-way ANOVA revealed significant differences between the 4 groups in the right CCA, $F(3,89)= 6.868$, $p= <0.001$; left CCA, $F(3,89)= 7.346$, $p= <0.001$; right ICA, $F(3,89)= 7.351$, $p= <0.001$; and left ICA, $F(3,89)= 3.022$, $p= 0.034$. Bonferroni post-hoc analyses indicate female controls had significantly lower BFV in the right CCA ($p= 0.023$) and right ICA ($p= 0.003$) when compared to male controls; and females with HOC had significantly lower BFV in the right CCA ($p < 0.001$) and right ICA ($p= 0.001$) when compared to male controls. In the left CCA and left ICA, female controls and females with HOC followed a similar trend (lower BFV) when compared to male controls (Figure 7). Another linear

regression analysis was conducted for each outcome variable with the 4 cohort groups, which adjusted for both HOC and sex (Table 4). Compared to male controls, female controls had significantly lower BFV in the right CCA (β= -84.88; *p*= 0.004); left CCA(β= -58.47; *p*= 0.048); and right ICA (β= -97.11; *p*= <0.001). Compared to male controls, females with HOC had significantly lower BFV in the right CCA (β= -137.26; *p*< 0.001); left CCA (β= -121.32; *p*= <0.001); right ICA (β= -121.39; *p*< 0.001) and left ICA (β= -67.78; *p*= 0.035).

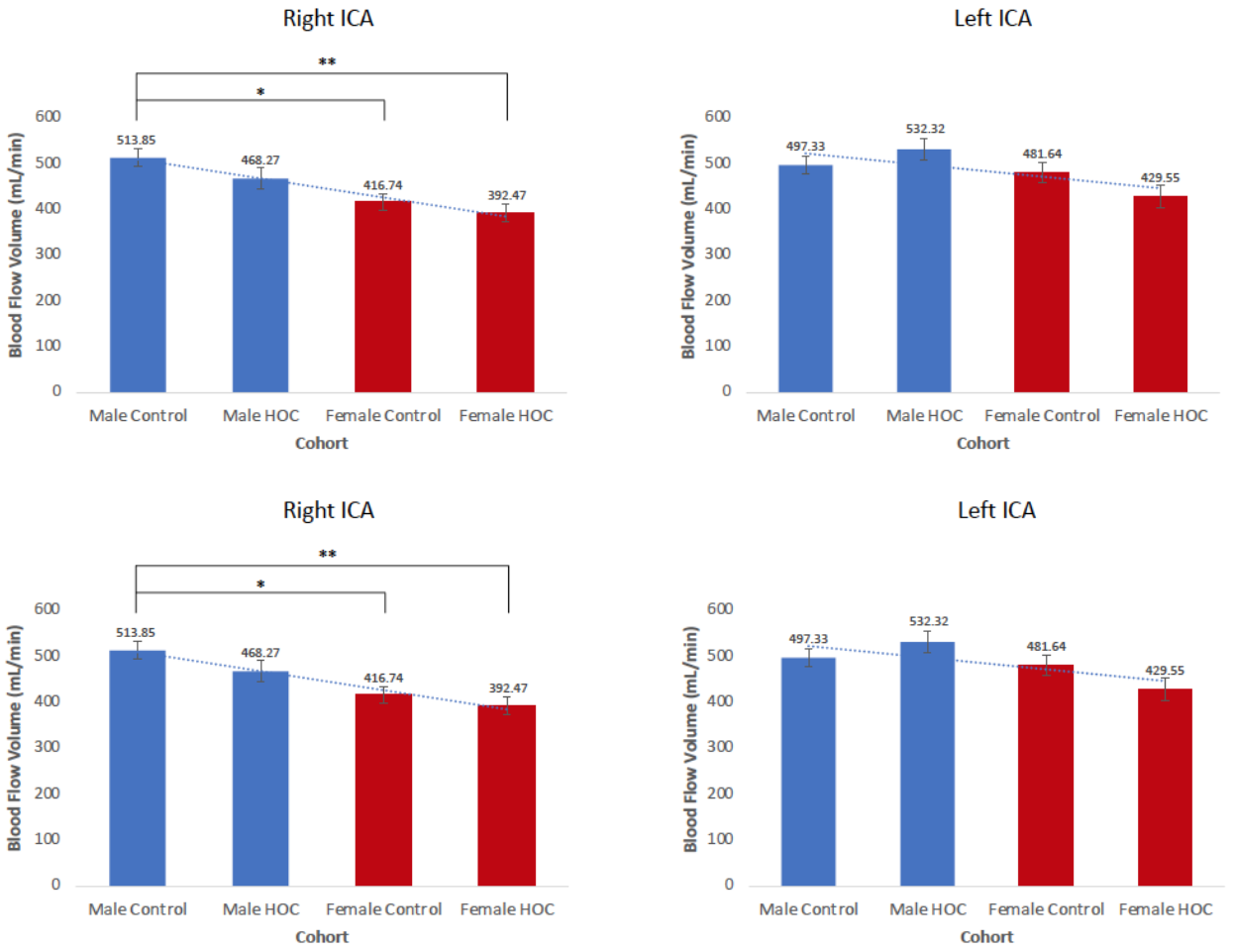

Figure 7. Differences in blood flow volume in the bilateral common carotid arteries (CCA) and bilateral internal carotid arteries (ICA) between male controls, males with HOC, female controls, and females with HOC. *=p<0.05, **=p<0.01. Error bars represent standard error of the mean.

Table 4: Linear regression analysis of blood flow volume in the bilateral common carotid, internal carotid, and vertebral arteries. Male Control served as the reference group.

Vessel	Male HOC			Female Control			Female HOC		
	β	95% CI	*p*	*β*	95% CI	*p*	*β*	95% CI	*p*
Right CCA	-46.42	-108.64 to 15.8	0.142	-84.88	-141.74 to -28.02	0.004	-137.26	-200.53 to -73.98	<0.001
Left CCA	27.78	-35.5 to 91.06	0.385	-58.47	-116.3 to -0.64	0.048	-121.32	-185.66 to -56.97	<0.001
Right ICA	-45.58	-103.18 to 12.02	0.119	-97.11	-149.75 to -44.47	<0.001	-121.39	-179.9 to -62.81	<0.001
Left ICA	34.99	-26.91 to 96.9	0.264	-15.69	-72.26 to 40.89	0.583	-67.78	-130.73 to -4.82	0.035
Right VA	7.07	-20.18 to 34.32	0.607	15.39	-9.43 to 40.21	0.221	-5.63	-33.36 to 22.1	0.687
Left VA	-21.06	-51.31 to 9.2	0.170	21.61	-5.91 to 49.13	0.122	8.8	-21.45 to 39.06	0.564

Abbreviations: HOC, history of concussion; CCA, common carotid artery; ICA, internal carotid artery; VA, vertebral artery; β, beta coefficient; CI, confidence interval

Discussion

This study evaluated the effects of HOC on BFV in the vessels contributing to CBF and found a trend toward lower BFV in athletes with HOC. Lower BFV was observed in the bilateral CCA, ICA and VA in athletes with HOC compared to controls. Observation of reduced CBF following concussion injury is consistent with both animal and human studies.[30-33,69] Multiple studies that have looked at sports-related concussions corroborate our findings of lower CBF following concussion injury[30-33,]; however these studies evaluate changes in CBF during the acute or subacute phases of concussion which can range from 24 hours to 30 days. Another study observing CBF in concussed athletes found that CBF was elevated in the early acute phase (1-3 days) but reduced in the late acute phase (5-7 days), underlining changes in CBF throughout the trajectory of a concussion injury.[29] Longitudinal studies examining CBF suggest hypoperfusion is present chronically in symptomatic patients following mTBI,[70] but there is limited research on

the long-term effects of concussion on CBF in asymptomatic individuals. The current study helps to fill this gap in the literature as it evaluates CBF in asymptomatic athletes, suggesting chronic hypoperfusion exists after the recovery period. Studies have observed differences between days to symptom recovery and days to cognitive recovery, indicating that symptom resolution or "asymptomatic" may not necessarily equal complete return to normal function.[14]

Secondary analysis of this study compared BFV in each vessel between the four cohorts. The results suggest that HOC may have sex-specific effects on BFV, as female controls had lower BFV compared to male controls, and females with HOC had even lower BFV compared to male controls in the right CCA and right ICA; and although not statistically significant, in the left CCA and left ICA. We made the decision to have male controls be the reference group because males have significantly larger vessel diameters compared to females[42]; we hypothesized male controls with no prior HOC would have the greatest BFV and we wanted to compare the other three cohorts to this reference group. Males with HOC did not reveal any significant differences when compared to male controls. Males with HOC did follow a similar trend to the female cohorts in the right CCA and right ICA (lower BFV); however, interestingly, elevated BFV in the left CCA and left ICA were found. Males had a higher standard deviation of BFV in the right CCA, left CCA, and right ICA compared to females. Perhaps these findings suggest greater overall variability in the effects of HOC on BFV for male athletes. The findings of this study are supported by literature which reports sex-related differences in concussion injury. Not only are females likely to exhibit greater symptoms following concussion injury, but they are also at greater risk for post-concussion syndrome.[71]

There are several different explanations for the observed effects of HOC on CBF. A study observing fluid percussion injuries in rodents found acute reductions in both number of capillaries and diameter at the site of injury, suggesting that compromised structural integrity of the vasculature may contribute to reduced CBF.[69] Another animal model study indicates that the observed effects may be a result of persistent long-term changes in neurometabolic activity, leading to a decline in CBF demand.[72]

Human studies have also found abnormalities in brain structure following concussion injury. There appears to be a positive relationship between reduced gray matter volume and lower CBF; this relationship is specifically observed in the insula which plays a role in homeostatic functions including cerebral autoregulation.[73] Studies have also observed volumetric reductions in the thalamus in asymptomatic individuals with HOC.[74] Additionally, athletes with HOC demonstrate lower CBF in the thalamus[75]; this may be explained by the volume loss in the thalamus leading to lower CBF demand.

It is worth noting that in the present study, there were no significant differences between the VA bilaterally when comparing athletes with HOC to controls; or when comparing the female cohort groups to male controls. The data demonstrates differences specifically in the anterior cervical vessels (CCA and ICA), suggesting soft tissue in the neck may be a mechanism influencing blood flow to the brain post-concussion injury. The sternocleidomastoid (SCM) muscle is a cervical flexor that runs obliquely across the neck. The SCM is superficial to the common carotid sheath,[40] and is commonly injured in those who have suffered a Whiplash Associated Disorder (WAD)[76]; the literature provides support for a relationship between WAD and concussion.[77–79] Based on the anatomical association between the head and neck, it is

rational to postulate the SCM may serve as protection for the CCA and ICA and that significant impact of the head may result in inertial loading of the neck or vice versa. Consequently, the soft tissue may be a mechanism influencing blood flow to the brain post-concussion injury; swelling of the injured SCM muscle may apply pressure on the carotid sheath, which may lead to changes in vessel diameter and subsequently blood flow. It is important to further explore the relationship between the SCM muscle and CBF due to the muscles' proximity to the anterior cervical vessels of the neck.

Limitations

There are a few limitations to consider in the current study. First, the findings presented were based on a cross-sectional design. Future research should include longitudinal studies that follow concussed athletes from early stages in their injury in order to observe and understand how acute pathophysiology unfolds into the CBF patterns observed months to years later.

Secondly, this study did not consider time since the most recent concussion as this information was not available. Although all athletes were asymptomatic at the time of testing, there is a possibility that BFV varied across athletes with HOC depending on when their most recent injury took place. HOC was also self-reported by athletes, which may result in recall bias.

This study also did not control for factors that may influence cerebral hemodynamics such as the use of medications; alcohol intake; caffeine consumption; exercise; and blood pressure. Nor did it measure the neck circumference of the participants, which would allow us to consider individual morphology and the effects of neck size on the cervical arteries. Studies have established females have smaller neck girth as well as weaker neck muscles,[53-54] which may influence cerebrovascular impairments.

Finally, DU is operator-dependent which calls into question the inter- and intra- rater reliability of the tool. A single sonographer was responsible for data collection on the DU in an effort to avoid operator bias. However, the intra-rater reliability still needs to be considered. Notwithstanding these limitations, DU has potential to be a valuable clinical tool for concussion injuries because it has low operational costs, is easily accessible and allows for bedside monitoring.[46]

Conclusion

The present study provides novel insights into the potentially long-term outcomes of concussion on CBF. The findings demonstrate a trend towards lower BFV with HOC; this trend is important because it demonstrates that CBF has potential as a measure to monitor changes in physiology following concussion injury. The results contribute to developing a better understanding of sex-related differences in concussion, as they provide evidence of sex-specific effects in physiology post-concussion injury. Given the vital role of CBF regulation, it is not yet clear whether these effects lead to health consequences; therefore, further investigation is warranted.

CHAPTER THREE: DISCUSSION & CONCLUSIONS

Changes in CBF Post-Concussion

The effects of concussion on cerebral blood flow (CBF) are shown to vary depending on the time since injury. During the acute stage of injury (24-48 hours), CBF is elevated in concussed athletes compared to non-concussed athletes.[28-29] But, there appears to be a shift in CBF following this initial period after which concussed athletes present lower CBF compared to non-concussed athletes.[29–33] This depression in CBF has shown to normalize within one month in some populations[80] whereas in others it may take longer.[31] However, multiple studies show that blood flow may not normalize in athletes with HOC as some have exhibited persistently lower CBF for months to years post-concussion.[29,32,75]

History of concussion and CBF

The current study is consistent with previous research showing athletes with a history of concussion (HOC) have lower CBF than athletes without HOC.[29,32,75] Further research shows that concussed athletes who are persistently symptomatic have elevated CBF,[81-82] whereas athletes who are asymptomatic have reduced CBF compared to healthy controls.[83-85] Elevated CBF in the dorsal anterior cingulate cortex is associated with greater physical symptoms[85] and in the frontal and occipital regions with total symptom presentation.[84] In asymptomatic athletes, lower CBF is observed in the temporal and parietal cortices,[84,86] as well as bilateral frontotemporal region.[29] It is plausible to consider symptom presentation may be related to CBF regulation, where the presence of symptoms increases the demand for CBF.

A possible explanation for the lower CBF presentation in athletes with HOC could be due to abnormalities in brain structure. Research shows that long-term decreases in CBF may be

associated with reduced gray matter volume, and as a result, there is less brain tissue demanding oxygen and nutrients.[73] This cortical volume loss is specifically found in the insula- a region which is part of the autonomic nervous system (ANS). Thus, atrophy in this region can lead to impaired cerebrovascular function, which may explain the CBF effects witnessed in athletes with HOC. Studies have also found abnormalities in white matter following concussion.[87–90] Axonal damage caused by concussion can lead to a reduction in white matter connectivity; reduced connectivity, and therefore reduced neuron functioning, allows gray matter to be more susceptible to apoptosis.

The thalamus is another area that has been found to be smaller in individuals with HOC.[74] Lower CBF is also observed in the thalamus in athletes with HOC and these reductions correlate with neuropsychological impairment.[75] The thalamus plays a vital role in relaying information and is responsible for brain processes including maintaining consciousness and higher cognitive functioning.[91] Given its diverse role, lower CBF in the thalamus could be associated with reduced function in various brain processes.

Another possible mechanism that may explain the lower CBF in athletes with HOC is the anatomical location of damage. If the biomechanical forces which result in concussion are also applied to the upper cervical spine, then elements of the ANS could be damaged.[92] The corticospinal tract is cited as being particularly vulnerable to the effects of a concussion[93] and there may be a disconnect between the cortex and brainstem. Previous research shows changes in the brainstem white matter and suggests that these irregularities can persist despite resolution of symptoms.[94] Studies observing heart-rate variability following concussion also suggest impairments may extend beyond clinical recovery,[95] however research beyond this

period is limited. Cerebral autoregulation should be evaluated in future studies due to its effects on CBF.

Sex Differences in CBF

Results from the current study suggest that differences in CBF for athletes with HOC compared to the reference group (male controls) are sex-specific. Females controls and females with HOC presented significantly lower blood flow volume in the bilateral CCA and right ICA compared to male controls, whereas males with HOC did not.

These sex-differences may be explained by differences in anatomy and biomechanics. Females have less head mass and neck girth compared to males, as well as weaker neck flexor and extensor muscles.[53] A study reported that in comparison to males, female neck flexors are 32% weaker and neck extensors are 20% weaker.[76] Lower neck strength is associated with increased risk of concussion,[96] and therefore weaker supporting muscles in the cervical spine make females more susceptible to injury. This may be because females experience greater acceleration of the head-neck complex during impact to the body due to the weaker neck stabilization muscles.[53-54] Greater acceleration of the head-neck complex may influence cerebrovascular changes in females.

The differences in blood flow were specifically in the anterior cervical vessels (CCA and ICA), suggesting soft tissue in the neck may be a mechanism influencing blood flow to the brain post-concussion. The sternocleidomastoid (SCM) muscle is a cervical flexor that runs obliquely across the neck and is superficial to the common carotid sheath.[40] The muscle is commonly injured in those who have suffered from whiplash-associated disorders (WAD).[76] Hypertrophy is observed in the cervical muscles in patients with chronic WAD; cross-sectional area of the SCM

is shown to be greater in individuals with WAD compared to healthy controls.[97-98] Taking into account the SCM proximity to the ICA, swelling and/or hypertrophy of the injured SCM muscle may apply pressure on the carotid sheath and consequently the arteries, leading to changes in blood flow. Previous research looking at the correlation between SCM volume and ICA blood flow found SCM volume to be negatively correlated with blood flow velocity of the ICA; an increase in SCM volume corresponded to a decrease in ICA velocity.[99] The literature provides support for a relationship between WAD and concussion.[77–79] It is reasonable to propose that significant impact to the head may result in inertial loading of the neck causing strain injury to the soft tissues of the cervical spine.[78] Hence, similar to the changes in blood flow in WAD, injury to the SCM muscle may explain changes in blood flow witnessed in concussion. A possible explanation for lower blood flow volume observed in females with HOC may be due to sex-related differences in SCM volume.[99] Females reportedly have smaller bilateral SCM volumes compared to males.[99] Research shows there is an association between cross-sectional area of the neck musculature and cervicogenic symptoms such as headaches.[100]

Research evaluating sex differences in CBF is scarce; and to our knowledge there is only one other study that has investigated sex-related differences in athletes with HOC.[101] The study observed lower CBF in males with HOC compared to male controls, and although these results are contrary to the results of the current study, one fact remains consistent- males and females demonstrate differences in the deficits following concussion injury. The differing results suggest that the response to concussion may be time- and subject- dependent, stressing the need for further research to better understand this highly variable cohort that is at high risk of concussion.

Future Directions

The focus of the majority of concussion research has been on acute and subacute stages of injury (within 30 days post-injury)[29,33] or long term in athletes with HOC (>10 years post injury).[102] There is limited data on the time points in-between and thus future research needs to evaluate concussion at multiple time points, from immediate injury to beyond clinical recovery, to better understand the recovery process.

There is also limited research on asymptomatic athletes, months to years post-concussion injury, and the current study shows that even asymptomatic athletes may be subject to potential long-term consequences, highlighting the importance of investigating the long-term effects of concussion in this population. Considering concussion is a heterogenous injury it is challenging to select standardized protocols and analysis methods.[103] Therefore, future studies should consider within-subject analysis by evaluating CBF changes from pre- and post-concussion.

Future studies should also consider the impact of different sports (non-contact sport vs. contact sport vs. collision sport) as this has been shown to influence concussion incidence rates.[13] In addition, consideration of the effects of diet, medication, alcohol intake, caffeine consumption, and exercise should be taken into account as they may have a direct effect on CBF.

There is also inadequate research on sex-related differences in CBF following concussion injury; there is a paucity of investigations in athletes with HOC in particular. Investigating sex-related differences in athletes with HOC can help examine differences in recovery patterns between males and females, which can in turn aid with concussion management and treatment

protocols. Studying sex-related differences may also help reveal differences in vulnerability to repeat injury; animal studies indicate that metabolic dysregulation may make the brain more vulnerable to repeat injury.[25,27]

Finally, it is important to further explore the relationship between the SCM muscle and CBF due to the muscles' proximity to the anterior cervical vessels of the neck. There is a lack of literature data on the relationship between this muscle and blood flow volume following concussion injury.

Conclusions

The current study provides novel insights into the potentially long-term outcomes of concussion on CBF. Currently, there is inadequate knowledge and understanding of sex-related differences in CBF following concussion injury. To our knowledge, this is one of few studies that examines sex differences in CBF in athletes with a history of concussion. The results contribute to developing a better understanding of sex-related differences in concussion, as they provide evidence of sex-specific effects in physiology post-concussion injury.

CBF biomarkers can help to characterize physiological aspects of concussion injury in hopes of improving current concussion management and recovery protocols. Symptom reporting is subjective and not always a reliable predictor of long-term outcomes.[14] Doppler ultrasound shows promise as an objective measure to monitor changes in physiology and help understand what is happening in the brain after a concussion.

CBF plays a fundamental role in regulating homeostasis in the brain by maintaining oxygen, nutrient, and hormone delivery. Without adequate perfusion, hypo-oxygenated brain tissue becomes vulnerable to damage.[28] Given the vital role of CBF regulation, it is not yet clear

whether the effects observed in this study lead to long-term health consequences. Hence, further investigation is warranted.

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