Electronic Thesis and Dissertation Repository

4-20-2017 12:00 AM

Impaired Dynamic Cerebrovascular Autoregulation in Adolescent Concussion

Marcy Erin Moir, *The University of Western Ontario*

Supervisor: Dr. J Kevin Shoemaker, *The University of Western Ontario*
A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Kinesiology
© Marcy Erin Moir 2017

Follow this and additional works at: https://ir.lib.uwo.ca/etd

Part of the Systems and Integrative Physiology Commons

Recommended Citation

Abstract

Although cerebrovascular impairments are believed to contribute to concussion symptoms, little information exists regarding brain vasomotor control in adolescent concussion, particularly during changes in arterial blood pressure (ABP). This research tested the hypothesis that adolescent concussion is marked by impaired dynamic cerebral autoregulation (dCA). Thirty concussed adolescents and thirty healthy controls completed two sit-to-stand trials. Cerebral blood flow velocity and ABP were measured continuously. Cerebrovascular resistance (CVR) was calculated and the rate of drop in CVR relative to the change in ABP provided the rate of regulation (RoR). The concussed adolescents were followed through rehabilitation for up to 12-weeks. At the first visit, the concussed adolescents demonstrated reduced RoR compared with the healthy controls (0.16 ± 0.04 vs. 0.21 ± 0.07 sec^{-1}; $P\leq0.001$). At the concussed adolescents final visit, RoR recovers to levels seen in the healthy controls (0.21 ± 0.08 vs. 0.21 ± 0.07 sec^{-1}; $P=0.93$). Concussed adolescents demonstrate an impairment in dCA that recovers with symptoms.

Keywords

Acknowledgments

Dr. Kevin Shoemaker, thank you for this incredible experience. I am so fortunate to have had the opportunity to work under your guidance. Since the first day of taking your undergraduate class, I was inspired by your passion for science, research, and teaching, motivating me to pursue this path. You have provided a positive, nurturing atmosphere, emphasizing the importance of personal growth as a researcher and as a person. I am truly grateful for your unending support and I look forward to the opportunity to continue working with you and learning much more from you. "People will forget what you said, people will forget what you did, but people will never forget how you made them feel" Maya Angelou.

Dr. Jim Dickey, thank you for your support throughout this process. Your enthusiasm about research and your empathy for students is admirable. I am grateful for your advice and encouragement, particularly throughout the writing process.

Dr. Lisa Fischer, thank you for your assistance with this project. Your expertise in concussion and your clinical experience was fundamental for the success of my project. Your benevolence and your passion for the advancement of concussion research is inspiring.

My lab mates, I simply could not have done this without each and every one of you. Steve, I could not have asked for a better friend to start this journey with. From day one, you have been someone I could always lean on for support and turn to for advice. You have encouraged me to think critically about my research and have always brought different perspectives to my projects. Chris, I am so happy that I got to work closely with you on our research projects. I admire your sincerity, graciousness, and drive and cannot thank you enough for always challenging me in my research and beyond. Thank you for always being someone I can turn to for support and share ideas with. To two of my greatest friends, Steve and Chris. I am so grateful for our friendship and I am excited to continue this journey together over the next four years. "The great thing about new friends is that they bring new energy to your soul" Shanna Rodriguez. Kole, thank you for showing me the ropes and guiding me throughout this process. You were a wonderful mentor who always pushed me to become better. Baraa, your strength and resilience are truly inspiring. Thank you for sharing your experiences with me, for your unrelenting encouragement, and for the warm,

Table of Contents

List of Tables

List of Figures

List of Appendices

List of Abbreviations

TBI   Traumatic Brain Injury

CBF   Cerebral Blood Flow

CA   Cerebral Autoregulation

CPP   Cerebral Perfusion Pressure

ABP   Arterial Blood Pressure

PCS   Post-Concussion Syndrome

ICP   Intracranial Pressure

MAP   Mean Arterial Pressure

CVR   Cerebrovascular Resistance

TCD   Transcranial Doppler

MCA   Middle Cerebral Artery

ACA   Anterior Cerebral Artery

PCA   Posterior Cerebral Artery

CBFV   Cerebral Blood Flow Velocity

CSA   Cross Sectional Area

$P_{ET}CO_2$ End Tidal Carbon Dioxide Partial Pressure

CO_2 Carbon Dioxide

$PaCO_2$ Partial Pressure of Arterial Carbon Dioxide

ANS	Autonomic Nervous System
K^+	Potassium
NO	Nitric Oxide
NE	Norepinephrine
ACh	Acetylcholine
SNS	Sympathetic Nervous System
PNS	Parasympathetic Nervous System
TFA	Transfer Function Analysis
MABP	Mean Arterial Blood Pressure
HUT	Head Up Tilt
LBNP	Lower Body Negative Pressure
RoR	Rate of Regulation
ARI	Autoregulatory Index
GCS	Glasgow Coma Scale
Mx	Mean Index of Autoregulation
FKSMC	Fowler Kennedy Sports Medicine Clinic
SCAT3	Sport Concussion Assessment Tool – 3rd Edition
GAD-7	Generalized Anxiety Disorder – 7 Item Scale
VAS	Visual Analogue Scale
ECG	Electrocardiogram

HR   Heart Rate

SV   Stroke Volume

CO   Cardiac Output

TPR   Total Peripheral Resistance

BMI   Body Mass Index

Chapter 1

1   Introduction

Concussion is a subset of traumatic brain injuries (TBI) (McCrory et al., 2013) and is a major concern among pediatric populations (Stewart, Gilliland, & Fraser, 2014). This subset of injuries is mild in severity (Grady, 2010; Signoretti, Lazzarino, Tavazzi, & Vagnozzi, 2011) and generally occurs without loss of consciousness (Karlin, 2011; Stewart et al., 2014). More than 50% of all TBI cases occur in children and adolescents with concussion being the most predominant (Choe, Babikian, DiFiori, Hovda, & Giza, 2012; Signoretti et al., 2011). Due to ongoing neurological development, concussive injuries sustained during maturation places children and adolescents at increased risk for prolonged recovery, persistent symptoms, and long-term difficulties in neurologic functioning (Choe et al., 2012; Grady, 2010; Karlin, 2011; McCrory et al., 2013; Pellman, Lovell, Viano, & Casson, 2006; Stewart et al., 2014).

Concussion is a complex process resulting in functional disturbances (McCrory et al., 2013). However, the underlying neurological and physiological processes that are affected are not well understood (Signoretti et al., 2011). Furthermore, structural injuries are not evident making concussions unidentifiable with standard imaging modalities (McCrory et al., 2013). Therefore, clinical management of concussion currently relies on subjective measures, largely the self-reporting of symptoms (McCrory et al., 2013), as objective markers remain elusive. Uncovering objective markers of concussion is imperative for improving the overall clinical care of concussion, and more importantly providing information on physiological recovery in turn aiding clinicians' decisions regarding readiness to return to activity and sport.

Regulation of cerebral blood flow (CBF) is a complex, integrative process that works through changes in vasomotor tone (Faraci & Heistad, 1998; Hughson, Edwards, O'Leary, & Shoemaker, 2001; Ursino & Lodi, 1998; Zauner & Muizelaar, 1997). Given the homeostatic nature of CBF control, we would expect to see alterations in cerebrovascular function following trauma to the brain. In fact, changes in

cerebrovascular function are manifested in a number of pathologies affecting the brain including stroke (Girouard & Iadecola, 2006; Meyer et al., 1973), Alzheimer's (Girouard & Iadecola, 2006; Kalaria, 1996), and moderate to severe head-injury (Bailey et al., 2013; Czosnyka, Smielewski, Piechnik, Steiner, & Pickard, 2001; Lang et al., 2003; Lee et al., 2001; M. S. Vavilala et al., 2004). However, while impaired cerebrovascular function is associated with moderate and severe head injuries, the evidence supporting these changes in mild TBI and concussion is less clear.

A common approach to evaluating cerebrovascular function is the assessment of cerebral autoregulation (CA) which regulates CBF in the face of fluctuating cerebral perfusion pressure (CPP) (N. A. Lassen, 1959). Through vasomotor adjustments, CA is able to maintain CBF at relatively constant levels within a CPP range of 50-150 mmHg (N. A. Lassen, 1959). Investigations of CA following head injury have largely focused on adults suffering from moderate to severe TBI and have found variably impaired CA (Bailey et al., 2013; Czosnyka et al., 2001; Lang et al., 2003; Lee et al., 2001). Pediatric populations and patients suffering from concussion have seldom been studied. Furthermore, the investigations into head-injury have employed static measures of CA (Czosnyka et al., 2001; Lang et al., 2003). Static CA looks only at the association between mean CBF and mean arterial blood pressure (ABP) following induced alterations or spontaneous changes in ABP (R. B. Panerai, 1998). With this approach, measures are taken in steady-state and the initial period of transition is ignored, when ABP and CBF are changing dynamically (R. B. Panerai, Dawson, Eames, & Potter, 2001; Tiecks, Lam, Aaslid, & Newell, 1995). Therefore, static CA provides information on the absolute change in CBF for a given change in ABP but overlooks the time course of the autoregulatory response (R. B. Panerai, 1998; R. B. Panerai et al., 2001; Tiecks et al., 1995). The use of static measures of CA may be one reason why previous investigations have found variably impaired CA. Homeostatic processes work to maintain a state of equilibrium and may seem relatively intact at rest and at low levels of stress. A previous investigation of cerebral hemodynamic function failed to uncover impairments at rest but deficiencies were witnessed when participants were subjected to hemodynamic stress (Bailey et al., 2013). Therefore, by challenging the adaptive dynamics of CA we may expose impaired cerebrovascular function.

      The primary **purpose** of this investigation was to examine underlying cerebrovascular outcomes associated with adolescent concussion. More specifically, application of a dynamic model would allow for the evaluation of dynamic autoregulatory function. Furthermore, it was of interest to assess the resolution of dynamic CA throughout clinical recovery. This research tested the **hypothesis** that adolescents diagnosed with a concussion would demonstrate impaired dynamic CA when compared to healthy adolescent controls.

Chapter 2

2   Literature Review

2.1   Concussion

Concussion is a subset of TBI characterized by low velocity injuries and caused by direct hits to the head or neck, or by indirect hits to the body where the force is transmitted to the head (Daneshvar, Nowinski, McKee, & Cantu, 2011; McCrory et al., 2013; Stewart et al., 2014). Despite the presence of impairments in functional neurological outcomes, structural injury is not evident following concussion with no abnormalities observed using standard imaging techniques (McCrory et al., 2013; Stewart et al., 2014). Typically, concussive injuries result in a rapid onset of symptoms though symptoms can develop gradually over minutes to hours (Daneshvar et al., 2011; McCrory et al., 2013; Stewart et al., 2014). Symptoms of concussion are widespread but can generally be grouped into clinical symptoms (i.e. headache, dizziness), physical signs (amnesia, loss of consciousness), behavioral changes (irritability, emotional), cognitive impairment (slowed reaction time), and sleep disturbances (Daneshvar et al., 2011; McCrory et al., 2013; Stewart et al., 2014). Generally, symptoms resolve spontaneously with the majority of individuals experiencing symptom resolution within 7-10 days (Daneshvar et al., 2011; McCrory et al., 2013; Stewart et al., 2014). However, in 10-15% of cases symptoms persist beyond this recovery period and may progress to Post-Concussion Syndrome (PCS) (McCrory et al., 2013). The definition of PCS varies largely though it has been recognized when symptoms persist beyond 42 days or 6 weeks (Willer & Leddy, 2006). The ability to define who will progress to PCS represents a key effort in concussion research; however, no markers of this progression are currently understood.

2.1.1   Pediatric Concussion

Concussion is a major concern among pediatric populations (Stewart et al., 2014). More than 50% of all TBI cases occur in children and adolescents with concussion being the most predominant form of injury (Choe et al., 2012; Signoretti et al., 2011; Stewart et al., 2014). A large contributor to the high incidence of concussion in youth is their

involvement in sports and recreational activities (Choe et al., 2012; Daneshvar et al., 2011; Grady, 2010; Karlin, 2011). In fact, sports- and recreation-related injuries are the most common mechanism for concussion in this population (Choe et al., 2012; Daneshvar et al., 2011; Grady, 2010; Karlin, 2011; Stewart et al., 2014). It has been suggested that child and adolescent brains may be more vulnerable to the functional outcomes of concussion (Karlin, 2011; Stewart et al., 2014). The reason for such vulnerability may be related to ongoing neurologic development at this age, placing children and adolescents at increased risk for prolonged recovery, persistent symptoms, and long-term impairments in neurologic functioning (Karlin, 2011; Stewart et al., 2014). Recovery in pediatric populations tends to be prolonged beyond the 7-10-day period typically seen in adult populations (Field, Collins, Lovell, & Maroon, 2003; Lovell et al., 2003; McCrory et al., 2013; Pellman et al., 2006). However, investigations have not yet reported a general timeline for symptom resolution in this population.

Following concussion, a period of cognitive and physical rest is prescribed in the initial stages of recovery and this period can often become prolonged in pediatric populations (McCrory et al., 2013; Purcell, 2014; Stewart et al., 2014). Once symptoms begin to improve, light cognitive activities are progressively introduced and, once tolerated, pediatric patients can begin modified school attendance (Purcell, 2014). Once patients are back to full attendance at school, light physical activity can be introduced (McCrory et al., 2013; Purcell, 2014). Persistent symptoms in the initial stages of rehabilitation often prolongs the period of rest in pediatric populations and this can be associated with negative impacts on their emotional and cognitive development (Stewart et al., 2014). With prolonged absence from school and physical activity, children and adolescents may experience social withdrawal, high levels of anxiety, irritability, and depression (Stewart et al., 2014). Furthermore, extended absenteeism from school and persistent difficulties with memory and concentration can negatively influence intellectual development (Stewart et al., 2014). Together, this demonstrates the importance of appropriate rehabilitation of pediatric concussions and the need for a collaborative approach to ensure optimal care (Purcell, 2014).

2.1.2   Pathophysiology of Concussion

Despite considerable efforts, many gaps remain in our knowledge of the pathophysiology of concussion (Signoretti et al., 2011). Much of the research in this area has relied on animal models of TBI which are accompanied by concerns with the applicability of these models to injury constructs in humans (Bazarian, Blyth, & Cimpello, 2006; Grady, 2010). The large majority of TBI research has applied four injury models to rodents including fluid percussion injuries, controlled cortical impact injuries, weight drop-impact acceleration injuries, and blast injuries (Xiong, Mahmood, & Chopp, 2013). With fluid percussion injuries, the severity of the injury depends on the strength of the pressure wave created in the fluid and transferred to the intact dura exposed through craniotomy (Xiong et al., 2013). With this model, there is little control over the biomechanical forces applied and fluid percussion injuries often induce intracranial hemorrhage, encephalitis, cortical contusions, and a progressive damage to the grey matter (Xiong et al., 2013) which are associated with more severe forms of TBI in humans. Controlled cortical impact injuries applies controlled impacts to the exposed, intact dura and can model a range a TBI severities through greater control of the biomechanical forces (Xiong et al., 2013). However, the damage reported with controlled cortical impact injuries are generally widespread and can involve tissue degeneration (Xiong et al., 2013). Furthermore, cognitive and emotional deficits associated with controlled cortical impact injuries can last a year following the injury (Xiong et al., 2013). This injury model may also be more applicable to moderate or severe forms of head-injury. Similar paradigms are seen with the weight-drop acceleration model where weight is dropped on the unprotected skull or the exposed dura (Xiong et al., 2013). This model commonly produces cortical contusions and hemorrhages (Xiong et al., 2013) and may relate to more severe forms of TBI. Considerations must also be made for the physiological differences between the rodent species and humans. Nonetheless, animal models have provided a groundwork for our understanding of the pathophysiology of TBI. Some human evidence has supported the occurrence of similar mechanical and metabolic changes following head injury (Bazarian et al., 2006; Choe et al., 2012; Grady, 2010; Willer & Leddy, 2006).

Functional neurological outcomes and concussion symptoms may relate to physical damage to nerves (Bazarian et al., 2006). Diffuse axonal injuries refers to the stretching of axons following shear, compression, or torsional forces to neural tissue (Bazarian et al., 2006; Grady, 2010; Willer & Leddy, 2006). With diffuse axonal injuries, changes in the arrangement of cytoskeletal elements of the axons are observed (Bazarian et al., 2006; Choe et al., 2012). In particular, axonal neurofilaments and microtubules become damaged which disrupts axonal transport (Bazarian et al., 2006; Choe et al., 2012). Shortly after the injury axonal swelling is detected and an accumulation of proteins, in particular the amyloid precursor protein, is noted at the site of injury (Bazarian et al., 2006; Choe et al., 2012; Grady, 2010). This protein is common in identifying areas of injury in autopsies and experimental models of TBI in rodents (Bazarian et al., 2006; Ciallella et al., 2002; Itoh et al., 2009).

Metabolism within nerves may also affect their function, producing neurologic symptoms. The metabolic changes that occur following TBI are often referred to as a complex, metabolic cascade (Signoretti et al., 2011; Willer & Leddy, 2006). Initial depolarization of neuronal membranes results in the release of several neurotransmitters, specifically excitatory amino acids (Grady, 2010; Signoretti et al., 2011; Willer & Leddy, 2006). Particularly, there is a considerable increase in the concentration of glutamate (Choe et al., 2012). The release of these neurotransmitters creates fluxes of potassium, sodium, and calcium across neuronal and vascular membranes (Choe et al., 2012; Grady, 2010; Willer & Leddy, 2006). The cells attempt to regain equilibrium, in turn, leading to a surge in glucose utilization (Willer & Leddy, 2006). However, influx of calcium into vascular smooth muscle leads to vasoconstriction thereby reducing CBF and glucose delivery (Grady, 2010; Willer & Leddy, 2006). What follows is a state of metabolic depression where there is a mismatch between the energy demand of the brain and the energy supply to the brain (Grady, 2010; Willer & Leddy, 2006). In this hypothesis, reactive changes in CBF become part of the problem.

Previous investigations have provided evidence of alterations in CBF following TBI. Largely, a reduction in CBF has been reported in severely head-injured patients (Adelson et al., 1997; Muizelaar et al., 1989; Obrist, Langfitt, Jaggi, Cruz, & Gennarelli,

1984). However, one previous investigation has also demonstrated a reduction in CBF following concussion in children and adolescents aged 11 to 15 years (Maugans, Farley, Altaye, Leach, & Cecil, 2012). In contrast, another investigation provided support for normal or below normal CBF values immediately following severe head injury that was followed by a period of hyperemia lasting between 1 and 14 days before returning to normal or below normal values (Overgaard & Tweed, 1974). Therefore, a state of reduced CBF appears to develop following head-injury of varying severities though the time course of these changes has not been elucidated. Given the state of increased energy demand, reduced CBF would have serious implications on cerebral perfusion (Grady, 2010; Willer & Leddy, 2006). Therefore, understanding alterations in CBF following concussion becomes an important piece of information in guiding concussion management (Grady, 2010).

2.2   Cerebral Blood Flow

Blood flow in the cerebral vasculature differs from systemic circulation largely due to the encompassment of the cerebral circulatory system in the skull (Czosnyka & Pickard, 2004; Czosnyka et al., 2001; Ursino & Lodi, 1998). With the cerebral vasculature enclosed in the skull, the vessels are naturally exposed to the influence of external sources of pressure termed intracranial pressure (ICP) (Czosnyka & Pickard, 2004; Ursino & Lodi, 1998). The main determinants of CBF are CPP and cerebral vascular resistance (CVR), with CPP determined as mean arterial pressure (MAP) minus ICP (Zauner & Muizelaar, 1997). Therefore, CBF is defined as CPP/CVR (Zauner & Muizelaar, 1997). Because the cerebral circulation is enclosed in the skull it is difficult to make direct measures of CBF in humans and research has largely relied on indirect measurements.

2.2.1   Methods of Measuring Cerebral Blood Flow

The first measurement of CBF came in 1945 when Kety and Schmidt applied the inert gas method to animals and humans. The method was based on the principles that i) the rate of gas uptake by the brain depends on the rate of blood flow to the brain and ii) the arterio-venous gas difference depends on the volume of blood flowing to the brain (Kety

& Schmidt, 1945). More specifically, the inert gas method often references the Fick equation which, when applied to a single organ, describes CBF as the ratio of the rate of gas uptake relative to the arterio-venous gas difference (Kety & Schmidt, 1945; N. A. Lassen, 1959). Following inhalation of an inert gas, blood samples are taken from a peripheral artery and the internal jugular vein (N. A. Lassen, 1959). Blood samples from a peripheral artery, such as the femoral artery utilized in the original work by Kety and Schmidt (1945), are believed to be a good representation of blood in the cerebral arteries (N. A. Lassen, 1959). The venous concentration of gas provides a measure of the rate of gas uptake per unit weight of the brain (N. A. Lassen, 1959). An estimation of total brain weight is required to provide a value of absolute cerebral blood flow (R. B. Panerai, 1998). Although the inert gas method relies on a number of assumptions, good agreement has been demonstrated between this method and direct measures of cerebral blood flow in monkeys (N. A. Lassen, 1959). However, jugular venous samples are difficult to perform in humans and are more invasive.

The inert gas method was further developed and the xenon clearance technique was first described in 1963 by Lassen and colleagues (Obrist, Thompson, King, & Wang, 1967). With this approach, an inert, diffusible radioisotope is injected into one of the internal carotid arteries (ICA) and monitored extra-cranially through gamma spectroscopy for 45 to 60 minutes (Obrist et al., 1967). The resulting clearance curves are subjected to a two-compartment analysis which provides separate blood flow for grey and white matter (Ingvar, Cronqvist, Ekberg, Risberg, & Høedt-Rasmussen, 1965; Obrist et al., 1967). This approach involves intra-ICA injections in addition to jugular venous sampling making this method invasive.

In addition, another method for the indirect measurement of CBF was introduced in 1947 (Gibbs, Maxwell, & Gibbs, 1947). Gibbs and colleagues (1947) developed the indicator injection method based on the Stewart Hamilton techniques for measuring flow (N. A. Lassen, 1959). A biologically inert indicator is injected into one of the ICA (N. A. Lassen, 1959). The concentration of the indicator subsequently measured in jugular venous samples is related to the rate of flow in the brain (Nylin & Blömer, 1955). Again,

this method involves intra-ICA injections and jugular venous samples making it a more invasive procedure.

In 1977, near-infrared spectroscopy (NIRS) was described as a non-invasive means of quantifying CBF (Jobsis, 1977). Near-infrared light is applied to the intact skull and transmitted through biological tissue (Wyatt et al., 1990). Brain tissue is relatively transparent in the near-infrared range allowing non-invasive measures of tissue oxygen saturation (Jobsis, 1977; Murkin & Arango, 2009; Wyatt et al., 1990). Near-infrared light can be transmitted through tissue up to a distance of 8 cm (Wyatt et al., 1990). Reflectance-mode NIRS is most commonly employed where receiving sensors are placed ipsilateral to the transmitter (Wyatt et al., 1990). The Beer-Lambert equation, using the difference between the intensity of the transmitted light and received light, provides regional tissue oxygen saturation. Total cerebral hemoglobin subsequently gives an estimation of blood flow velocity (Wyatt et al., 1990).

Presently, Transcranial Doppler ultrasound (TCD) has become the most prevalent method of measuring cerebral blood flow in conscious humans due to its ability to provide continuous information in a non-invasive manner. This method is discussed in detail below.

2.2.1.1   Transcranial Doppler Ultrasound

Doppler ultrasound was first used to measure blood flow velocity in the extracranial vessels in 1965 (Miyazaki & Kato, 1965). The technique was available to measure blood flow velocity in the intracranial vessels as well, though it was limited to use during surgical procedures due to the thickness of the skull and the subsequent attenuation of the sound waves (Aaslid, Markwalder, & Nornes, 1982). However, in 1982, Aaslid and colleagues demonstrated that insonation of the major cerebral arteries was possible within the frequency range of 1-2 MHz and through the thinner regions of the skull termed acoustic windows (Aaslid et al., 1982). Four acoustic windows have been identified and applied in TCD examinations which include the transtemporal, transocular, suboccipital, and transcondylar windows (Aaslid et al., 1982; R. B. Panerai, 2009; C. Willie et al., 2011). The transtemporal window is most commonly utilized in TCD examinations as the

middle cerebral artery (MCA), anterior cerebral artery (ACA), and posterior cerebral artery (PCA) can be insonated with this approach (R. B. Panerai, 2009; C. Willie et al., 2011). The MCA, ACA, and PCA are vessels of the circle of Willis, which plays an important role in absorbing pressure and protecting the cerebral microcirculation (Vrselja, Brkic, Mrdenovic, Radic, & Curic, 2014). The circle of Willis originates from two major sources, the vertebral arteries and the internal carotid arteries (Iqbal, 2013; Vrselja et al., 2014). The vertebral arteries anastomose forming the basilar artery which in turn branches into the two PCA (Iqbal, 2013; Vrselja et al., 2014). The internal carotid arteries branch into the MCA, ACA, and the posterior communicating arteries with the two ACA joined by the anterior communicating artery (Iqbal, 2013; Vrselja et al., 2014). Together the PCA, posterior communicating arteries, MCA, ACA, and anterior communicating artery form the circle of Willis (Iqbal, 2013; Vrselja et al., 2014) (Figure 2.1).

Blood flow throughout the major arteries of the circulatory system is believed to be predominantly laminar leading to a parabolic flow profile, particularly during peak systole, with the highest velocity in the center of the vessel and the lowest velocities along the walls of the vessel (R. B. Panerai, 2009; C. Willie et al., 2011). TCD provides continuous information on these velocity profiles and subsequently calculates a variety of metrics such as the instantaneous peak velocity and mean velocity profiles. The peak velocity profiles represent the maximum velocity at any moment in time while the mean velocity profiles represent the intensity weighted mean of a cross section of the vessel (Newell, Aaslid, Lam, Mayberg, & Winn, 1994; R. B. Panerai, 2009; C. Willie et al., 2011). Mean velocity profiles require a high signal-to-noise ratio and slight movements of the sample volume can lead to inaccurate estimations of flow (Newell et al., 1994). In contrast, slight movement of the sample volume has little effect on peak velocity profiles (Newell et al., 1994). Therefore, mean velocity profiles may be more representative of overall CBF though peak velocity profiles may be more dependable indexes of CBF, particularly in cases where you cannot visualize the position of the Doppler window within the artery as is the case with TCD (Newell et al., 1994).

The Doppler probe emits sound waves that are reflected off the red blood cells travelling through the insonated artery (R. B. Panerai, 2009; C. Willie et al., 2011). The reflected sound waves are detected by the probe and the shift in frequency between the transmitted and reflected waves corresponds to the blood flow velocity (C. Willie et al., 2011). Changes in cerebral blood flow velocity (CBFV) are proportional to changes in CBF provided the cross-sectional area (CSA) of the insonated vessel remains constant (Aaslid, Lindegaard, Sorteberg, & Nornes, 1989; Clark et al., 1996; Giller, Bowman, Dyer, Mootz, & Krippner, 1993; R. B. Panerai, 1998; Serrador, Picot, Rutt, Shoemaker, & Bondar, 2000; C. Willie et al., 2011). Whether the larger basal arteries of the brain undergo diameter changes with alterations in ABP and end tidal carbon dioxide partial pressure ($P_{ET}CO_2$) has been studied extensively. However, the results of the investigations remain equivocal, with some studies arguing no change in vessel diameter (Aaslid et al., 1989; Aaslid, Newell, Stooss, Sorteberg, & Lindegaard, 1991; Bishop, Powell, & Rutt, 1986; Giller et al., 1993; Larsen, Olsen, Hansen, Paulson, & Knudsen, 1994; Lindegaard et al., 1987; Newell et al., 1994; Serrador et al., 2000; Sorteberg et al., 1989; Ter Minassian et al., 1998; Valdueza et al., 1997) and other studies providing evidence of diameter changes (Coverdale, Gati, Opalevych, Perrotta, & Shoemaker, 2014; Dahl, Russell, Nyberg-Hansen, & Rootwelt, 1989; Faraci & Heistad, 1990; Madsen et al., 1993).

The CSA of the insonated artery has been shown to remain unaltered, at least in a statistical sense, with modest variations in both ABP and $P_{ET}CO_2$ (Bishop et al., 1986; Giller et al., 1993; Larsen et al., 1994; Lindegaard et al., 1987; Serrador et al., 2000; Valdueza et al., 1997). Following alterations in ABP via drug-infusion, measures of CBFV with TCD and CBF with xenon clearance techniques or electromagnetic flowmeters were collected (Larsen et al., 1994; Lindegaard et al., 1987). The measures of CBFV and CBF were highly correlated suggesting a constant vessel diameter (Larsen et al., 1994; Lindegaard et al., 1987). Similarly, following alterations in $P_{ET}CO_2$ via the administration of a 5% carbon dioxide (CO_2) gas mixture, CBFV and CBF as measured with TCD and xenon clearance techniques, respectively, were strongly correlated (Bishop et al., 1986). Furthermore, a small number of studies have employed magnetic resonance imaging (MRI) to measure vessel diameter (Serrador et al., 2000; Valdueza et al., 1997).

Serrador and colleagues (2000) employed lower-body negative pressure (LBNP) to produce changes in ABP and, in separate conditions, altered $P_{ET}CO_2$ with the administration of a CO_2 gas mixture and induced hyperventilation. Vessel CSA remained statistically similar under all conditions (Serrador et al., 2000). Valdueza and colleagues (1997) also produced alterations in $P_{ET}CO_2$ via induced hyperventilation and similarly found no change in vessel CSA. One investigation used craniotomy procedures to make direct measures of MCA CSA (Giller et al., 1993). With the infusion of nitroprusside, ABP dropped 30 ± 16 mmHg and a 2.5% change in MCA CSA was observed (Giller et al., 1993). Although a small change was witnessed, it has been argued that this change in CSA was not significant enough to result in differences between CBFV and CBF measures (Giller et al., 1993; Newell et al., 1994).

Despite strong evidence supporting constant vessel diameter, other investigations have reported changes in vessel CSA with alterations in ABP or $P_{ET}CO_2$ (Coverdale et al., 2014; Dahl et al., 1989; Faraci & Heistad, 1990; Madsen et al., 1993). In fact, when similar techniques were employed to measure CBFV and CBF, disparities were witnessed between the measures suggesting a change in the CSA of the vessel (Dahl et al., 1989). Following the administration of nitroglycerin, CBFV, measured with TCD, decreased (Dahl et al., 1989). However, no significant changes were observed in CBF measured with xenon clearance and single-photon emission computed tomography (Dahl et al., 1989). Therefore, conduit vessel CSA must have increased to maintain CBF. In addition, a recent MRI study measured vessel CSA following alterations in $P_{ET}CO_2$ (Coverdale et al., 2014). An increase in $P_{ET}CO_2$ of approximately 10 mmHg from normocapnia resulted in an increase in MCA CSA of 0.9 mm^2 (Coverdale et al., 2014). During a decrease in $P_{ET}CO_2$ of 13 mmHg from normocapnia, the MCA CSA decreased by 0.5 mm^2 (Coverdale et al., 2014).

The discrepancies between investigations is likely due to the methodological techniques employed and the magnitude of stimuli induced. For instance, the MRI studies that reported no change in MCA CSA were performed at 1.5 Tesla (Serrador et al., 2000; Valdueza et al., 1997) while the more recent MRI study demonstrating changes in MCA CSA used 3 Tesla strength with greater spatial resolution (Coverdale et al., 2014).

Furthermore, the changes in ABP between investigations ranged from 2-30 mmHg and the changes in $P_{ET}CO_2$ between investigations ranged from 8-13 mmHg. Therefore, future investigations must consider the level of stimulus and methodological techniques in the experimental design to minimize the potential of vessel CSA changes.
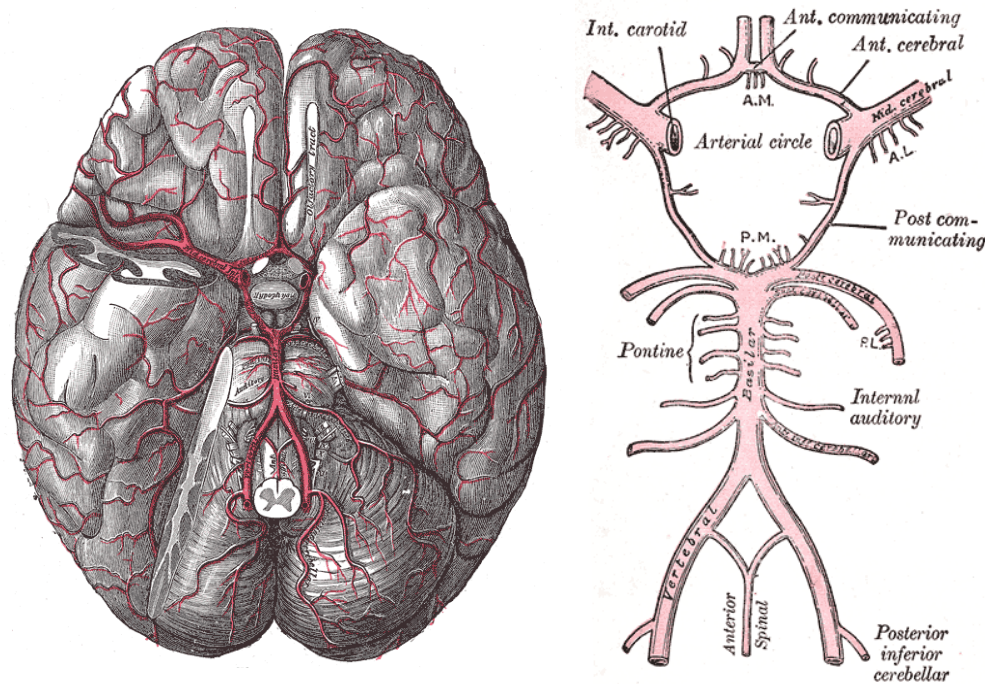
Figure 2.1: The arteries at the base of the brain. The arterial anastomoses were described in detail by Thomas Willis and are commonly referred to as the circle of Willis. Reproduction of a lithograph plate from Gray's Anatomy from the 20th U.S. edition of Gray's Anatomy of the Human Body, originally published in 1918.

2.3   Cerebral Blood Flow Regulation

The brain has a high metabolic demand (Van Beek, Claassen, Rikkert, & Jansen, 2008; C. K. Willie, Tzeng, Fisher, & Ainslie, 2014), receiving approximately 15% of total cardiac output and accounting for roughly 20% of overall oxygen consumption (Jain, Langham, & Wehrli, 2010; Y.-C. Tzeng & Ainslie, 2014; Udomphorn, Armstead, & Vavilala, 2008). In addition, the brain has a limited capacity for storage of substrates

(Y.-C. Tzeng & Ainslie, 2014). Together, this creates a need for the precise regulation of CBF leading to complex and integrative mechanisms of control (Udomphorn et al., 2008; C. K. Willie et al., 2014). To sustain appropriate levels of brain perfusion, CBF is tightly regulated by the partial pressure of arterial carbon dioxide ($PaCO_2$), cerebral metabolism, the autonomic nervous system (ANS), and ABP (C. K. Willie et al., 2014). These contributors are discussed in detail below.

2.3.1   Carbon Dioxide

The cerebral vasculature has a high sensitivity to $PaCO_2$ that is unique to the cerebral circulation (Brugniaux, Hodges, Hanly, & Poulin, 2007; Ursino & Lodi, 1998). An increase in $PaCO_2$ leads to dilation of the cerebral vasculature and an increase in CBF, while a decrease in $PaCO_2$ leads to vasoconstriction and a decrease in CBF (Brugniaux et al., 2007). With each mmHg increase in $PaCO_2$, CBF generally increases 3-4% reaching peak flow approximately 10-20 mmHg above normocapnia (Brugniaux et al., 2007). With decreases in $PaCO_2$, CBF is generally decreased 2-3% per mmHg to a minimum level that is reached within the range of 10-15 mmHg (Brugniaux et al., 2007).

It is quite clear that alterations in $PaCO_2$ are accompanied by changes in CBF (Brugniaux et al., 2007; Ursino & Lodi, 1998). However, for some time, it was unknown if the CBF response was a result of $PaCO_2$ itself, or possibly changes in pH that accompany the changes in $PaCO2$ (C. K. Willie et al., 2014). Under conditions where $PaCO_2$ was maintained and blood pH was altered, CBF was not influenced in animals (Harper & Bell, 1963) or in humans (Lambertsen, Semple, Smyth, & Gelfand, 1961). However, changes in vasomotor tone were witnessed when extravascular pH was altered in animal experiments (Kontos, Raper, & Patterson, 1977; Kontos, Wei, Raper, & Patterson, 1977; Wahl, Deetjen, Thurau, Ingvar, & Lassen, 1970). These observations led to the understanding that CO_2 molecules diffuse across the blood-brain barrier leading to alterations in extravascular pH and consequently influencing vasomotor tone (Lambertsen et al., 1961; N. Lassen, 1968). Animal studies (Apkon, Weed, & Boron, 1997; Koehler & Traystman, 1982; Kontos, Raper, et al., 1977; Kontos, Wei, et al., 1977; Peng, Ivarsen, Nilsson, & Aalkjær, 1998; Wahl et al., 1970) and *in vivo* vascular preparations (Dabertrand, Nelson, & Brayden, 2012) have provided further evidence

supporting this view. Therefore, the CBF response appears to result from an interaction between $PaCO_2$ and pH.

What remains unclear are the mechanisms that alter vasomotor tone in response to changes in $PaCO_2$ and extravascular pH. Potassium (K^+) channels are suggested to play a role in the $PaCO_2$ and extravascular pH effect on vasomotor tone. Several K^+ channels have been identified in cerebral blood vessels with four main types being recognized including calcium-activated K^+ channels, delayed rectifier K^+ channels, inward rectifier K^+ channels, and ATP-sensitive K^+ channels (Kitazono, Faraci, Taguchi, & Heistad, 1995). Specifically, delayed rectifier, calcium-activated, and ATP-sensitive K^+ channels are believed to contribute to hypercapnia-induced vasodilation (Kitazono et al., 1995; Lindauer, Vogt, Schuh-Hofer, Dreier, & Dirnagl, 2003). Animal research demonstrated the attenuation of hypercapnia-induced vasodilation following the inhibition of the calcium-activated K^+ channels (Lindauer et al., 2003). Furthermore, a complete suppression of hypercapnia-induced vasodilation occurred following inhibition of both the calcium-activated and ATP-sensitive K^+ channels (Lindauer et al., 2003).

The potent dilator nitric oxide (NO) is also thought to play a role in the cerebral vascular response to $PaCO_2$ and extravascular pH. Infusion of a NO donor, sodium nitroprusside, provoked a reduction in MCA constriction following hypocapnia and an increase in MCA dilation following hypercapnia (Lavi, Egbarya, Lavi, & Jacob, 2003). Furthermore, the dilatory response of the MCA to hypercapnia was lower following the infusion of a NO synthase inhibitor, N^G-Monomethyl-L-arginine (L-NMMA), and this effect was subsequently reversed with the infusion of L-arginine (Schmetterer et al., 1997). In contrast, Ide et al. (2007) found no difference in the hypercapnia induced increase in MCA flow velocity with or without the infusion of L-NMMA. This would suggest that L-NMMA did not attenuate the dilatory effect of NO providing evidence that NO does not influence the vasomotor response to $PaCO_2$ and extravascular pH (Kojiro Ide, Worthley, Anderson, & Poulin, 2007). However, changes in flow velocity reflect downstream events and it may be that the NO effect of $PaCO_2$ only influences large cerebral arteries. Animal research has further demonstrated a role of NO, specifically

produced by neuronal NO synthase, in hypercapnia-induced vasodilation (Lindauer et al., 2001; Wang, Paulson, & Lassen, 1992).

From this evidence, we can conclude that K^+ channels and NO are, in some way, mediators of the $PaCO_2$ and extravascular pH vasomotor effect. However, there are still many questions that remain unanswered in the control of carbon dioxide on CBF.

2.3.2   Metabolic Control

Neurovascular coupling refers to the robust balance between local blood flow and cerebral metabolism and was first recognized by Mosso in 1880 (Iadecola, 2004). Neuronal activity produces local vasodilation and subsequently a functional hyperemic response (Iadecola, 1993, 2004; Roy & Sherrington, 1890). The tight coupling has been attributed to the anatomical interactions and complex signaling between the neurons, astrocytes and blood vessels (Iadecola, 2004). In fact, the CBF response to neural activity is very rapid and is restricted to the region of activation (Iadecola, 2004). The cellular mechanisms that are responsible for this activation-flow coupling are not entirely understood due, in part, to the complex signaling mechanisms between the structures of the neurovascular unit (Iadecola, 2004). One view that has long been a focus of understanding the activation-flow coupling, is the release of vasoactive agents (Iadecola, 1993, 2004). Furthermore, the release of neurotransmitters that are not vasoactive have been linked to the production of vasodilators such as NO (Bhardwaj et al., 2000; Faraci & Breese, 1993) and adenosine (Iliff, D'Ambrosio, Ngai, & Winn, 2003). Attempts to uncover the predominant mediators of the hyperemic response have been unsuccessful as inhibition of any one mediator may lessen, but not eradicate, the hyperemic response (Iadecola, 1993, 2004). Furthermore, it has been noted that the attenuation of the CBF response with the inhibition of mediators varies by the region of the brain studied (Gotoh et al., 2001; Hayashi et al., 2002; Yang, Chen, Ebner, & Iadecola, 1999; Yang, Zhang, Ross, & Iadecola, 2003).

Recently, strong support for the importance of astrocytes in the activation-flow coupling has emerged (Zonta et al., 2003). The rapid CBF response can be attributed to the functional coupling of neurons and astroctyes and the direct contact of the astrocytic

end-feet with the smooth muscle cells of the penetrating arterioles and the pericytes of the capillaries (Figure 2.2; (Iadecola, 2004). The astrocytic end-feet release several vasoactive agents including, but not limited to, potent vasodilators such as NO and adenosine (Nedergaard, Ransom, & Goldman, 2003; Paulson & Newman, 1987). Furthermore, Zonta and colleagues (2003) observed the production of calcium in astrocytes that was linked to the release of glutamate from neurons. The calcium travelled to the astrocytic end-feet where subsequent vascular dilation was witnessed (Zonta et al., 2003).
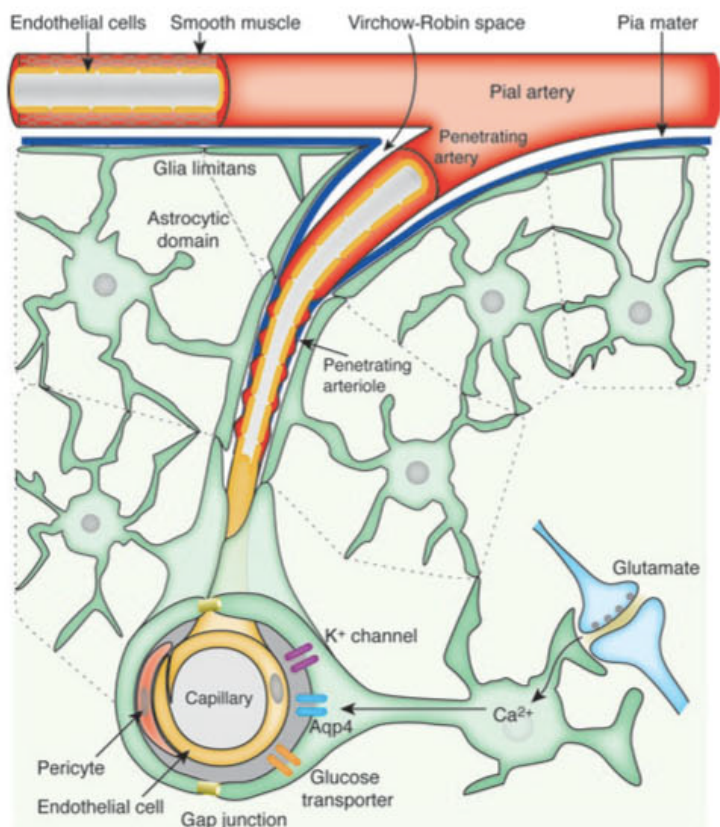
Figure 2.2: The interaction between astrocytes and the cerebral arterioles. The astrocytic end-feet are in direct contact with the smooth muscle cells of the penetrating arterioles and the pericytes of the capillaries. As a result, the astrocytes exert a high level of control over the vasomotor tone of the cerebral vessels and consequently, the regulation of CBF. Iadecola & Nedergaard (2007) Glial regulation of the cerebral microvasculature. *Nature Neuroscience* 10 (11): 1369-1376.

2.3.3   Neural Control

The cerebral vasculature is densely innervated by adrenergic and cholinergic fibres that originate from extrinsic or intrinsic sources, depending on the vessel location (Bleys, Cowen, Groen, Hillen, & Ibrahim, 1996; Hamel, 2006). Extrinsic origins include the superior cervical ganglion, sphenopalatine and otic ganglia, or the trigeminal ganglion (Hamel, 2006). The superior cervical ganglion yields sympathetic nerve fibres releasing norepinephrine (NE) and neuropeptide Y (Hamel, 2006). In contrast, the sphenopalatine and otic ganglia yield parasympathetic nerve fibres releasing acetylcholine (ACh) and NO along with other vasoactive agents (Hamel, 2006). The trigeminal ganglion provides sensory nerves that releases, among others, calcitonin gene-related peptide, a potent vasodilator (Hamel, 2006). This pathway is thought to act as a protective mechanism returning vessel tone to resting levels following vasoconstrictor stimuli (Hamel, 2006). Intrinsic origins include the nucleus basalis, locus coeruleus, and the raphe nucleus (Hamel, 2006). The nucleus basalis releases the parasympathetic mediator ACh and the locus coeruleus provides the sympathetic mediator NE (Hamel, 2006). The raphe nucleus releases 5-HT, or serotonin, which plays a major role in microvascular tone, largely appearing to have a vasoconstrictor effect (Cohen, Bonvento, Lacombe, & Hamel, 1996; Hamel, 2006).

Human studies have evaluated the role of the sympathetic nervous system (SNS) in CBF though they have been limited to various patient populations requiring ganglionectomy or the use of various ganglion blockade techniques in patient populations and in healthy adults (C. K. Willie et al., 2014). Investigations employing ganglion excision in patients have demonstrated increases in CBF (Jeng, Yip, Huang, & Kao, 1999; Shenkin, 1969; Shenkin, Cabieses, & Van Den Noordt, 1951). However, with local cervical ganglion blockades, the results were equivocal with some studies showing an increase in CBF (K Ide et al., 2000; Treggiari et al., 2003; Umeyama et al., 1995; Yokoyama, Kishida, & Sugiyama, 2004), other studies showing no change in CBF (Harmel, Hafkenschiel, Austin, Crumpton, & Kety, 1949; Ohta, Hadeishi, & Suzuki, 1990; Scheinberg, 1950), and one investigation showing a decrease in CBF (Kang et al., 2010). The inconsistency in the results of the ganglion blockade investigations could be

explained by the variations in populations investigated or by the use of subcutaneous blockade strategies where it is difficult to obtain full blocks of the targeted ganglia (C. K. Willie et al., 2014). Despite the equivocal data of the ganglion blockade studies, all investigations employing ganglionectomy showed increased CBF clearly demonstrating the influence of the SNS on CBF, at least under baseline conditions (C. K. Willie et al., 2014). Additionally, animal experiments (Cassaglia, Griffiths, & Walker, 2008a, 2008b, 2009) and human studies (Ainslie, 2009; Mayhan, Werber, & Heistad, 1987; Y.-C. Tzeng et al., 2010) have provided evidence for the importance of the SNS during alterations in ABP, particularly under conditions of increased CPP. In situations of high CPP, sympathetic constriction of the large cerebral arteries protects downstream circulation (Mayhan et al., 1987).

The level of influence the parasympathetic nervous system (PNS) exerts over CBF has rarely been studied. One previous investigation employed the stimulation of the basal forebrain, observing an increase in CBF (Hamel, 2006). However, after blockade of the muscarinic M5 receptors or following the inhibition of NO synthase, the increase in CBF witnessed with basal forebrain stimulation was attenuated (Hamel, 2006). The results of this investigation provide some evidence of PNS influence over CBF.

2.3.4 Blood Pressure and Cerebral Autoregulation

CA is a homeostatic mechanism that works to preserve CBF in the face of fluctuations in CPP (N. A. Lassen, 1959). Through vasomotor adjustments, CA is able to maintain CBF at relatively constant levels when CPP is within the range of 50 mmHg to 150 mmHg (N. A. Lassen, 1959). Beyond the lower and upper boundaries of autoregulation, vasomotor adjustments can no longer occur and CBF passively follows changes in CPP (Len & Neary, 2011; R. B. Panerai, 1998; Udomphorn et al., 2008). The autoregulation boundaries are not completely fixed and shifts in the boundaries are thought to take place in conditions where changes in metabolism occur such as altering $PaCO_2$ and chronic hypertension (R. B. Panerai, 1998; Van Beek et al., 2008).

Regulation of CA occurs through myogenic, neurogenic and metabolic mechanisms (R. B. Panerai, 2008; Rangel-Castilla, Gasco, Nauta, Okonkwo, &

Robertson, 2008; Y.-C. Tzeng & Ainslie, 2014; Vrselja et al., 2014). The myogenic response is initiated from the vascular smooth muscle in response to changes in transmural pressure across the vessel wall (Rangel-Castilla et al., 2008; Y.-C. Tzeng & Ainslie, 2014). Following increases in blood pressure, the vascular smooth muscle is stretched leading to the activation of stretch-sensitive ion channels (Clifford, 2011; Y.-C. Tzeng & Ainslie, 2014). Activation of the ion channels initiates membrane depolarization and the movement of calcium into the vascular smooth muscle through voltage-gated calcium channels consequently leading to constriction of the vessel (Clifford, 2011; Y.-C. Tzeng & Ainslie, 2014). In contrast, with decreases in blood pressure the opposite occurs resulting in vasodilation (Clifford, 2011; Y.-C. Tzeng & Ainslie, 2014). To test the role of extracellular calcium in the myogenic response, studies have employed calcium channel blockades, via nimodipine and nicardipine, during oscillatory lower body suction to induce pressure-dependent oscillations in CBF (Tan, Hamner, & Taylor, 2013; Y. C. Tzeng, Chan, Willie, & Ainslie, 2011). Using transfer function analysis (TFA), Windkessel modeling, and regression analytical techniques, the investigations found diminished autoregulatory responses following calcium channel blockade (Tan et al., 2013; Y. C. Tzeng et al., 2011). These data would suggest that the myogenic mechanism does exert influence over CA.

Investigations evaluating the neural control of CA have largely focused on animal models though, more recently human investigations have supported the role of neurogenic factors in CA (Y.-C. Tzeng & Ainslie, 2014). Kimmerly and colleagues (2003) evaluated CA following the infusion of both norepinephrine and phentolamine. The infusion of norepinephrine, an alpha-adrenergic receptor agonist, increased MAP though MCA velocity remained relatively unchanged demonstrating intact autoregulation (Kimmerly et al., 2003). However, phentolamine, an alpha-adrenergic receptor antagonist, co-infused with norepinephrine caused a significant drop in MAP and MCA flow velocity, indicating an impairment to CA (Kimmerly et al., 2003). This evidence for impaired CA following alpha-adrenergic receptor blockade suggests a role of the SNS in CA (Kimmerly et al., 2003). Furthermore, Zhang and colleagues (2002) evaluated dynamic CA before and after ganglion blockade with trimethaphan. Following ganglion blockade, a large increase in transfer function gain and a reduction in the phase lead of

CBFV to ABP occurred suggesting an impairment in CA (Zhang et al., 2002). Though, ganglion blockades disrupt both sympathetic and parasympathetic nerve activity so it is not clear whether sympathetic and/or cholinergic influences affected CA in this model (Zhang et al., 2002).

Metabolites in the vascular endothelium have also been recognized to contribute to the autoregulatory response (R. B. Panerai, 2008; Y.-C. Tzeng & Ainslie, 2014; Van Beek et al., 2008). In particular, NO has garnered much attention given its role as a potent dilatory stimulus in the cerebral circulation (Faraci & Brian, 1994; Faraci & Heistad, 1998). The infusion of L-NMMA to block endothelial NO production resulted in a weakened autoregulatory response suggesting a contribution of endothelial NO to CA (White, Vallance, & Markus, 2000). Furthermore, animal research using L-NMMA infusion found similar results (Faraci & Brian, 1994). However, previous investigations have also provided evidence that inhibition of endothelial NO has no effect on CA (Faraci & Brian, 1994; Y.-C. Tzeng & Ainslie, 2014). Given the equivocal results, the exact role of NO remains unclear though it is considered to exert some level of influence over CA (Faraci & Brian, 1994; Rangel-Castilla et al., 2008; Y.-C. Tzeng & Ainslie, 2014).

Unfortunately, the cerebral vasculature cannot be entirely isolated from other influences preventing investigators from determining the relative contribution of the myogenic mechanism, neurogenic factors, and metabolic factors in CA (Y.-C. Tzeng & Ainslie, 2014).

2.3.4.1   Static Cerebral Autoregulation

Static CA looks at the association between mean CBF and mean arterial blood pressure (MABP) following induced alterations or spontaneous changes in ABP (R. B. Panerai, 1998). With this approach measures are taken in steady-state (Tiecks et al., 1995). Static CA studies are the foundation for the classical autoregulation curve (N. A. Lassen, 1959). Lassen discovered the autoregulation curve after combining the results from multiple studies where the species investigated and the methodology employed varied (R. B. Panerai, 1998; Van Beek et al., 2008). Nonetheless, the classical autoregulation curve

continues to be the dominant model of cerebral autoregulation (R. B. Panerai, 1998). Few studies have since evaluated the relationship between ABP and indices of CPP over long durations and, with various statistical techniques, have found autoregulation curves similar to that outlined by Lassen (Czosnyka et al., 2001; Tan, 2012).

Static CA evaluates steady-state ABP and CBF before and after inducing a change in ABP (R. B. Panerai, 1998; Rangel-Castilla et al., 2008; Tiecks et al., 1995). By this approach, the initial period following the alteration in ABP is ignored, when ABP and CBF are changing dynamically (R. B. Panerai et al., 2001; Tiecks et al., 1995). As a result, static CA provides information on the absolute change in CBF for a given change in ABP but overlooks the time course of the autoregulatory response (R. B. Panerai, 1998; R. B. Panerai et al., 2001; Tiecks et al., 1995). Techniques commonly used to induce changes in ABP include drug infusion, head-up-tilt (HUT), and lower body negative pressure (LBNP) (R. B. Panerai, 1998). Because steady-state ABP and CBF are required, static CA assessments tend to be time-consuming (Tiecks et al., 1995). Therefore, static CA may not be the most practical method for assessing cerebrovascular function in clinical settings.

2.3.4.2   Dynamic Cerebral Autoregulation

Dynamic CA evaluates dynamic changes in CBFV in response to rapid, transient changes in ABP (Aaslid et al., 1989). Assessment of dynamic CA is only possible with continuous measures of CBFV from TCD providing information on the adaptive period following alterations in ABP (Aaslid et al., 1989). Therefore, dynamic CA provides information on the time course of the autoregulatory response (Tiecks et al., 1995; M. Vavilala et al., 2002). Previous research has proposed that the time course of the autoregulatory response is affected first in various pathological conditions suggesting the importance of dynamic CA measures (Tiecks et al., 1995). The rapid nature of dynamic CA assessments may be useful in evaluating cerebrovascular function in clinical settings given the proper tools and analysis processes are accessible.

Appropriate methodology is necessary to induce rapid, transient changes in ABP. The thigh-cuff model was derived by Aaslid and colleagues (1989) and has been most

commonly employed by investigations evaluating dynamic CA (R. B. Panerai, 1998). The thigh-cuff model involves the inflation of two large blood pressure cuffs around both thighs to suprasystolic levels (Aaslid et al., 1989). The thigh-cuffs remain inflated for two minutes before being deflated rapidly thereby producing a rapid drop in ABP (Aaslid et al., 1989). The thigh-cuff model requires a number of repeated trials due to high variability in the ABP drop (Sorond, Serrador, Jones, Shaffer, & Lipsitz, 2009). Repeated cuff inflations are often quite painful for participants making it difficult to complete the desired number of trials (Lipsitz, Mukai, Hamner, Gagnon, & Babikian, 2000; Sorond et al., 2009). Due to the limited clinical applicability of the thigh-cuff model, particularly in certain populations, additional models have recently been developed as an alternative (Sorond et al., 2009). One model that was developed is the sit-to-stand model (Lipsitz et al., 2000). The sit-to-stand model, as originally described by Lipsitz et al. (2000), involves sitting in a straight-backed chair with legs at 90 degrees for five minutes followed by standing for one minute. The sit-to-stand test is well-tolerated and can be performed effectively in elderly populations (Sorond, Khavari, Serrador, & Lipsitz, 2005; Sorond et al., 2009). This model induces a consistent and reproducible drop in MAP, similar to that of a successful thigh-cuff trial (Sorond et al., 2009). In addition, the sit-to-stand protocol is a more relevant physiological stimulus and may be more suitable for clinical use particularly in certain populations (Sorond et al., 2005; Sorond et al., 2009).

Dynamic CA can be evaluated using the rate of regulation (RoR) or the dynamic autoregulatory index (ARI) (Aaslid et al., 1989; Tiecks et al., 1995). The RoR was originally derived from the thigh-cuff model and represents the per-second adjustment of the CVR response necessary to compensate for the change in ABP (Aaslid et al., 1989). Following the deflation of the thigh-cuffs, a drop in ABP, CBFV, and CVR occur. The rate of change in CVR ($\Delta CVR/\Delta T$) is calculated during the time interval from 1 to 3.5 seconds following cuff deflation (Aaslid et al., 1989). The change in ABP (ΔABP) is determined as the difference between the control ABP, defined by the mean during the 4 seconds prior to cuff release, and the mean ABP during the interval from 1 to 3.5 seconds (Aaslid et al., 1989). This value is then divided by the control ABP (Aaslid et al., 1989). The RoR is then defined as the rate of change in CVR relative to the change in ABP (($\Delta CVR/\Delta T)/\Delta ABP$) (Aaslid et al., 1989). Though this approach was originally derived

from the thigh-cuff deflation model, the sit-to-stand model produces similar responses (Sorond et al., 2009); therefore, RoR theoretically should be applicable to both models.

The dynamic ARI was also derived from the thigh-cuff deflation model (Tiecks et al., 1995). This approach is based on a computer model that calculated ten hypothetical curves for the CBFV responses to a 30 second drop in ABP. First, a hypothetical curve for CBFV was computed assuming autoregulation was absent and was associated with an ARI value of 0 (Tiecks et al., 1995). Nine additional curves were computed, each assigned an ARI value of 1 through 9 (Tiecks et al., 1995). The actual CBFV response witnessed in the experiment is fitted to one of the ten curves and the associated ARI values signifies the strength of the autoregulatory response with 0 being absent autoregulation and 9 being optimal autoregulation (Tiecks et al., 1995). Normal ARI values were reported to be 5 ± 1 (Tiecks et al., 1995). This approach has also been applied in the sit-to-stand model (Sorond et al., 2009).

2.3.4.3   Cerebral Autoregulation and Carbon Dioxide

Similar to the cerebral circulation, PaCO2 has a profound effect on CA that is witnessed for static and dynamic CA. With respect to static CA, the boundaries of the autoregulation curve are adjusted in response to alterations in PaCO2 (Aaslid et al., 1989; Edwards, Shoemaker, & Hughson, 2002; Meng & Gelb, 2015; R. Panerai, Deverson, Mahony, Hayes, & Evans, 1999; R. B. Panerai, 1998; Ursino & Lodi, 1998). During hypercapnia, the autoregulation curve is shifted upwards to a higher CBF (R. Panerai et al., 1999; R. B. Panerai, 1998; Ursino & Lodi, 1998) and the upper and lower limits are shifted leftwards and rightwards, respectively, narrowing the plateau region of the curve (Meng & Gelb, 2015; R. B. Panerai, 1998). At very high levels of PaCO2, the plateau region dissipates and cerebral autoregulation is no longer functional (R. Panerai et al., 1999; R. B. Panerai, 1998). In contrast, the autoregulation curve is shifted downwards during hypocapnia (Meng & Gelb, 2015; Ursino & Lodi, 1998) and the plateau region widens (R. Panerai et al., 1999). One investigation has reported that the upper limit shifts rightwards and the lower limit shifts leftwards (R. Panerai et al., 1999). However, a second investigation has described only moderate changes in the lower limit with changes in the upper limit unclear (Meng & Gelb, 2015). Though the exact alterations in the

boundaries of autoregulation with hypocapnia remain unknown, low PaCO2 does shift the autoregulatory curve down (Aaslid et al., 1989; Edwards et al., 2002; R. Panerai et al., 1999).

With respect to dynamic CA, Aaslid and colleagues (1989) evaluated the effect of PaCO2 on RoR in a group of healthy adults. Hypercapnia produced a reduction in autoregulatory capacity from normocapnia slowing the RoR (Aaslid et al., 1989). In contrast, hypocapnia enhanced the autoregulatory response, increasing the RoR (Aaslid et al., 1989).

2.3.4.4   Cerebral Autoregulation and Head Injury

Cerebral autoregulatory outcomes following head injury have become an area of interest in recent decades, particularly with recent increases in concussion incidence (Stewart et al., 2014). Many investigations have focused on adults suffering from moderate to severe TBI (Bailey et al., 2013; Czosnyka et al., 2001; Lang et al., 2003; Lee et al., 2001). In contrast, pediatric populations and patients suffering from concussion have seldom been studied. Furthermore, the investigation of CA in head injury have largely employed static measures of CA (Czosnyka et al., 2001; Lang et al., 2003; Lee et al., 2001).

One of the earlier investigations into CA following head injury was conducted in adults admitted to the hospital for minor head injury (Jünger et al., 1997). Minor head injury was defined by a Glasgow Coma Scale (GCS) score of 13 to 15 and participants were treated with bed rest, analgesic medications, and intravenous administration of crystalloid solutions (Jünger et al., 1997). Loss of consciousness was not reported in this investigation (Jünger et al., 1997). Participants completed dynamic CA testing with the thigh-cuff deflation model within 48 hours of the concussive injury (Jünger et al., 1997). To evaluate CA, dynamic ARI was calculated and similar ARI values were witnessed for the head-injured patients and the healthy controls (Jünger et al., 1997). However, the head-injured patients appeared to present as two distinct groups, one with relatively normal ARI values and one with low ARI values (Jünger et al., 1997). Therefore, impairments in dynamic CA were witnessed in some but not all adults with minor head injury (Jünger et al., 1997).

In 2001, Czosnyka and colleagues examined the associations between CA and ABP, CPP, and ICP in adults suffering from severe TBI with an average GCS value of 6 (Czosnyka et al., 2001). Recordings of ABP, CPP, and ICP waveforms were captured over a period from 20 minutes to 120 minutes (Czosnyka et al., 2001). To evaluate static CA, the mean index of autoregulation (Mx) was calculated as a Pearson correlation coefficient of CPP and CBFV with positive associations indicating impaired autoregulation (Czosnyka et al., 2001). The relationship between Mx and ABP was described by a U-shaped curve with weaker autoregulation observed at low ABP and at high ABP (Czosnyka et al., 2001). Similarly, a U-shaped curve was also seen for the relationship between Mx and CPP with weaker autoregulation observed at low CPP and at high CPP (Czosnyka et al., 2001). Autoregulation and ICP were inversely related, such that the autoregulatory response was weakened with increasing ICP (Czosnyka et al., 2001). The results of this investigation provided information on a range of ABP, CPP, and ICP within which static CA was optimized in severe TBI (Czosnyka et al., 2001).

Lee and colleagues (2001) measured static CA in moderate to severely head-injured patients presenting with a mean GCS score of 7. An elevation in ABP was induced following the infusion of phenylephrine (Lee et al., 2001). The average ABP and CBFV before and after phenylephrine infusion were used to estimate CVR (Lee et al., 2001). A pressure autoregulation index was calculated as the percent change in estimated CVR divided by the percent change in MAP (Lee et al., 2001). Similar to the investigation by Jünger and colleagues (1997), a variable impairment was observed in static CA (Lee et al., 2001). In addition, participants were assessed over the course of six days (Lee et al., 2001). No differences were seen in static CA between time points though there did appear to be a trend towards improvement after four days' post-injury (Lee et al., 2001).

Lang and colleagues (2003) also evaluated the temporal profile of static CA in adults suffering from moderate to severe TBI. The participants presented with an average GCS score of 7 and measures of ABP and CBFV were collected over a period of approximately 18 minutes (Lang et al., 2003). To evaluate static CA, Mx was calculated and impaired CA was defined as values greater than 0.3 (Lang et al., 2003). No

impairments in static CA were witnessed though there appeared to be a trend towards deficits in autoregulation immediately after injury. This remained throughout the first three days of recovery and improvements began between four and six days post-injury (Lang et al., 2003).

More recently, static and dynamic CA were examined in professional boxers who experienced repeated sub-concussive head impacts (Bailey et al., 2013). The control participants were matched with the boxers for age and physical fitness levels (Bailey et al., 2013). This investigation utilized two methods of evaluating dynamic CA; RoR and ARI were calculated following thigh-cuff deflation (Bailey et al., 2013). In addition, static CA was measured with TFA (Bailey et al., 2013). For TFA, steady-state ABP and CBFV were collected over a period of 5 minutes (Bailey et al., 2013). No differences were observed in static CA between the boxers and the healthy controls (Bailey et al., 2013). However, with the thigh-cuff deflation model, the boxers demonstrated lower RoR and ARI values when compared with the healthy controls (Bailey et al., 2013). Therefore, although static CA remained intact in the boxers, impairments in dynamic CA were observed (Bailey et al., 2013). This investigation demonstrates the importance of employing dynamic measures of CA in head-injured patients.

As previously mentioned, the pediatric population remains poorly studied with respect to CA and head-injury. To date, only two studies have investigated static CA in pediatric TBI (Muizelaar et al., 1989; M. S. Vavilala et al., 2004). Muizelaar and colleagues (1989) measured static CA in severely head-injured children presenting with an average GCS score of 5.5. Following the infusion of phenylephrine, ABP was increased by approximately 30% (Muizelaar et al., 1989). In some instances, if CBF was normal or high and if motor evoked potentials could be monitored continuously, ABP was lowered with the infusion of trimethaphan camsylate (Muizelaar et al., 1989). Static CA was evaluated as %CPP/%CVR and impaired CA was defined as a value greater than 2 (Muizelaar et al., 1989). On the other hand, Vavilala and colleagues (2004) assessed three groups of children that were divided based on injury severity using the GCS. Group one included patients with severe TBI defined by a GCS score below 9, group two included moderate TBI patients defined by a GCS score of 9-12, and group three

included mild TBI patients defined by GCS score of 13-15 (M. S. Vavilala et al., 2004). Participants were under general anesthesia for extracranial surgical procedures (M. S. Vavilala et al., 2004). Phenylephrine was infused over a period of 3 to 5 minutes and ABP was raised to specific criteria based on age (M. S. Vavilala et al., 2004). Static CA was evaluated using static ARI calculated as %ΔCVR/%ΔMAP, where 0 represents absent autoregulation and 1 represents optimal autoregulation (M. S. Vavilala et al., 2004). In this investigation, intact autoregulation was defined as an ARI $\geq$ 0.4 (M. S. Vavilala et al., 2004). Both investigations found only variably impaired static CA in children and adolescents following mild to severe TBI (Muizelaar et al., 1989; M. S. Vavilala et al., 2004). To date, measures of static or dynamic CA have not been performed in adolescent concussion.

2.4   Summary and Purpose

Concussion is a major concern in adolescent populations though very little data exist on cerebrovascular outcomes following concussive injuries in this population. Therefore, there is a strong need to evaluate CA in adolescent concussion provided the importance of this homeostatic mechanism in CBF regulation and consequently maintenance of cerebral perfusion to meet the metabolic demands of the brain. Previous investigations of CA in the pediatric population have largely employed static measures (Muizelaar et al., 1989; M. S. Vavilala et al., 2004). However, as the investigation by Bailey et al. (2013) demonstrated, it is important to evaluate the dynamic, adaptive responses of the cerebral circulation to hemodynamic stress. Therefore, the **purpose** of this investigation was to evaluate dynamic cerebral autoregulatory function in adolescents diagnosed with a concussion. This study tested the **hypothesis** that concussed adolescents would be marked by impaired dynamic autoregulatory responses.

Chapter 3

3   Methods

3.1   Participants

Thirty adolescents diagnosed with a concussion (15 ± 1 years; 20 females) and thirty healthy controls (14 ± 2 years; 15 females) participated in the current investigation. Participants were between the ages of 12 and 18 years and were actively involved in sports and recreational activities. In total, eleven participants were on various medications for asthma and exercise-induced asthma (Ventolin, n=7; Flovent, n=1), depression (Cipralax, n=1), ADHD (Strattera, n=1), hyperthyroidism (Tapazole, n=1), headaches (Amitriptyline, n=1), and anxiety (Sertraline, n=1). In addition, a total of six female participants were on oral contraceptives.

Concussed adolescents were recruited upon diagnosis by a physician at the Fowler Kennedy Sports Medicine Clinic (FKSMC) and control participants were recruited from various athletic organizations within the community. Control participants with a previous history of concussion were included provided the injury was not within 6 months of their initial visit and they were no longer experiencing persistent symptoms (n = 8).

Potential participants were excluded if they had existing bone or muscle problems that could impact balance or gait, diagnosis of a pre-existing heart disease, use of beta-blockers or anticonvulsants or any other medications that affect heart or blood pressure control, significant respiratory disease or illness, any pre-existing neurological disorders, metabolic disorders such as diabetes, a history of significant neck injury, Rheumatoid arthritis, vertebral basilar artery insufficiency, primary or metastatic bone tumor, severe osteoporosis, inability to understand English, diagnosis of post-traumatic stress disorder, and/or pregnancy.

All participants provided written informed consent and received detailed explanations of all experimental protocols prior to participation. The study was approved by the Health Sciences Research Ethics Board at Western University.

3.2   Experimental Protocols

Healthy controls participated in up to two laboratory sessions separated by at least one week. Concussed adolescents participated in laboratory sessions weekly or biweekly, for up to six visits or until medical clearance was granted by a physician at FKSMC. Identical testing procedures were followed at each laboratory session.

At the initial laboratory session, participants provided a detailed medical history. Concussed adolescents further provided detailed information concerning the injury including etiology and initial symptomology. Participants with a previous history of concussion provided similar details of past injuries along with specific information on the length of recovery and the history of symptoms following recovery. At each subsequent visit, participants provided a medical update documenting any changes to their health or medications. For the concussed adolescents, details about their progress as well as current rehabilitative approaches being followed were documented.

To outline their symptom profile at the time of testing, participants completed the Sport Concussion Assessment Tool - 3rd edition (SCAT3) self-report symptom evaluation. The SCAT3 symptom evaluation includes a total of 22 symptoms that are rated on a scale of 0 to 6 with a maximum symptom severity score of 132. A Child SCAT3 is completed for children 12 and younger and differs from the SCAT3 in the number of symptoms, the description of symptoms, and in the severity scale ranging from 0 to 3. Additionally, participants completed the Generalized Anxiety Disorder 7-item Scale (GAD-7) to provide details on the levels of anxiety generally experienced over the two-week period prior to the testing session. Participants also completed a visual analogue scale (VAS) of anxiety to identify levels of anxiety at the time of testing. The VAS utilized in the present investigation was developed by our laboratory in conjunction with the FKSMC and is shown in Figure 3.1. To provide information on pubertal development, participants completed the Tanner Puberty Stages Questionnaire. Female

participants answered additional question regarding menstrual cycle including age of menarche, frequency of menstruation, day of menstrual cycle, and whether participants were on oral contraceptives. Furthermore, anthropometric measures were recorded and three baseline blood pressure measurements were collected in a seated position with a manual sphygmomanometer.

Participants were instrumented with a standard 3-lead electrocardiogram (ECG) for continuous measures of heart rate (HR) and a finger photoplethysmograph (Finometer, Finapres Medical Systems BV, Amsterdam, The Netherlands) for continuous measures of ABP. Stroke volume (SV) and cardiac output (CO) were obtained using the Finometer Modelflow algorithm that accounts for participant sex, age, height, and weight. Breathing frequency and $P_{ET}CO_2$ were monitored continuously with a respiratory strain gauge and a gas analyzer (ML206, ADInstruments, Colorado Springs, CO, USA), respectively.

Following completion of a 5-minute baseline recording period in the supine position, participants were assisted into a seated position in a chair for the sit-to-stand protocol. Participants were fitted with a sling to maintain the right arm in a fixed position at the level of the heart thereby eliminating hydrostatic pressure effects during the postural changes (Lipsitz et al., 2000). Participants were equipped with a bilateral monitoring headband device (Neurovision 500M, Neurovision TOC2M, Multigon Industries, Elmsford, CA, USA) and the right MCA was insonated through the transtemporal window and CBFV recorded (2 MHz PW Doppler probe, Neurovision 500M, Neurovision TOC2M, Multigon Industries, Elmsford, CA, USA). Mean flow velocity was collected for twenty-seven participants (13 concussed adolescents and 14 healthy controls) and peak flow velocity was collected for thirty-three participants (17 concussed adolescents and 16 healthy controls).

For the sit-to-stand protocol, participants rested in the seated position for three minutes then transitioned to a standing position for two minutes. Participants then resumed the seated position and the protocol was repeated. The transition to standing and sitting was done without the use of hands.
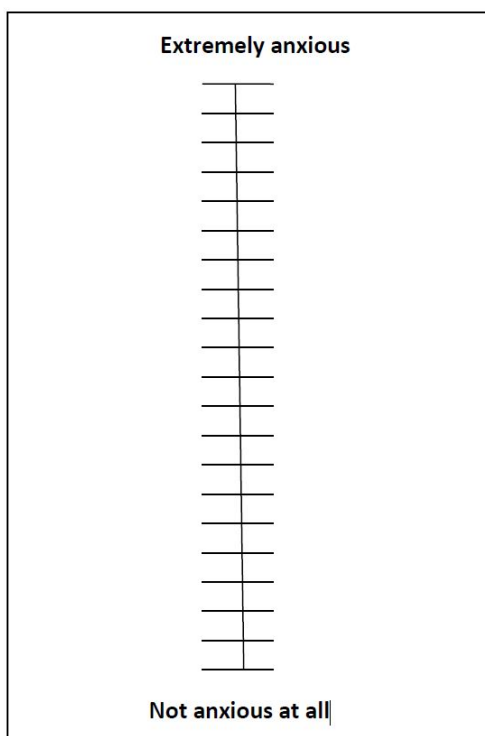
Figure 3.1: Visual analogue scale of anxiety. The scale was used to measure the participants' levels of anxiety at the time of testing.

3.3   Data Analysis

The brachial blood pressure waveform was corrected to manual sphygmomanometric values. Hemodynamic and respiratory measures were evaluated in the supine, sitting, and standing postures. Measures of steady-state ABP, HR, CO, SV, total peripheral resistance (TPR), $P_{ET}CO_2$, and respiration frequency were averaged during the five-minute supine period, the full three-minute sitting periods, and the full two-minute standing periods. TPR was calculated as mean arterial pressure (MAP) divided by CO. Measures of steady-state CBFV and CVR were averaged only during the full three-minute sitting periods and the full two-minute standing periods as this data was not collected during the supine posture.

In addition, the initial dynamic changes in the hemodynamic and respiratory parameters with standing were assessed (Figure 3.2). The control values for each hemodynamic

parameter was defined as the mean value in the four seconds of sitting prior to standing. The four seconds of sitting prior to standing was representative of the full sitting period for all hemodynamic and respiratory parameters. The absolute change in MABP, CBFV, CVR, SV, and TPR, was calculated as the difference between the control value and the nadir value after standing. For HR and CO, the absolute change was calculated as the difference between the control value and the peak value after standing. Upon transitioning to standing, $P_{ET}CO_2$ continues to fall throughout the remainder of the standing period. Therefore, the absolute change in $P_{ET}CO_2$ was evaluated as the difference between the control value and the mean value during the interval in which the reduction in CVR occurred.
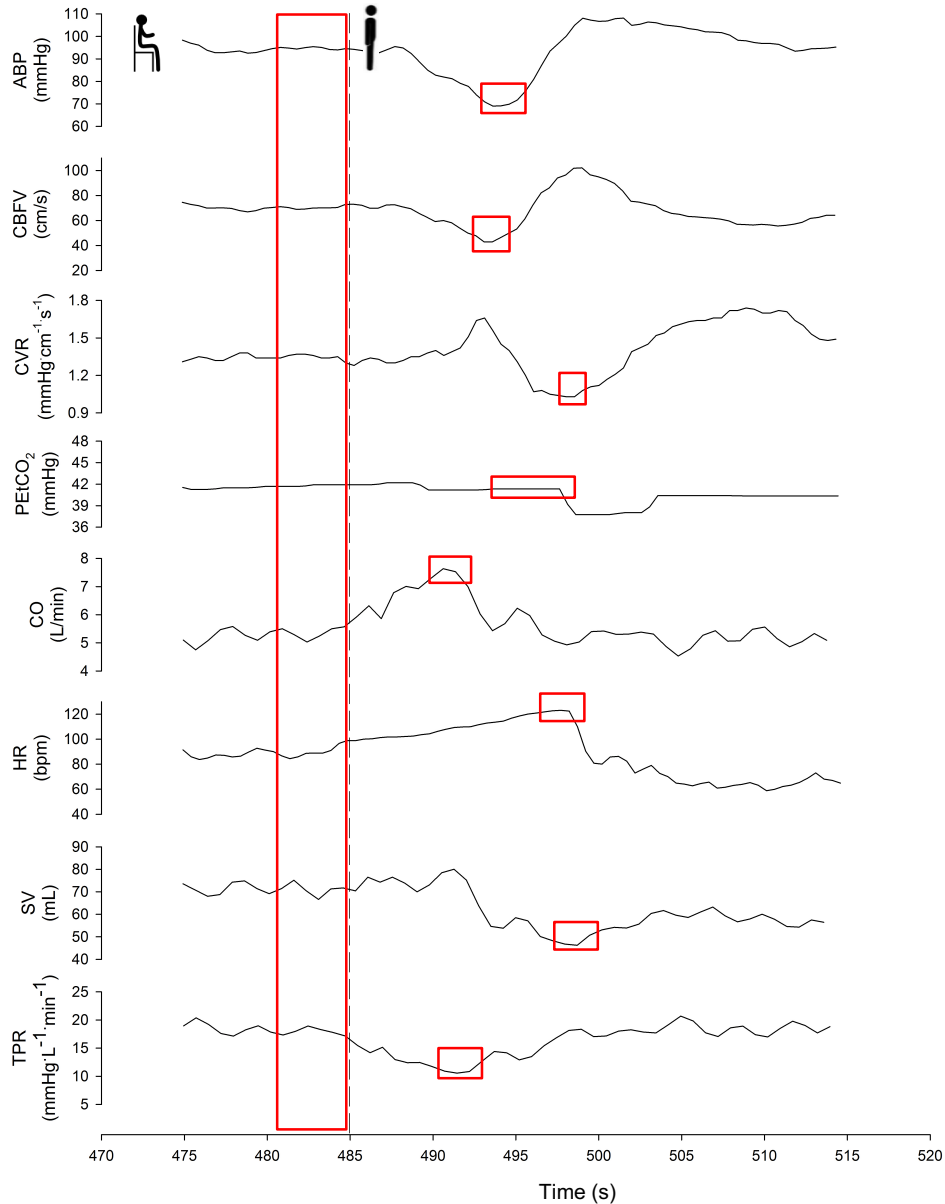
Figure 3.2: Measurement of dynamic changes in the hemodynamic and respiratory parameters with standing. Sample data from concussed participant. ABP, arterial blood pressure; CBFV, cerebral blood flow velocity; CVR, cerebral vascular resistance; PETCO$_2$, end tidal carbon dioxide partial pressure; CO, cardiac output; HR, heart rate; SV, stroke volume; TPR, total peripheral resistance. The control values were defined as the mean value in the four seconds of sitting prior to standing. Initial absolute changes were evaluated as the difference between the control values and the nadir values (ABP, CBFV, CVR, SV, TPR) or peak values (CO, HR). The difference in PETCO$_2$ was evaluated using the mean value during the interval in which CVR is reduced.

The time course of the changes in the hemodynamic parameters with standing was evaluated (Figure 3.3). The time to nadir was assessed for MABP, CBFV, CVR, and TPR by measuring the time from standing to the nadir value. In contrast, the time to peak HR was evaluated by calculating the time from standing to the peak value. In addition, the time to peak MABP and CBFV was evaluated by calculating the time from standing to the peak value following the initial reduction. Furthermore, the delay in the cerebrovascular response was evaluated. The time to CVR drop was assessed as the time from standing to the initiation of the CVR drop.
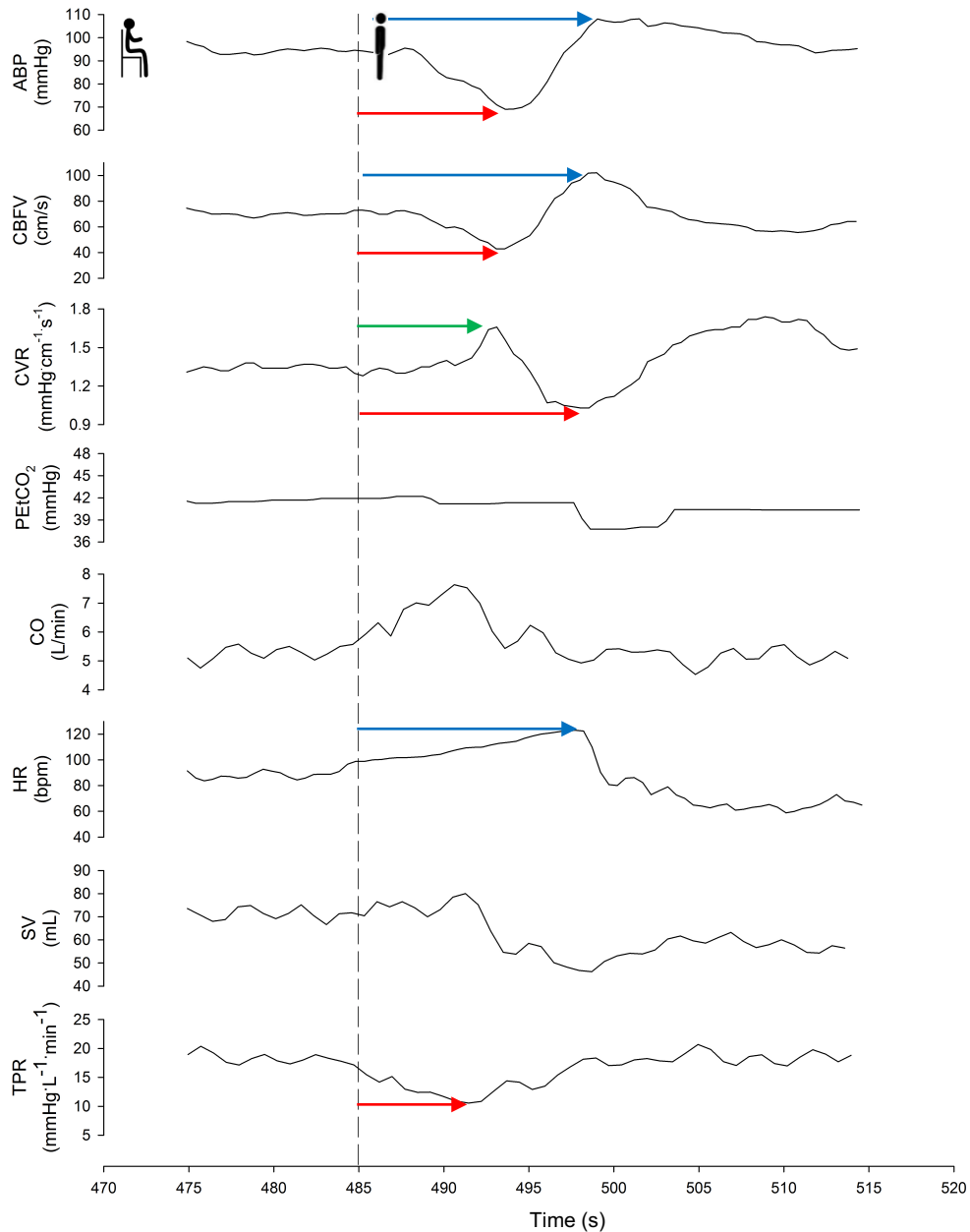
Figure 3.3: Measurement of the time course of changes in the hemodynamic parameters with standing. Sample data from concussed participant. ABP, arterial blood pressure; CBFV, cerebral blood flow velocity; CVR, cerebral vascular resistance; $P_{ET}CO_2$, end tidal carbon dioxide partial pressure; CO, cardiac output; HR, heart rate; SV, stroke volume; TPR, total peripheral resistance. Time to nadir was measured from the time of standing to the nadir value (red arrows) and time to peak was measured from the time of standing to the peak value (blue arrows). The time to CVR drop was measured from the time of standing to the initiation of the drop in CVR (green arrow).

To evaluate cerebral autoregulation, RoR was calculated as previously described by Aaslid et al. (1989) with modifications for application to the sit-to-stand model (Figure 3.4). Control values of ABP and CBFV were defined by their means in the four seconds of sitting prior to standing. Subsequent changes in ABP and CBFV with standing were calculated relative to the control values. The CVR was determined as ABP divided by CBFV. Upon standing, a drop in CVR occurred and the change in CVR was measured from the initiation of the drop, or the maximum value, to the nadir value (ΔCVR). A regression line was computed and the slope of the line defined the rate of change in CVR (ΔCVR/ΔT). RoR represents the per second adjustment of the CVR response required to fully compensate for the drop in ABP. Thus, RoR is defined as the rate of change in CVR relative to the change in ABP ((ΔCVR/ΔT)/ΔABP). The change in ABP (ΔABP) is calculated by subtracting the control ABP from the mean ABP during the interval in which CVR is reduced; this value is then divided by the control ABP.

Sit-to-stand trials were excluded if the drop in MABP was less than 12 mmHg (M. Vavilala et al., 2002), if the ABP or CBFV signals were lost during the protocol, or if the CBFV signal was uninterpretable. Noisy CBFV tracings with signal spikes or dropouts were smoothed through a low-pass filter (10-50 Hz). Two sit-to-stand trials were completed and participants with two adequate sit-to-stand trials were included in the data analysis. In total thirty-eight participants were excluded from analysis.
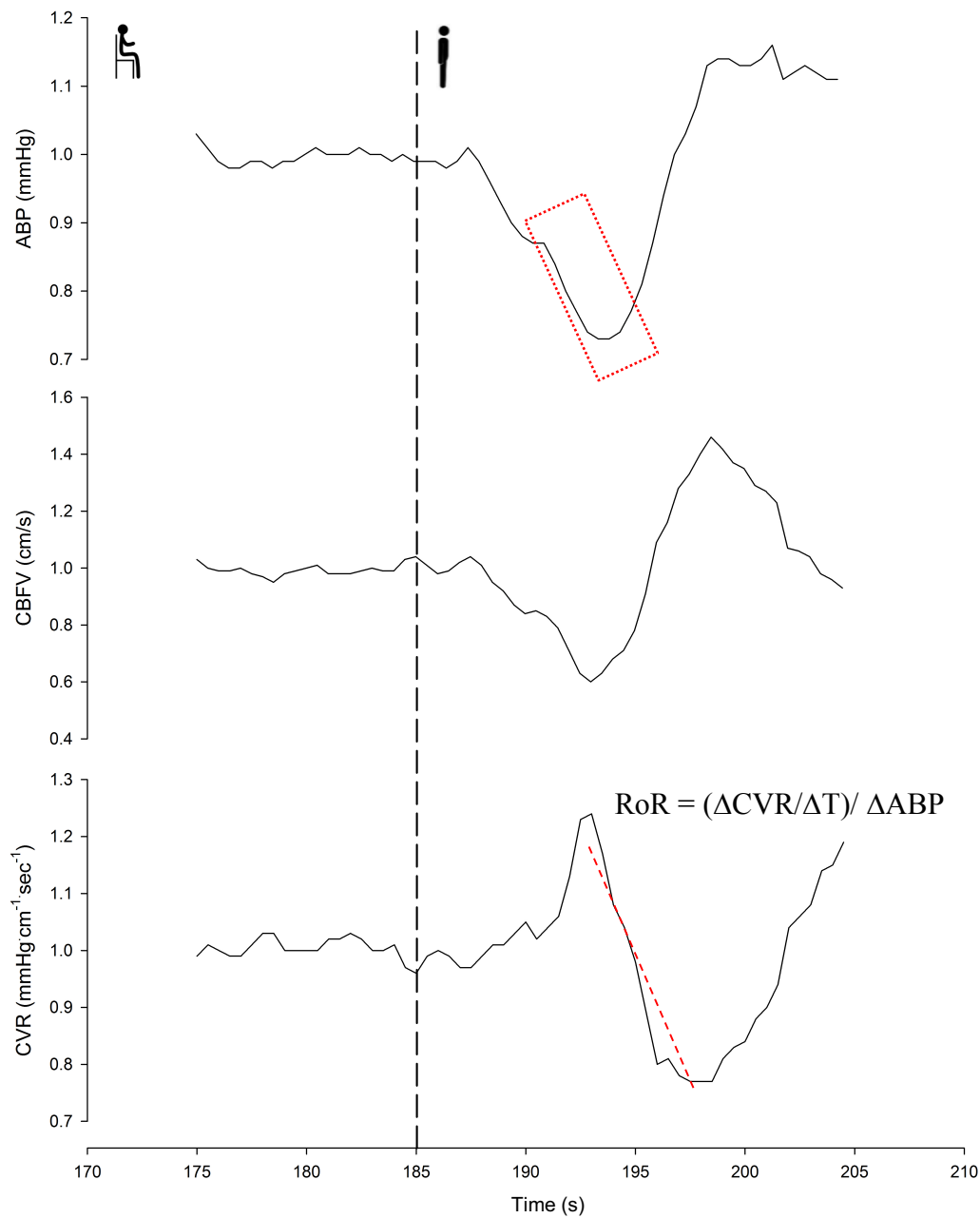
Figure 3.4: Measurement of the rate of regulation with a sit-to-stand model. Sample data from concussed participant. ABP, arterial blood pressure; CBFV, cerebral blood flow velocity; CVR, cerebral vascular resistance. Relative changes in ABP and CBFV were calculated by dividing ABP and CBFV by the control values in the four seconds of sitting prior to standing. CVR is calculated by dividing ABP by CBFV. The change in CVR is measured and the slope of the line (ΔCVR/ΔT) is divided by the change in ABP (ΔABP) to provide RoR ((ΔCVR/ΔT)/ΔABP).

3.4   Statistical Analysis

Data are presented as mean ± SD, unless otherwise indicated. SigmaPlot 12.5 and SPSS Statistics 23 were used for statistical analysis. Participant characteristics were evaluated between the healthy controls and the concussed adolescents using unpaired t-tests. Further, supine hemodynamic and respiratory parameters were compared between the healthy controls and the concussed adolescents using unpaired t-tests. Comparisons of steady-state sitting and standing hemodynamic and respiratory parameters between the healthy controls and concussed adolescents were made with a two-way repeated measures ANOVA. The initial absolute changes in the hemodynamic parameters with standing and the time course of these changes were compared between the two groups using unpaired t-tests. Comparison of RoR between the healthy controls and the concussed adolescents were made with unpaired t-tests. In this approach, the healthy controls acted as a reference group to which the concussed adolescents were compared at the first and final visit and a Bonferroni correction was applied. Significance was set as $P \leq 0.05$.

Chapter 4

4   Results

4.1   Participant Characteristics and Supine Hemodynamic and Respiratory Parameters

Participant physical characteristics and supine hemodynamic and respiratory data are presented in Table 4.1. Participant physical characteristics, including age, height, weight, and body mass index (BMI), were not statistically different between the concussed adolescents and healthy controls. The concussed adolescents sustained their injury through participation in sports and recreational activities and attended the laboratory for their initial visit, on average, 26 ± 19 days' post-injury. Notably, the concussed adolescents were largely in the acute phase of recovery at the time of their initial visit (17 ± 12 days post-injury) with the exception of six participants whose initial visit was greater than 42 days' post-injury classifying them as PCS (58 ± 11 days post-injury) (Willer & Leddy, 2006). Compared with the healthy controls, the concussed adolescents collectively scored higher on the SCAT-3 symptom evaluation at the initial visit for both the number of symptoms experienced ($P \leq 0.001$) and the severity of symptoms ($P \leq 0.001$). One control participant was excluded from the SCAT-3 analysis as they completed a Child SCAT-3 evaluation. When the concussed adolescents were grouped by days' post-injury, those in the acute phase of recovery scored similarly on the SCAT3 to those suffering from PCS for both the symptom score (11 ± 6 vs. 10 ± 6; $P = 0.62$) and severity score (25 ± 23 vs. 20 ± 20; $P = 0.77$). Participants' general anxiety levels over the 2-week period prior to the initial visit, as measured through the GAD-7, was not statistically different between the healthy controls and the concussed adolescents. Furthermore, GAD-7 scores were also not significantly different between the acutely concussed adolescents and PCS adolescents (5 ± 4 vs. 3 ± 4; $P = 0.15$). In addition, the participants' anxiety at the time of testing, as assessed via the VAS of anxiety, was not statistically different between the healthy controls and the concussed adolescents (17 ± 13 vs. 16 ± 23; $P = 0.34$). The VAS of anxiety was developed following the start of the investigation and was completed in fifteen healthy controls and ten concussed

adolescents. One participant was on medication for anxiety and was included in the analysis as results remained the same when the participant was removed from analysis.

Supine hemodynamic parameters, including MABP, systolic BP, diastolic BP, HR, SV, and CO were not significantly different between the healthy controls and the concussed adolescents. Compared with the healthy controls, the concussed adolescents demonstrated reduced TPR in the supine posture ($P = 0.02$). Furthermore, respiration rate was higher in the concussed adolescents in the supine posture ($P = 0.04$). However, supine $P_{ET}CO_2$ was not different between the two groups. Supine $P_{ET}CO_2$ was recorded in a smaller sample of participants that included twelve healthy controls and fourteen concussed adolescents. There was no difference in supine respirations between the participants with $P_{ET}CO_2$ collected and those without $P_{ET}CO_2$ collected in both groups. Therefore, we can assume that the supine $P_{ET}CO_2$ reported is representative of the entire sample.

Table 4.1: Participant characteristics and supine hemodynamic and respiratory parameters.

	CTRL	CONC
Physical Characteristics		
n, males/females	30, 15/15	30, 10/20
Age (y)	14 ± 2	15 ± 1
Height (cm)	170 ± 8	170 ± 9
Weight (kg)	66 ± 17	64 ± 14
BMI (kg/m^2)	22 ± 5	22 ± 4
SCAT-3 Symptom Score (22)	6 ± 5	11 ± 6*
SCAT-3 Severity Score (132)	9 ± 11	24 ± 22*
GAD-7 Score	4 ± 3	5 ± 4
Supine Hemodynamic & Respiratory Parameters		
MABP (mmHg)	86 ± 9	86 ± 11
Systolic BP (mmHg)	115 ± 10	114 ± 14
Diastolic BP (mmHg)	70 ± 9	70 ± 10
HR (bpm)	66 ± 9	68 ± 10
SV (mL)	61 ± 17	68 ± 19
CO (L·min^{-1})	4.0 ± 1.1	4.6 ± 1.4
TPR (mmHg·L^{-1}·min^{-1})	24 ± 7	20 ± 6*
Respiration (breaths·min^{-1})	16 ± 3	17 ± 3*
PETCO$_2$ (mmHg)	41 ± 3	39 ± 3

Values are mean ± SD. CTRL, control; CONC, concussed; BMI, body mass index; MABP, mean arterial blood pressure; BP, blood pressure; HR, heart rate; SV, stroke volume; CO, cardiac output; TPR, total peripheral resistance; PETCO$_2$, end tidal carbon dioxide partial pressure. *Significantly different from CTRL, $P \leq 0.05$.

4.2   Hemodynamic and Respiratory Responses to Standing

The steady-state hemodynamic data for the sitting and standing postures are presented in Table 4.2. There was a main effect of posture on MABP ($P \leq 0.001$), CVR ($P = 0.04$), HR ($P \leq 0.001$), and $P_{ET}CO_2$ ($P \leq 0.001$). Compared with the sitting posture, MABP and $P_{ET}CO_2$ were lower in the standing posture while CVR and HR were higher. Sitting and standing $P_{ET}CO_2$ was also collected in a smaller sample of participants including eleven healthy controls and fourteen concussed adolescents. Further, there was a main effect of posture on peak CBFV ($P \leq 0.001$) and mean CBFV ($P \leq 0.001$). Peak and mean CBFV were lower in the standing posture than in the sitting posture. There was a main effect of group on respiration ($P = 0.02$) with the concussed adolescents breathing at a higher respiration rate than the healthy controls. A posture x group interaction was detected for SV ($P \leq 0.001$) and CO ($P = 0.002$). Specifically, the concussed adolescents experienced a larger decrease in SV from the sitting posture to the standing posture. Furthermore, the concussed adolescents experienced a decrease in CO from the sitting posture to the standing posture while the healthy controls maintained CO at similar levels between the two postures. There was no effect of posture or group on TPR.

Table 4.2: Steady-state hemodynamic and respiratory parameters during sitting and standing postures.

	CTRL		CONC	
Sit-Stand Hemodynamic Parameters	Sitting	Standing	Sitting	Standing
MABP (mmHg) *	89 ± 13	87 ± 13	87 ± 13	84 ± 14
Peak CBFV (cm·s^{-1}) *	69 ± 14	65 ± 13	70 ± 14	66 ± 14
Mean CBFV (cm·s^{-1}) *	30 ± 4	29 ± 4	31 ± 4	30 ± 4
CVR (V) *	2.15 ± 1.01	2.18 ± 0.96	1.96 ± 0.87	2.01 ± 0.89
HR (bpm) *	72 ± 11	86 ± 11	76 ± 12	93 ± 16
SV (mL) * ‡	56 ± 17	48 ± 15	60 ± 19	47 ± 16
CO (L·min^{-1}) ‡	4.0 ± 1.2	4.0 ± 1.3	4.5 ± 1.4	4.3 ± 1.4
TPR (mmHg·L^{-1}·min^{-1})	24 ± 7	24 ± 8	21 ± 6	22 ± 8
Respiration (breaths·min^{-1}) †	16 ± 3	16 ± 3	18 ± 2	17 ± 2
PETCO$_2$ (mmHg) *	39 ± 2	38 ± 2	38 ± 3	36 ± 3

Values are mean ± SD. CTRL, control; CONC, concussed; MABP, mean arterial blood pressure; CBFV, cerebral blood flow velocity; CVR, cerebrovascular resistance; HR, heart rate; SV, stroke volume; CO, cardiac output; TPR, total peripheral resistance; PETCO$_2$, end tidal carbon dioxide partial pressure. * Main effect of posture, † Main effect of group, ‡ Posture x group interaction, $P \leq 0.05$.

In contrast to the steady-state data presented above, changes in hemodynamic parameters during the transition phase between sitting and standing are presented in Figure 4.1. With standing, MABP drops which elicits a fall in CBFV. The absolute drop in MABP was statistically similar between the healthy controls and the concussed adolescents (-24 ± 7 vs. -24 ± 8 mmHg; $P = 0.97$; Figure 4.1A). The healthy controls demonstrated an absolute decrease in peak CBFV of 19 ± 8 cm/s and the concussed adolescents an absolute decrease of 21 ± 4 cm/s ($P = 0.45$; Figure 4.1B). The absolute drop in mean CBFV was not statistically different between the healthy controls and the concussed adolescents (- 8 ± 3 vs. -8 ± 2 cm/s; $P = 0.95$). Furthermore, the fall in CBFV produces a reactive reduction in CVR that is also not statistically different between the healthy controls and the concussed adolescents (-0.5 ± 0.4 vs. -0.4 ± 0.3 mmHg·cm^{-1}·sec^{-1}; $P = 0.53$; Figure 4.1C). Initially with standing, a small decline in $P_{ET}CO_2$ occurs. The healthy controls demonstrated an absolute fall in $P_{ET}CO_2$ of 0.4 ± 0.6 mmHg and the concussed adolescents an absolute fall of 0.5 ± 1.0 mmHg ($P = 0.66$; Figure 4.1D). In addition, a drop in SV occurs with standing that was not significantly different between the healthy controls and the concussed adolescents (-7.9 ± 20.4 vs. -13.4 ± 15.0 mL; $P = 0.24$; Figure 4.1G). The decrease in SV was offset by a rapid increase in HR resulting in a marked initial increase in CO. The healthy controls HR increased by 26 ± 9 bpm and the concussed adolescents HR increased by 29 ± 7 bpm ($P = 0.18$; Figure 4.1F). The healthy controls exhibited an absolute increase in CO of 1.8 ± 0.7 L/min and the concussed adolescents' CO increased by 1.5 ± 1.4 L/min ($P = 0.34$; Figure 4.1E). Finally, a decrease in TPR occurred in the initial phase of standing that was not statistically different between the healthy controls and the concussed adolescents (-10.6 ± 4.6 vs. -9.2 ± 4.4 mmHg·L^{-1}·min^{-1}; $P = 0.27$; Figure 4.1H).

The time course of the initial changes in hemodynamic parameters with standing are presented in Figure 4.2. The healthy controls took 6.2 ± 1.3 seconds to reach nadir MABP and the concussed adolescents took 6.9 ± 1.1 seconds ($P = 0.02$; Figure 4.2A). The time to reach peak MABP was also longer in the concussed adolescents compared with the healthy controls (15.4 ± 2.6 vs. 14.1 ± 2.3 sec; $P = 0.02$; Figure 4.2B). Compared with the healthy controls, the concussed adolescents took longer to reach

CBFV nadir (4.6 ± 1.9 vs. 5.9 ± 1.4 sec; $P = 0.004$; Figure 4.2C). However, the time to reach peak CBFV was not statistically different between the healthy controls and the concussed adolescents (11.1 ± 1.9 vs. 12.0 ± 1.6 sec; $P = 0.07$; Figure 4.2D). The time course of the CVR response was not significantly different between the two groups. With respect to the time to the initiation of the CVR drop, the healthy controls took 2.5 ± 1.6 seconds and the concussed adolescents took 2.6 ± 1.7 seconds ($P = 0.74$; Figure 4.2E). The time to nadir CVR was 8.8 ± 1.7 seconds in the healthy controls and 9.6 ± 1.8 seconds in the concussed adolescents ($P = 0.08$; Figure 4.2F). Compared with the healthy controls, the concussed adolescents took longer to reach the peak HR (9.5 ± 1.9 vs. 10.3 ± 2.4 sec; $P = 0.04$; Figure 4.2G). Finally, the healthy controls and the concussed adolescents did not demonstrate a significant difference in the time to nadir TPR (5.2 ± 1.3 vs. 5.1 ± 1.4 sec; $P = 0.72$; Figure 4.2H).

Figure 4.1: Initial hemodynamic and respiratory changes with standing. Values are mean ± SD. CTRL, control; CONC, concussed; MABP, mean arterial blood pressure; CBFV, cerebral blood flow velocity; CVR, cerebral vascular resistance; PETCO2, end-tidal carbon dioxide partial pressure; CO, cardiac output; HR, heart rate; SV, stroke volume; TPR, total peripheral resistance. *Significantly different from CTRL, $P \leq 0.05$.

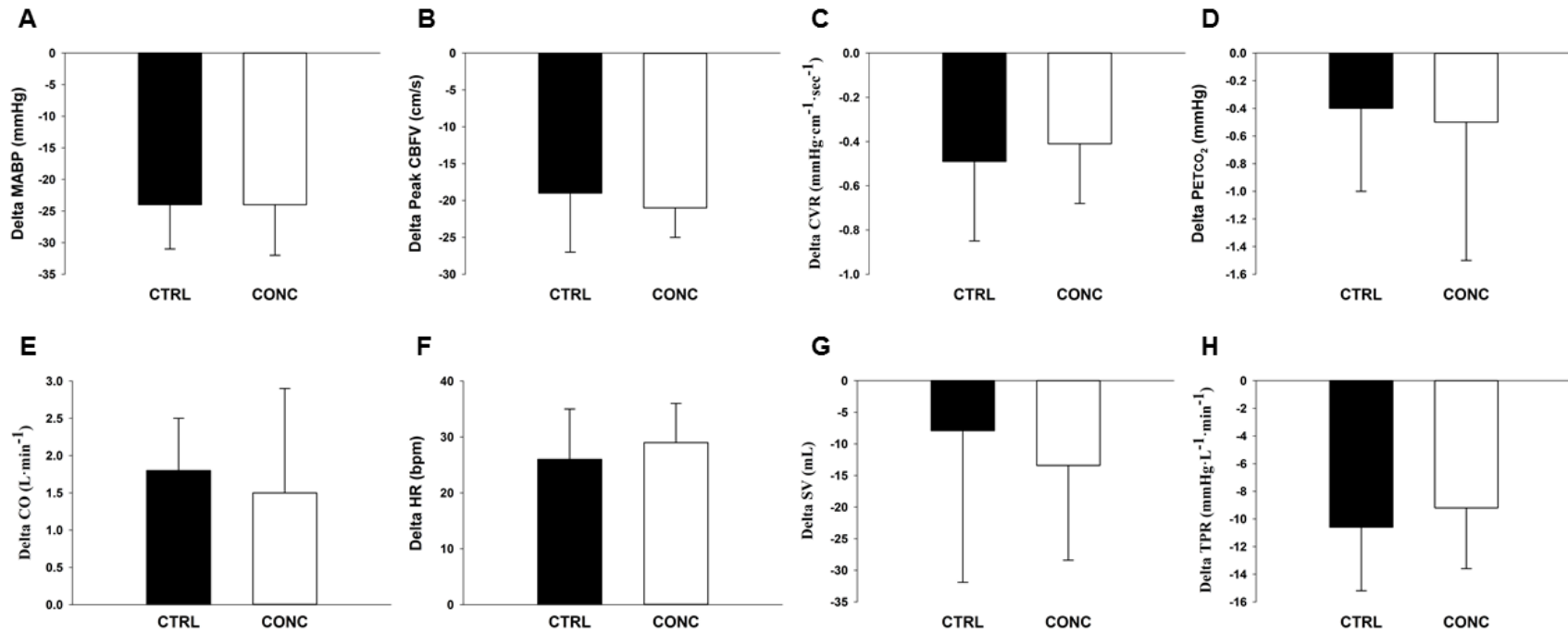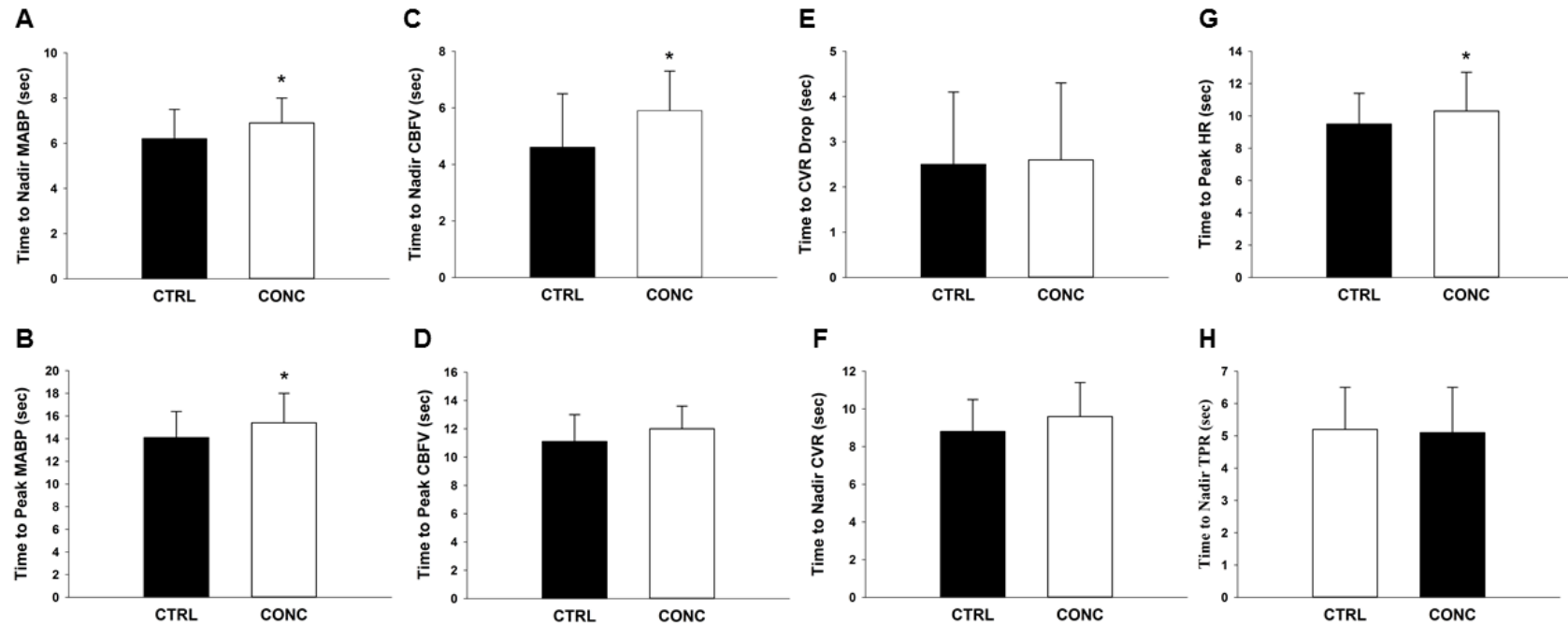**Figure 4.2:** Time course of hemodynamic responses with standing. Values are mean ± SD. CTRL, control; CONC, concussed; MABP, mean arterial blood pressure; CBFV, cerebral blood flow velocity; CVR, cerebral vascular resistance; HR, heart rate. *Significantly different from CTRL, $P \leq 0.05$.

4.3   Dynamic Cerebral Autoregulation

The values for RoR calculated from mean CBFV and peak CBFV were not significantly different for both the concussed adolescents (0.15 ± 0.04 vs. 0.17 ± 0.05 sec^{-1}; $P = 0.32$) and the healthy controls (0.21 ± 0.07 vs. 0.22 ± 0.07 sec^{-1}; $P = 0.62$). Therefore, we combined the RoR data from the two signal types. The healthy controls RoR was not statistically different between the first and second visits (0.23 ± 0.08 vs. 0.26 ± 0.13 sec^{-1}; $P = 0.43$). Following transient drops in MABP induced by postural adjustments, RoR was reduced in the concussed adolescents compared with the healthy controls (0.16 ± 0.04 vs. 0.21 ± 0.07 sec-1; $P \leq 0.001$; Figure 4.3). In fact, the concussed adolescents demonstrated a decrease in RoR of 24% from the healthy controls. When the concussed adolescents were grouped based on day post-injury, there was no difference in RoR between the acutely concussed and the adolescents with PCS (0.15 ± 0.04 vs. 0.18 ± 0.06, $P = 0.22$). Sex differences were not observed in the present investigation with concussed males exhibiting a RoR of 0.15 sec^{-1} and females exhibiting a RoR of 0.16 sec^{-1} ($P = 0.75$). Furthermore, a RoR of 0.22 sec^{-1} and 0.20 sec^{-1} were witnessed for healthy males and females, respectively ($P = 0.44$). Furthermore, analysis showed no correlation between age and RoR.

A previous history of concussions may influence values of RoR in a subsequent injury. The healthy controls could be grouped into pure controls who had never sustained a concussion and controls with a previous history of concussion. There was no difference in the autoregulatory response between the two groups with the pure controls exhibiting a RoR of 0.21 sec^{-1} and the previously concussed controls a RoR of 0.23 sec^{-1} ($P = 0.53$). The concussed adolescents could also be grouped into participants undergoing care for their first concussion and those with a previous history of concussions. Again, no differences were witnessed in dynamic CA with both groups demonstrating a RoR of 0.16 sec^{-1} ($P = 0.76$).

The current investigation followed the concussed adolescents over the course of their rehabilitation, for up to 12 weeks. Of the 30 concussed adolescents, 13 participants provided RoR data for the first and last visits. Furthermore, the participants were either

medically cleared by their last visit, or rated themselves 100% of baseline, or scored 2 or fewer symptoms on the SCAT3 symptom evaluation. At the time of the concussed adolescents last visit, RoR recovered in accordance with clinical symptoms. In fact, no significant differences were observed between the healthy controls and the concussed adolescents at their last visit (0.21 ± 0.07 vs. 0.21 ± 0.08 sec^{-1}; $P = 0.80$; Figure 4.3).
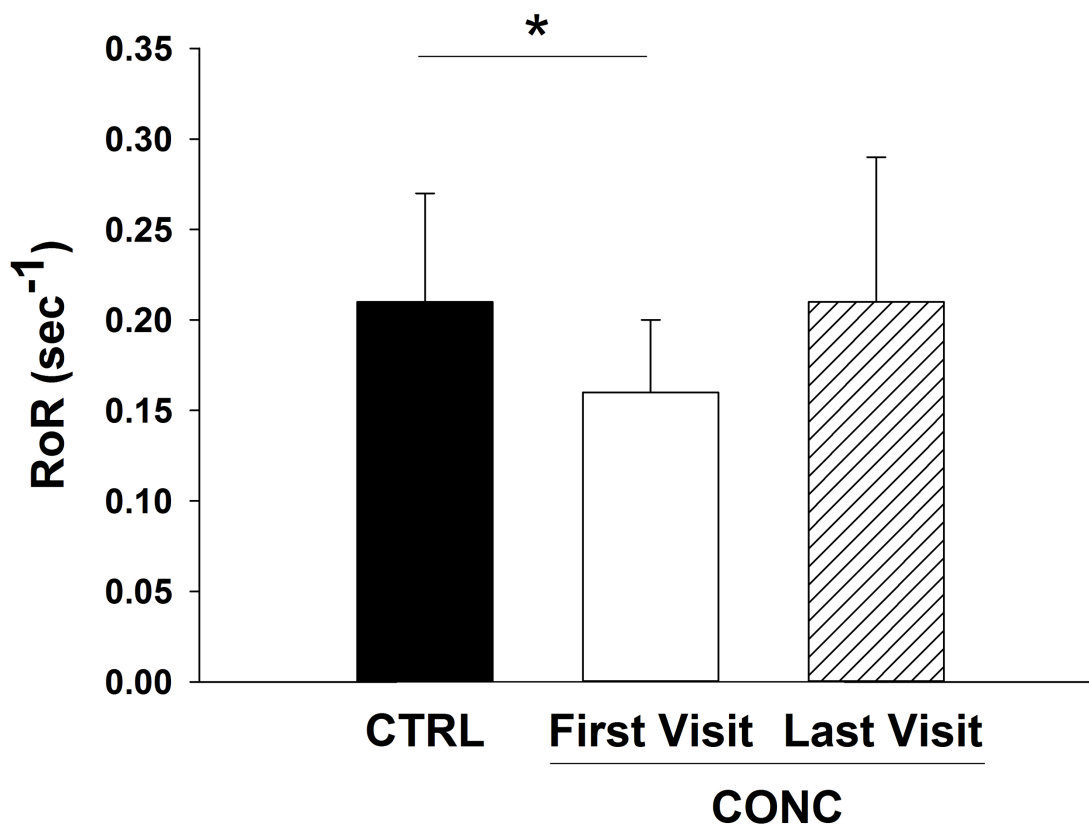
Figure 4.3: Rate of regulation during a sit-to-stand task. Values are mean ± SD. CTRL, control; CONC, concussed; RoR, rate of regulation. *P ≤ 0.025.

Chapter 5

5 Discussion

The main findings of the current investigation are as follows: (1) that adolescents diagnosed with a concussion demonstrate impaired dynamic CA, indicated by reduced RoR, and (2) the impairment in dynamic CA recovers in accordance with clinical symptoms. Therefore, the present data support the hypothesis that adolescent concussion is marked by impairments in dynamic CA.

The role of head injury on cerebrovascular control has been shown in experimental models of head-injury (Abdul-Muneer et al., 2013; DeWitt et al., 1992; Ellison, Erb, Kontos, & Povlishock, 1989; Hayward et al., 2011; Wei, Kontos, Dietrich, Povlishock, & Ellis, 1981) and in severely-head injured adults (Czosnyka et al., 2001; Lang et al., 2003; Lee et al., 2001) but its presence in concussion remains uncertain. Further, studies in humans are normally based on steady-state conditions such as average blood flow observed in the supine or seated position. Two previous investigations have evaluated static CA in children and adolescents over a range of TBI severity (Muizelaar et al., 1989; M. S. Vavilala et al., 2004). Using phenylephrine- or trimethaphan camsylate-induced changes in ABP both investigations found only variably impaired static CA in children and adolescents following TBI. However, the use of anesthesia, the variations in TBI severity, and the age range of participants complicate the interpretation of these studies. These differences, along with the use of static CA, limits comparison of the results from the presents study with previous investigations in head-injured adolescents.

In this study, we addressed the possibility that dynamic, rather than steady-state, cerebrovascular patterns may expose abnormal cerebrovascular control. Therefore, the present investigation was the first to describe dynamic CA impairments in adolescents with a concussion diagnosis. The emphasis on dynamic autoregulatory control in the current investigation was based on a previous speculation by Tiecks et al. (1995) who acknowledged that measures of dynamic responses may be of particular importance in

head-injury, suggesting that the latency of the autoregulatory response is impacted. The diminished RoR value in the current investigation confirms the speculation by Tiecks et al. (1995) and suggests that concussion, in some way, impairs the cerebrovascular response.

Many factors contribute to the cerebrovascular response, including the significant impact of $PaCO_2$ on vasomotor tone. Levels of $P_{ET}CO_2$ at baseline and their change during the sit-to-stand maneuver could have an effect on the RoR in the concussed adolescents as states of elevated $P_{ET}CO_2$, or hypercapnia, negatively impacts the RoR in healthy adults (Aaslid et al., 1989). Furthermore, impairments in cerebrovascular reactivity to alterations in $P_{ET}CO_2$ were reported following head-injury (Becelewski & Pierzchała, 2002; Enevoldsen & Jensen, 1978; Lee et al., 2001). In the current investigation, $P_{ET}CO_2$ levels were not significantly different between the concussed adolescents and the healthy controls in the supine, sitting, and standing postures. In addition, a decline in $P_{ET}CO_2$ was witnessed upon standing that was also witnessed in previous investigations employing a sit-to-stand model (Sorond et al., 2009). However, the decline was small and was not statistically different between the two groups. Therefore, it appears that the reduced RoR witnessed in the concussed adolescents could not be explained by $P_{ET}CO_2$ levels. Unfortunately, we were not able to study cerebrovascular reactivity to CO_2 in these patients due to potential symptom exacerbation with administration of a CO_2 gas mixture.

An additional factor that could influence the cerebrovascular response is the astrocyte-vascular interactions. Normally, astrocytes play a critical role in linking neuronal metabolic activity with the local vascular response (Iadecola, 2004). Recently, Augustine et al. (2014) used an endothelial cell-astrocyte co-culture to examine the effects of simulated blast injury on the coupling of these two tissues. Following the air blast, the astrocytic end-feet moved away from the vasculature which could compromise neurovascular coupling and lead to impaired vascular responses (Augustine, Cepinskas, Fraser, & Group, 2014). Recently, an investigation reported the involvement of astrocytes in pressure and flow evoked changes in vasomotor tone (Kim et al., 2015). This observation supports the findings from Augustine et al. (2014) that retraction of the

astrocytes may impair the cerebrovascular response particularly during changes in pressure and flow. However, relating the cell culture model employed in the study by Augustine et al. (2014) to human concussion must be considered carefully as concussions are a mild form of injury whereas the air blast to the cell culture may be more representative of severe forms of TBI. In fact, data from the present investigation suggests the cerebrovascular response was not compromised in adolescent concussion. With standing, the initial reduction in CVR was not statistically different between the concussed adolescents and the healthy controls. This observation opposes the investigation conducted by Bailey et al. (2014) which reported a larger relative reduction in CVR in the TBI patients compared with the healthy controls. One likely reason for this difference is that the current investigation evaluated primarily acutely concussed patients while the study by Bailey and colleagues (2013) evaluated chronic TBI patients who experience repetitive sub-concussive head-impacts. Thus, these two populations differ significantly in the severity of the injury and would likely exhibit varying levels of CA impairment. In fact, the current investigation showed a 24% reduction in RoR between the healthy controls and concussed adolescents while the boxers demonstrated a 48% reduction in RoR from the healthy controls. In addition, the time course of the changes in CVR in the present investigation further demonstrate intact vascular responses. The healthy controls and concussed adolescents showed no significant difference in the time to the initiation of the CVR drop or in the time to the nadir CVR suggesting the cerebrovascular response does not appear to be compromised at least in the concussion spectrum of disorders. Therefore, we must consider additional factors that could contribute to RoR including central hemodynamic responses.

Central hemodynamics could influence the RoR through variations in the components of the RoR calculation or in the rate of the underlying physiologic responses (Figure 5.1). In the current investigation, the initial absolute drop in MABP was statistically similar between the concussed adolescents and the healthy controls. This is consistent with a previous report employing the thigh-cuff model in TBI patients and healthy controls (Bailey et al., 2013). However, Jünger et al. (1997) found the MABP drop to be of smaller magnitude in mild TBI patients compared with the healthy controls. The inconsistent results between the current study and that of Jünger et al. (1997) may be

due to the use of the thigh-cuff model which results in highly variable drops in ABP with each trial (Sorond et al., 2009). The drop in MABP observed in the current investigation is similar to previous investigations using sit-to-stand models for the evaluation of dynamic CA (Lipsitz et al., 2000; Sorond et al., 2009). However, in assessing the time course of the MABP response in the present investigation, the concussed adolescents took longer, by approximately 10%, to reach nadir MABP when compared with the healthy controls. A group effect also emerged in the time course of the HR response with the concussed adolescents taking longer, by approximately 8%, to reach peak HR compared with the healthy controls. The HR response may be prolonged in the concussed adolescents due to dysregulation in autonomic function. The increase in HR with standing has been attributed to rapid vagal withdrawal (Ewing et al., 1980). In fact, it has been reported that concussion is marked by cardiac autonomic dysregulation, specifically measures of vagal activity (Gall, Parkhouse, & Goodman, 2004; Su, Kuo, Kuo, Lai, & Chen, 2005). In as much as the HR response influences the duration of the drop in MABP with standing, the impaired neural control of HR may translate into cerebrovascular dynamics. Therefore, the present observations suggest that sluggish central hemodynamics may contribute to the reduced RoR in the concussed adolescents. It is noted, however, that duration of the fall in CBFV with standing was approximately 28% longer in the concussed adolescents and does not appear to be accounted for completely by the slower central hemodynamics. Therefore, while these data point to the integrative nature of studying CBF control, more studies are needed to understand, for example, the interactions between the neural control of HR and cerebrovascular autoregulation.
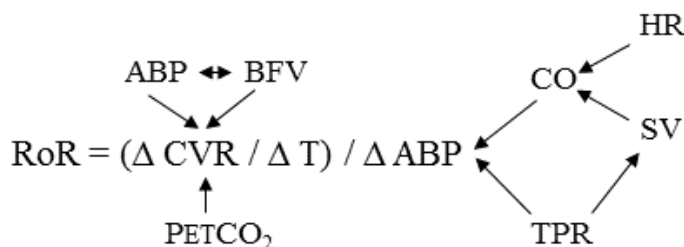

Figure 5.1: Schematic of the rate of regulation calculation and potential contributors to cerebral autoregulation.

As mentioned, the present investigation was the first to employ dynamic measures of CA in concussed adolescents. One previous investigation has studied dynamic CA in healthy adolescents using the thigh-cuff method and witnessed lower dynamic ARI values in the adolescents compared with the healthy adults (M. Vavilala et al., 2002). This finding was not replicated in a later study which demonstrated similar static ARI values between children and adults under general anesthesia (M. Vavilala et al., 2003). From these findings, it appears that age may play a role in dynamic CA while static CA is seemingly unaffected by age. However, observations from the present investigation provides evidence that RoR, analogous to dynamic ARI, is similar between adolescent and adult populations. The values observed for the healthy controls in the current investigation are in line with the values reported by Aaslid and colleagues (1989) in healthy adults. Specifically, the healthy adolescents in the current investigation demonstrated a RoR of 0.21 sec^{-1} whereas the healthy adults in the study by Aaslid and colleagues (1989) exhibited a RoR of 0.20 sec^{-1}. Furthermore, using the thigh-cuff method, Bailey et al. (2013) demonstrated absolute values of RoR in adults following head-injury that are almost identical with the concussed adolescents in the current investigation (0.18 vs. 0.16 sec-^{1}). Therefore, indices of autoregulation, both static and dynamic, are shown to be similar across a range of ages.

We must acknowledge that different models were utilized to induce a drop in ABP between the current investigation and the investigations conducted by Aaslid et al. (1989) and Bailey et al. (2013). In the previous investigations, the thigh-cuff model was employed to produce a rapid, transient drop in ABP (Aaslid et al., 1989; Bailey et al., 2013). However, due to the high number of trials that are required and the painful nature of the test (Sorond et al., 2009), it is not a suitable protocol to employ in a young population with concussion. Therefore, we opted to use a sit-to-stand model as it has provided similar results to the thigh-cuff model and is a more tolerable test (Sorond et al., 2009). Sorond and colleagues (2009) performed both the sit-to-stand and thigh-cuff deflation tests in a group of healthy participants. The two models produced similar absolute drops in ABP and CBFV although slower declines were seen in both parameters in the sit-to-stand model (Sorond et al., 2009). A slightly larger drop in $PETCO_2$ was witnessed in the sit-to-stand model (Sorond et al., 2009). However, at the time of CBFV

nadir, the drop in $P_{ET}CO_2$ was similar between the two models so it was proposed that CA would likely not differ significantly between the two models (Sorond et al., 2009). In fact, Sorond and colleagues (2009) found similar dynamic ARI values with both models. The sit-to-stand model was also associated with a higher between-subjects' variability but lower within-subject variability and a higher intraclass correlation coefficient (Sorond et al., 2009). Therefore, the sit-to-stand model is a reliable alternative to the thigh-cuff model.

The current investigation was also the first to describe the temporal resolution of dynamic CA throughout clinical recovery in concussed adolescents. A few studies have investigated temporal resolution of CA in adults suffering from moderate to severe TBI (Lang et al., 2003; Lee et al., 2001). These investigations witnessed an impairment in CA that began improving between four and six days' post-injury (Lang et al., 2003; Lee et al., 2001). Recovery has been shown to vary significantly between adults and adolescents limiting the comparison of the results from the present study to the investigations of temporal resolution in adults (Field et al., 2003; Lovell et al., 2003; Pellman et al., 2006). Nonetheless, the observation that the impairment in dynamic CA recovered with symptom improvement suggests that this measure reflects injury status.

5.1   Limitations

This investigation does involve various methodological considerations and limitations that must be acknowledged. First, the use of TCD only permits the estimation of CBF from measures of CBFV (Clark et al., 1996; Lindegaard et al., 1987; Newell et al., 1994; Valdueza et al., 1997). True quantification of CBF requires a measure of CBFV and vessel CSA (Kontos, 1989). However, previous investigations have provided evidence for stable vessel CSA in the face of altered ABP proving the validity of TCD to quantify CBF (Aaslid et al., 1989; Aaslid et al., 1991; Giller et al., 1993; Larsen et al., 1994; Lindegaard et al., 1987; Newell et al., 1994; Serrador et al., 2000).

We must also take into consideration the adolescent population studied in the current investigation and acknowledge the ongoing maturation that occurs in this population. Previous research has demonstrated alterations in cerebral hemodynamics

with age (Biagi et al., 2007; Hales, Kawadler, Aylett, Kirkham, & Clark, 2014; Leung, Kosinski, Croal, & Kassner, 2016). However, regression analysis showed no significant correlation between age and RoR in the present investigation. Furthermore, it has been previously reported that static ARI values are similar between children and adults (M. Vavilala et al., 2003) and the data from the current investigation support this finding with similar RoR values witnessed between the adolescents from the present study and adults from previous investigations (Aaslid et al., 1989; Bailey et al., 2013). Nonetheless, it is important to consider the factor of age and development in studies of cerebral autoregulation in the adolescent population.

5.2 Conclusion and Impact

This study was the first to demonstrate impaired dynamic CA in adolescents diagnosed with a concussion. This finding has significant impact on characterizing the physiological changes that occur with adolescent concussion. Specifically, it is of major importance to understand the cerebrovascular outcomes of concussion to help guide concussion rehabilitation and management. The present investigation also demonstrated that the impaired dynamic CA recovers in accordance with clinical symptoms. Therefore, RoR appears to be an objective marker that scales to the injury. Dynamic CA may provide a useful clinical tool that can ensure accurate diagnosis, guide rehabilitation, and aid in decisions regarding patient's readiness to return to sport.

References

Aaslid, R., Lindegaard, K.-F., Sorteberg, W., & Nornes, H. (1989). Cerebral autoregulation dynamics in humans. *Stroke, 20*(1), 45-52.

Aaslid, R., Markwalder, T.-M., & Nornes, H. (1982). Noninvasive transcranial Doppler ultrasound recording of flow velocity in basal cerebral arteries. *Journal of neurosurgery, 57*(6), 769-774.

Aaslid, R., Newell, D. W., Stooss, R., Sorteberg, W., & Lindegaard, K.-F. (1991). Assessment of cerebral autoregulation dynamics from simultaneous arterial and venous transcranial Doppler recordings in humans. *Stroke, 22*(9), 1148-1154.

Abdul-Muneer, P., Schuetz, H., Wang, F., Skotak, M., Jones, J., Gorantla, S., . . . Haorah, J. (2013). Induction of oxidative and nitrosative damage leads to cerebrovascular inflammation in an animal model of mild traumatic brain injury induced by primary blast. *Free Radical Biology and Medicine, 60*, 282-291.

Adelson, P. D., Clyde, B., Kochanek, P., Wisniewski, S., Marion, D., & Yonas, H. (1997). Cerebrovascular response in infants and young children following severe traumatic brain injury: a preliminary report. *Pediatric neurosurgery, 26*(4), 200-207.

Ainslie, P. N. (2009). Have a safe night: intimate protection against cerebral hyperperfusion during REM sleep. *Journal of applied physiology, 106*(4), 1031-1033.

Apkon, M., Weed, R. A., & Boron, W. (1997). Motor responses of cultured rat cerebral vascular smooth muscle cells to intra-and extracellular pH changes. *American Journal of Physiology-Heart and Circulatory Physiology, 273*(1), H434-H445.

Augustine, C., Cepinskas, G., Fraser, D. D., & Group, C. C. C. T. B. (2014). Traumatic injury elicits JNK-mediated human astrocyte retraction in vitro. *Neuroscience, 274*, 1-10.

Bailey, D. M., Jones, D. W., Sinnott, A., Brugniaux, J. V., New, K. J., Hodson, D., . . . Ainslie, P. N. (2013). Impaired cerebral haemodynamic function associated with chronic traumatic brain injury in professional boxers. *Clinical Science, 124*(3), 177-189.

Bazarian, J. J., Blyth, B., & Cimpello, L. (2006). Bench to bedside: evidence for brain injury after concussion—looking beyond the computed tomography scan. *Academic Emergency Medicine, 13*(2), 199-214.

Becelewski, J., & Pierzchała, K. (2002). Cerebrovascular reactivity in patients with mild head injury. *Neurologia i neurochirurgia polska, 37*(2), 339-350.

Bhardwaj, A., Northington, F. J., Carhuapoma, J. R., Falck, J. R., Harder, D. R., Traystman, R. J., & Koehler, R. C. (2000). P-450 epoxygenase and NO synthase inhibitors reduce cerebral blood flow response toN-methyl-d-aspartate. *American Journal of Physiology-Heart and Circulatory Physiology, 279*(4), H1616-H1624.

Biagi, L., Abbruzzese, A., Bianchi, M. C., Alsop, D. C., Del Guerra, A., & Tosetti, M. (2007). Age dependence of cerebral perfusion assessed by magnetic resonance continuous arterial spin labeling. *Journal of Magnetic Resonance Imaging, 25*(4), 696-702.

Bishop, C., Powell, S., & Rutt, D. (1986). Transcranial Doppler measurement of middle cerebral artery blood flow velocity: a validation study. *Stroke, 17*(5), 913-915.

Bleys, R. L., Cowen, T., Groen, G. J., Hillen, B., & Ibrahim, N. B. (1996). Perivascular nerves of the human basal cerebral arteries: I. Topographical distribution. *Journal of Cerebral Blood Flow & Metabolism, 16*(5), 1034-1047.

Brugniaux, J. V., Hodges, A. N., Hanly, P. J., & Poulin, M. J. (2007). Cerebrovascular responses to altitude. *Respiratory physiology & neurobiology, 158*(2), 212-223.

Cassaglia, P. A., Griffiths, R. I., & Walker, A. M. (2008a). Sympathetic nerve activity in the superior cervical ganglia increases in response to imposed increases in arterial pressure. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 294*(4), R1255-R1261.

Cassaglia, P. A., Griffiths, R. I., & Walker, A. M. (2008b). Sympathetic withdrawal augments cerebral blood flow during acute hypercapnia in sleeping lambs. *Sleep, 31*(12), 1729-1734.

Cassaglia, P. A., Griffiths, R. I., & Walker, A. M. (2009). Cerebral sympathetic nerve activity has a major regulatory role in the cerebral circulation in REM sleep. *Journal of applied physiology, 106*(4), 1050-1056.

Choe, M. C., Babikian, T., DiFiori, J., Hovda, D. A., & Giza, C. C. (2012). A pediatric perspective on concussion pathophysiology. *Current opinion in pediatrics, 24*(6), 689-695.

Ciallella, J. R., Ikonomovic, M. D., Paljug, W. R., Wilbur, Y. I., Dixon, C. E., Kochanek, P. M., . . . DeKosky, S. T. (2002). Changes in expression of amyloid precursor protein and interleukin-1β after experimental traumatic brain injury in rats. *Journal of neurotrauma, 19*(12), 1555-1567.

Clark, J. M., Skolnick, B. E., Gelfand, R., Farber, R. E., Stierheim, M., Stevens, W. C., . . . Lambertsen, C. J. (1996). Relationship of 133Xe cerebral blood flow to middle cerebral arterial flow velocity in men at rest. *Journal of Cerebral Blood Flow & Metabolism, 16*(6), 1255-1262.

Clifford, P. S. (2011). Local control of blood flow. *Advances in physiology education, 35*(1), 5-15.

Cohen, Z., Bonvento, G., Lacombe, P., & Hamel, E. (1996). Serotonin in the regulation of brain microcirculation. *Progress in neurobiology, 50*(4), 335-362.

Coverdale, N. S., Gati, J. S., Opalevych, O., Perrotta, A., & Shoemaker, J. K. (2014). Cerebral blood flow velocity underestimates cerebral blood flow during modest hypercapnia and hypocapnia. *Journal of applied physiology, 117*(10), 1090-1096.

Czosnyka, M., & Pickard, J. D. (2004). Monitoring and interpretation of intracranial pressure. *Journal of Neurology, Neurosurgery & Psychiatry, 75*(6), 813-821.

Czosnyka, M., Smielewski, P., Piechnik, S., Steiner, L. A., & Pickard, J. D. (2001). Cerebral autoregulation following head injury. *Journal of neurosurgery, 95*(5), 756-763.

Dabertrand, F., Nelson, M. T., & Brayden, J. E. (2012). Acidosis Dilates Brain Parenchymal Arterioles by Conversion of Calcium Waves to Sparks to Activate BK ChannelsNovelty and Significance. *Circulation Research, 110*(2), 285-294.

Dahl, A., Russell, D., Nyberg-Hansen, R., & Rootwelt, K. (1989). Effect of nitroglycerin on cerebral circulation measured by transcranial Doppler and SPECT. *Stroke, 20*(12), 1733-1736.

Daneshvar, D. H., Nowinski, C. J., McKee, A. C., & Cantu, R. C. (2011). The epidemiology of sport-related concussion. *Clinics in sports medicine, 30*(1), 1-17.

DeWitt, D. S., Prough, D. S., Taylor, C. L., Whitley, J. M., Deal, D. D., & Vines, S. M. (1992). Regional cerebrovascular responses to progressive hypotension after traumatic brain injury in cats. *American Journal of Physiology-Heart and Circulatory Physiology, 263*(4), H1276-H1284.

Edwards, M. R., Shoemaker, J. K., & Hughson, R. L. (2002). Dynamic modulation of cerebrovascular resistance as an index of autoregulation under tilt and controlled Pet CO2. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 283*(3), R653-R662.

Ellison, M. D., Erb, D. E., Kontos, H. A., & Povlishock, J. T. (1989). Recovery of impaired endothelium-dependent relaxation after fluid-percussion brain injury in cats. *Stroke, 20*(7), 911-917.

Enevoldsen, E. M., & Jensen, F. T. (1978). Autoregulation and CO2 responses of cerebral blood flow in patients with acute severe head injury. *Journal of neurosurgery, 48*(5), 689-703.

Ewing, D., Hume, L., Campbell, I., Murray, A., Neilson, J., & Clarke, B. (1980). Autonomic mechanisms in the initial heart rate response to standing. *Journal of applied physiology, 49*(5), 809-814.

Faraci, F. M., & Breese, K. R. (1993). Nitric oxide mediates vasodilatation in response to activation of N-methyl-D-aspartate receptors in brain. *Circulation Research, 72*(2), 476-480.

Faraci, F. M., & Brian, J. (1994). Nitric oxide and the cerebral circulation. *Stroke, 25*(3), 692-703.

Faraci, F. M., & Heistad, D. D. (1990). Regulation of large cerebral arteries and cerebral microvascular pressure. *Circulation Research, 66*(1), 8-17.

Faraci, F. M., & Heistad, D. D. (1998). Regulation of the cerebral circulation: role of endothelium and potassium channels. *Physiological reviews, 78*(1), 53-97.

Field, M., Collins, M. W., Lovell, M. R., & Maroon, J. (2003). Does age play a role in recovery from sports-related concussion? A comparison of high school and collegiate athletes. *The Journal of pediatrics, 142*(5), 546-553.

Gall, B., Parkhouse, W., & Goodman, D. (2004). Heart rate variability of recently concussed athletes at rest and exercise. *Medicine and science in sports and exercise, 36*, 1269-1274.

Gibbs, F. A., Maxwell, H., & Gibbs, E. (1947). Volume flow of blood through the human brain. *Archives of Neurology & Psychiatry, 57*(2), 137-144.

Giller, C. A., Bowman, G., Dyer, H., Mootz, L., & Krippner, W. (1993). Cerebral arterial diameters during changes in blood pressure and carbon dioxide during craniotomy. *Neurosurgery, 32*(5), 737-742.

Girouard, H., & Iadecola, C. (2006). Neurovascular coupling in the normal brain and in hypertension, stroke, and Alzheimer disease. *Journal of applied physiology, 100*(1), 328-335.

Gotoh, J., Kuang, T.-Y., Nakao, Y., Cohen, D. M., Melzer, P., Itoh, Y., . . . Sokoloff, L. (2001). Regional differences in mechanisms of cerebral circulatory response to neuronal activation. *American Journal of Physiology-Heart and Circulatory Physiology, 280*(2), H821-H829.

Grady, M. F. (2010). Concussion in the adolescent athlete. *Current problems in pediatric and adolescent health care, 40*(7), 154-169.

Hales, P. W., Kawadler, J. M., Aylett, S. E., Kirkham, F. J., & Clark, C. A. (2014). Arterial spin labeling characterization of cerebral perfusion during normal maturation from late childhood into adulthood: normal 'reference range' values and their use in clinical studies. *Journal of Cerebral Blood Flow & Metabolism, 34*(5), 776-784.

Hamel, E. (2006). Perivascular nerves and the regulation of cerebrovascular tone. *Journal of applied physiology, 100*(3), 1059-1064.

Harmel, M., Hafkenschiel, J., Austin, G., Crumpton, C., & Kety, S. (1949). The effect of bilateral stellate ganglion block on the cerebral circulation in normotensive and hypertensive patients. *Journal of Clinical Investigation, 28*(3), 415.

Harper, A. M., & Bell, R. A. (1963). The effect of metabolic acidosis and alkalosis on the blood flow through the cerebral cortex. *Journal of Neurology, Neurosurgery & Psychiatry, 26*(4), 341-344.

Hayashi, T., Katsumi, Y., Mukai, T., Inoue, M., Nagahama, Y., Oyanagi, C., . . . Fukuyama, H. (2002). Neuronal nitric oxide has a role as a perfusion regulator and a synaptic modulator in cerebellum but not in neocortex during somatosensory stimulation—an animal PET study. *Neuroscience research, 44*(2), 155-165.

Hayward, N. M. E. A., Tuunanen, P. I., Immonen, R., Ndode-Ekane, X. E., Pitkänen, A., & Gröhn, O. (2011). Magnetic resonance imaging of regional hemodynamic and cerebrovascular recovery after lateral fluid-percussion brain injury in rats. *Journal of Cerebral Blood Flow & Metabolism, 31*(1), 166-177.

Hughson, R. L., Edwards, M. R., O'Leary, D. D., & Shoemaker, J. K. (2001). Critical analysis of cerebrovascular autoregulation during repeated head-up tilt. *Stroke, 32*(10), 2403-2408.

Iadecola, C. (1993). Regulation of the cerebral microcirculation during neural activity: is nitric oxide the missing link? *Trends in neurosciences, 16*(6), 206-214.

Iadecola, C. (2004). Neurovascular regulation in the normal brain and in Alzheimer's disease. *Nature Reviews Neuroscience, 5*(5), 347-360.

Ide, K., Boushel, R., Sørensen, H., Fernandes, A., Cai, Y., Pott, F., & Secher, N. (2000). Middle cerebral artery blood velocity during exercise with beta-1 adrenergic and unilateral stellate ganglion blockade in humans. *Acta physiologica Scandinavica, 170*(1), 33-38.

Ide, K., Worthley, M., Anderson, T., & Poulin, M. J. (2007). Effects of the nitric oxide synthase inhibitor L-NMMA on cerebrovascular and cardiovascular responses to hypoxia and hypercapnia in humans. *The Journal of physiology, 584*(1), 321-332.

Iliff, J. J., D'Ambrosio, R., Ngai, A. C., & Winn, H. R. (2003). Adenosine receptors mediate glutamate-evoked arteriolar dilation in the rat cerebral cortex. *American Journal of Physiology-Heart and Circulatory Physiology, 53*(5), H1631.

Ingvar, D., Cronqvist, S., Ekberg, R., Risberg, J., & Høedt-Rasmussen, K. (1965). Normal values of regional cerebral blood flow in man, including flow and weight estimates of gray and white matter. *Acta Neurologica Scandinavica, 41*(S14), 72-78.

Iqbal, S. (2013). A comprehensive study of the anatomical variations of the circle of Willis in adult human brains. *Journal of clinical and diagnostic research: JCDR, 7*(11), 2423.

Itoh, T., Satou, T., Nishida, S., Tsubaki, M., Hashimoto, S., & Ito, H. (2009). Expression of amyloid precursor protein after rat traumatic brain injury. *Neurological research, 31*(1), 103-109.

Jain, V., Langham, M. C., & Wehrli, F. W. (2010). MRI estimation of global brain oxygen consumption rate. *Journal of Cerebral Blood Flow & Metabolism, 30*(9), 1598-1607.

Jeng, J.-S., Yip, P.-K., Huang, S.-J., & Kao, M.-C. (1999). Changes in hemodynamics of the carotid and middle cerebral arteries before and after endoscopic sympathectomy in patients with palmar hyperhidrosis: preliminary results. *Journal of neurosurgery, 90*(3), 463-467.

Jobsis, F. F. (1977). Noninvasive, infrared monitoring of cerebral and myocardial oxygen sufficiency and circulatory parameters. *Science, 198*(4323), 1264-1267.

Jünger, E. C., Newell, D. W., Grant, G. A., Avellino, A. M., Ghatan, S., Douville, C. M., . . . Winn, H. R. (1997). Cerebral autoregulation following minor head injury. *Journal of neurosurgery, 86*(3), 425-432.

Kalaria, R. N. (1996). Cerebral vessels in ageing and Alzheimer's disease. *Pharmacology & therapeutics, 72*(3), 193-214.

Kang, C.-K., Oh, S.-T., Chung, R. K., Lee, H., Park, C.-A., Kim, Y.-B., . . . Cho, Z.-H. (2010). Effect of Stellate Ganglion Block on the Cerebrovascular SystemMagnetic Resonance Angiography Study. *The Journal of the American Society of Anesthesiologists, 113*(4), 936-944.

Karlin, A. M. (2011). Concussion in the pediatric and adolescent population:"different population, different concerns". *PM&R, 3*(10), S369-S379.

Kety, S. S., & Schmidt, C. F. (1945). The determination of cerebral blood flow in man by the use of nitrous oxide in low concentrations. *American Journal of Physiology--Legacy Content, 143*(1), 53-66.

Kim, K. J., Iddings, J. A., Stern, J. E., Blanco, V. M., Croom, D., Kirov, S. A., & Filosa, J. A. (2015). Astrocyte contributions to flow/pressure-evoked parenchymal arteriole vasoconstriction. *Journal of Neuroscience, 35*(21), 8245-8257.

Kimmerly, D., Tutungi, E., Wilson, T., Serrador, J., Gelb, A., Hughson, R., & Shoemaker, J. (2003). Circulating norepinephrine and cerebrovascular control in conscious humans. *Clinical physiology and functional imaging, 23*(6), 314-319.

Kitazono, T., Faraci, F. M., Taguchi, H., & Heistad, D. D. (1995). Role of potassium channels in cerebral blood vessels. *Stroke, 26*(9), 1713-1723.

Koehler, R. C., & Traystman, R. J. (1982). Bicarbonate ion modulation of cerebral blood flow during hypoxia and hypercapnia. *American Journal of Physiology-Heart and Circulatory Physiology, 243*(1), H33-H40.

Kontos, H. A. (1989). Validity of cerebral arterial blood flow calculations from velocity measurements. *Stroke, 20*(1), 1-3.

Kontos, H. A., Raper, A. J., & Patterson, J. L. (1977). Analysis of vasoactivity of local pH, Pco2 and bicarbonate on pial vessels. *Stroke, 8*(3), 358-360.

Kontos, H. A., Wei, E. P., Raper, A., & Patterson, J. L. (1977). Local mechanism of CO2 action of cat pial arterioles. *Stroke, 8*(2), 226-229.

Lambertsen, C., Semple, S., Smyth, M., & Gelfand, R. (1961). H+ and pCO2 as chemical factors in respiratory and cerebral circulatory control. *Journal of applied physiology, 16*(3), 473-484.

Lang, E. W., Yip, K., Griffith, J., Lagopoulos, J., Mudaliar, Y., & Dorsch, N. W. (2003). Hemispheric asymmetry and temporal profiles of cerebral pressure autoregulation in head injury. *Journal of clinical neuroscience, 10*(6), 670-673.

Larsen, F. S., Olsen, K. S., Hansen, B. A., Paulson, O. B., & Knudsen, G. M. (1994). Transcranial Doppler is valid for determination of the lower limit of cerebral blood flow autoregulation. *Stroke, 25*(10), 1985-1988.

Lassen, N. (1968). Brain extracellular pH: the main factor controlling cerebral blood flow: Taylor & Francis.

Lassen, N. A. (1959). Cerebral blood flow and oxygen consumption in man. *Physiological reviews, 39*(2), 183-238.

Lavi, S., Egbarya, R., Lavi, R., & Jacob, G. (2003). Role of nitric oxide in the regulation of cerebral blood flow in humans. *Circulation, 107*(14), 1901-1905.

Lee, J. H., Kelly, D. F., Oertel, M., McArthur, D. L., Glenn, T. C., Vespa, P., . . . Martin, N. A. (2001). Carbon dioxide reactivity, pressure autoregulation, and metabolic suppression reactivity after head injury: a transcranial Doppler study. *Journal of neurosurgery, 95*(2), 222-232.

Len, T., & Neary, J. (2011). Cerebrovascular pathophysiology following mild traumatic brain injury. *Clinical physiology and functional imaging, 31*(2), 85-93.

Leung, J., Kosinski, P. D., Croal, P. L., & Kassner, A. (2016). Developmental trajectories of cerebrovascular reactivity in healthy children and young adults assessed with magnetic resonance imaging. *The Journal of physiology*.

Lindauer, U., Kunz, A., Schuh-Hofer, S., Vogt, J., Dreier, J. P., & Dirnagl, U. (2001). Nitric oxide from perivascular nerves modulates cerebral arterial pH reactivity. *American Journal of Physiology-Heart and Circulatory Physiology, 281*(3), H1353-H1363.

Lindauer, U., Vogt, J., Schuh-Hofer, S., Dreier, J. P., & Dirnagl, U. (2003). Cerebrovascular vasodilation to extraluminal acidosis occurs via combined activation of ATP-sensitive and Ca2+-activated potassium channels. *Journal of Cerebral Blood Flow & Metabolism, 23*(10), 1227-1238.

Lindegaard, K.-F., Lundar, T., Wiberg, J., Sjøberg, D., Aaslid, R., & Nornes, H. (1987). Variations in middle cerebral artery blood flow investigated with noninvasive transcranial blood velocity measurements. *Stroke, 18*(6), 1025-1030.

Lipsitz, L. A., Mukai, S., Hamner, J., Gagnon, M., & Babikian, V. (2000). Dynamic regulation of middle cerebral artery blood flow velocity in aging and hypertension. *Stroke, 31*(8), 1897-1903.

Lovell, M. R., Collins, M. W., Iverson, G. L., Field, M., Maroon, J. C., Cantu, R., . . . Fu, F. H. (2003). Recovery from mild concussion in high school athletes. *Journal of neurosurgery, 98*(2), 296-301.

Madsen, P. L., Sperling, B. K., Warming, T., Schmidt, J., Secher, N., Wildschiodtz, G., . . . Lassen, N. (1993). Middle cerebral artery blood velocity and cerebral blood flow and O2 uptake during dynamic exercise. *Journal of applied physiology, 74*(1), 245-250.

Maugans, T. A., Farley, C., Altaye, M., Leach, J., & Cecil, K. M. (2012). Pediatric sports-related concussion produces cerebral blood flow alterations. *Pediatrics, 129*(1), 28-37.

Mayhan, W. G., Werber, A. H., & Heistad, D. D. (1987). Protection of cerebral vessels by sympathetic nerves and vascular hypertrophy. *Circulation, 75*(1 Pt 2), I107-112.

McCrory, P., Meeuwisse, W. H., Aubry, M., Cantu, B., Dvořák, J., Echemendia, R. J., . . . Raftery, M. (2013). Consensus statement on concussion in sport: the 4th International Conference on Concussion in Sport held in Zurich, November 2012. *British journal of sports medicine, 47*(5), 250-258.

Meng, L., & Gelb, A. W. (2015). Regulation of cerebral autoregulation by carbon dioxide. *The Journal of the American Society of Anesthesiologists, 122*(1), 196-205.

Meyer, J. S., Shimazu, K., Fukuuchi, Y., Ohuchi, T., Okamoto, S., Koto, A., & Ericsson, A. D. (1973). Impaired neurogenic cerebrovascular control and dysautoregulation after stroke. *Stroke, 4*(2), 169-186.

Miyazaki, M., & Kato, K. (1965). Measurement of cerebral blood flow by ultrasonic Doppler technique: theory. *Japanese Circulation Journal, 29*(4), 375-382.

Muizelaar, J. P., Marmarou, A., DeSalles, A. A., Ward, J. D., Zimmerman, R. S., Li, Z., . . . Young, H. F. (1989). Cerebral blood flow and metabolism in severely head-injured children: Part 1: Relationship with GCS score, outcome, ICP, and PVI. *Journal of neurosurgery, 71*(1), 63-71.

Murkin, J., & Arango, M. (2009). Near-infrared spectroscopy as an index of brain and tissue oxygenation. *British journal of anaesthesia, 103*(suppl 1), i3-i13.

Nedergaard, M., Ransom, B., & Goldman, S. A. (2003). New roles for astrocytes: redefining the functional architecture of the brain. *Trends in neurosciences, 26*(10), 523-530.

Newell, D. W., Aaslid, R., Lam, A., Mayberg, T. S., & Winn, H. R. (1994). Comparison of flow and velocity during dynamic autoregulation testing in humans. *Stroke, 25*(4), 793-797.

Nylin, G., & Blömer, H. (1955). Studies on distribution of cerebral blood flow with thorium B-labeled erythrocytes. *Circulation Research, 3*(1), 79-85.

Obrist, W. D., Langfitt, T. W., Jaggi, J. L., Cruz, J., & Gennarelli, T. A. (1984). Cerebral blood flow and metabolism in comatose patients with acute head injury: relationship to intracranial hypertension. *Journal of neurosurgery, 61*(2), 241-253.

Obrist, W. D., Thompson, H. K., King, H., & Wang, H. S. (1967). Determination of regional cerebral blood flow by inhalation of 133-Xenon. *Circulation Research, 20*(1), 124-135.

Ohta, S., Hadeishi, H., & Suzuki, M. (1990). Effect of stellate ganglion block on cerebral blood flow in normoxemic and hyperoxemic states. *Journal of neurosurgical anesthesiology, 2*(4), 272-279.

Overgaard, J., & Tweed, W. A. (1974). Cerebral circulation after head injury: part 1: cerebral blood flow and its regulation after closed head injury with emphasis on clinical correlations. *Journal of neurosurgery, 41*(5), 531-541.

Panerai, R., Deverson, S., Mahony, P., Hayes, P., & Evans, D. (1999). Effect of CO2 on dynamic cerebral autoregulation measurement. *Physiological measurement, 20*(3), 265.

Panerai, R. B. (1998). Assessment of cerebral pressure autoregulation in humans-a review of measurement methods. *Physiological measurement, 19*(3), 305.

Panerai, R. B. (2008). Cerebral autoregulation: from models to clinical applications. *Cardiovascular Engineering, 8*(1), 42-59.

Panerai, R. B. (2009). Transcranial Doppler for evaluation of cerebral autoregulation. *Clinical autonomic research, 19*(4), 197-211.

Panerai, R. B., Dawson, S. L., Eames, P. J., & Potter, J. F. (2001). Cerebral blood flow velocity response to induced and spontaneous sudden changes in arterial blood pressure. *American Journal of Physiology-Heart and Circulatory Physiology, 280*(5), H2162-H2174.

Paulson, O. B., & Newman, E. A. (1987). Does the release of potassium from astrocyte endfeet regulate cerebral blood flow? *Science (New York, NY), 237*(4817), 896.

Pellman, E. J., Lovell, M. R., Viano, D. C., & Casson, I. R. (2006). Concussion in professional football: recovery of NFL and high school athletes assessed by computerized neuropsychological testing—part 12. *Neurosurgery, 58*(2), 263-274.

Peng, H. L., Ivarsen, A., Nilsson, H., & Aalkjær, C. (1998). On the cellular mechanism for the effect of acidosis on vascular tone. *Acta Physiologica, 164*(4), 517-525.

Purcell, L. K. (2014). Sport-related concussion: Evaluation and management. *Paediatrics & child health, 19*(3), 1.

Rangel-Castilla, L., Gasco, J., Nauta, H. J., Okonkwo, D. O., & Robertson, C. S. (2008). Cerebral pressure autoregulation in traumatic brain injury. *Neurosurgical focus, 25*(4), E7.

Roy, C. S., & Sherrington, C. S. (1890). On the regulation of the blood-supply of the brain. *The Journal of physiology, 11*(1-2), 85.

Scheinberg, P. (1950). Cerebral blood flow in vascular disease of the brain: With observations on the effects of stellate ganglion block. *The American journal of medicine, 8*(2), 139-147.

Schmetterer, L., Findl, O., Strenn, K., Graselli, U., Kastner, J., Eichler, H.-G., & Wolzt, M. (1997). Role of NO in the O2 and CO2 responsiveness of cerebral and ocular circulation in humans. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 273*(6), R2005-R2012.

Serrador, J. M., Picot, P. A., Rutt, B. K., Shoemaker, J. K., & Bondar, R. L. (2000). MRI measures of middle cerebral artery diameter in conscious humans during simulated orthostasis. *Stroke, 31*(7), 1672-1678.

Shenkin, H. A. (1969). Cervical sympathectomy on patients with occlusive cerebrovascular disease. *Archives of Surgery, 98*(3), 317-320.

Shenkin, H. A., Cabieses, F., & Van Den Noordt, G. (1951). The effect of bilateral stellectomy upon the cerebral circulation of man. *Journal of Clinical Investigation, 30*(1), 90.

Signoretti, S., Lazzarino, G., Tavazzi, B., & Vagnozzi, R. (2011). The pathophysiology of concussion. *PM&R, 3*(10), S359-S368.

Sorond, F. A., Khavari, R., Serrador, J. M., & Lipsitz, L. A. (2005). Regional cerebral autoregulation during orthostatic stress: age-related differences. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 60*(11), 1484-1487.

Sorond, F. A., Serrador, J. M., Jones, R. N., Shaffer, M. L., & Lipsitz, L. A. (2009). The sit-to-stand technique for the measurement of dynamic cerebral autoregulation. *Ultrasound in medicine & biology, 35*(1), 21-29.

Sorteberg, W., Lindegaard, K.-F., Rootwelt, K., Dahl, A., Russell, D., Nyberg-Hansen, R., & Nornes, H. (1989). Blood velocity and regional blood flow in defined cerebral artery systems. *Acta neurochirurgica, 97*(1-2), 47-52.

Stewart, T. C., Gilliland, J., & Fraser, D. D. (2014). An epidemiologic profile of pediatric concussions: identifying urban and rural differences. *Journal of trauma and acute care surgery, 76*(3), 736-742.

Su, C.-F., Kuo, T. B., Kuo, J.-S., Lai, H.-Y., & Chen, H. I. (2005). Sympathetic and parasympathetic activities evaluated by heart-rate variability in head injury of various severities. *Clinical Neurophysiology, 116*(6), 1273-1279.

Tan, C. O. (2012). Defining the characteristic relationship between arterial pressure and cerebral flow. *Journal of applied physiology, 113*(8), 1194-1200.

Tan, C. O., Hamner, J., & Taylor, J. A. (2013). The role of myogenic mechanisms in human cerebrovascular regulation. *The Journal of physiology, 591*(20), 5095-5105.

Ter Minassian, A., Melon, E., Leguerinel, C., Lodi, C. A., Bonnet, F., & Beydon, L. (1998). Changes in cerebral blood flow during PaCO2 variations in patients with severe closed head injury: comparison between the Fick and transcranial Doppler methods. *Journal of neurosurgery, 88*(6), 996-1001.

Tiecks, F. P., Lam, A. M., Aaslid, R., & Newell, D. W. (1995). Comparison of static and dynamic cerebral autoregulation measurements. *Stroke, 26*(6), 1014-1019.

Treggiari, M. M., Romand, J.-A., Martin, J.-B., Reverdin, A., Rüfenacht, D. A., & De Tribolet, N. (2003). Cervical sympathetic block to reverse delayed ischemic neurological deficits after aneurysmal subarachnoid hemorrhage. *Stroke, 34*(4), 961-967.

Tzeng, Y.-C., & Ainslie, P. N. (2014). Blood pressure regulation IX: cerebral autoregulation under blood pressure challenges. *European journal of applied physiology, 114*(3), 545-559.

Tzeng, Y.-C., Willie, C. K., Atkinson, G., Lucas, S. J., Wong, A., & Ainslie, P. N. (2010). Cerebrovascular regulation during transient hypotension and hypertension in humans. *Hypertension, 56*(2), 268-273.

Tzeng, Y. C., Chan, G. S., Willie, C. K., & Ainslie, P. N. (2011). Determinants of human cerebral pressure–flow velocity relationships: new insights from vascular modelling and Ca2+ channel blockade. *The Journal of physiology, 589*(13), 3263-3274.

Udomphorn, Y., Armstead, W. M., & Vavilala, M. S. (2008). Cerebral blood flow and autoregulation after pediatric traumatic brain injury. *Pediatric neurology, 38*(4), 225-234.

Umeyama, T., Kugimiya, T., Ogawa, T., Kandori, Y., Ishizuka, A., & Hanaoka, K. (1995). Changes in cerebral blood flow estimated after stellate ganglion block by single photon emission computed tomography. *Journal of the autonomic nervous system, 50*(3), 339-346.

Ursino, M., & Lodi, C. A. (1998). Interaction among autoregulation, CO2 reactivity, and intracranial pressure: a mathematical model. *American Journal of Physiology-Heart and Circulatory Physiology, 274*(5), H1715-H1728.

Valdueza, J. M., Balzer, J. O., Villringer, A., Vogl, T. J., Kutter, R., & Einhäupl, K. M. (1997). Changes in blood flow velocity and diameter of the middle cerebral artery during hyperventilation: assessment with MR and transcranial Doppler sonography. *American Journal of Neuroradiology, 18*(10), 1929-1934.

Van Beek, A. H., Claassen, J. A., Rikkert, M. G. O., & Jansen, R. W. (2008). Cerebral autoregulation: an overview of current concepts and methodology with special focus on the elderly. *Journal of Cerebral Blood Flow & Metabolism, 28*(6), 1071-1085.

Vavilala, M., Lee, L., Lee, M., Graham, A., Visco, E., & Lam, A. (2003). Cerebral autoregulation in children during sevoflurane anaesthesia. *British journal of anaesthesia, 90*(5), 636-641.

Vavilala, M., Newell, D., Junger, E., Douville, C., Aaslid, R., Rivara, F., & Lam, A. (2002). Dynamic cerebral autoregulation in healthy adolescents. *Acta anaesthesiologica scandinavica, 46*(4), 393-397.

Vavilala, M. S., Lee, L. A., Boddu, K., Visco, E., Newell, D. W., Zimmerman, J. J., & Lam, A. M. (2004). Cerebral autoregulation in pediatric traumatic brain injury. *Pediatric Critical Care Medicine, 5*(3), 257-263.

Vrselja, Z., Brkic, H., Mrdenovic, S., Radic, R., & Curic, G. (2014). Function of circle of Willis. *Journal of Cerebral Blood Flow & Metabolism, 34*(4), 578-584.

Wahl, M., Deetjen, P., Thurau, K., Ingvar, D., & Lassen, N. (1970). Micropuncture evaluation of the importance of perivascular pH for the arteriolar diameter on the brain surface. *Pflügers Archiv European Journal of Physiology, 316*(2), 152-163.

Wang, Q., Paulson, O. B., & Lassen, N. A. (1992). Effect of nitric oxide blockade by N G-nitro-l-arginine on cerebral blood flow response to changes in carbon dioxide tension. *Journal of Cerebral Blood Flow & Metabolism, 12*(6), 947-953.

Wei, E. P., Kontos, H. A., Dietrich, W. D., Povlishock, J. T., & Ellis, E. F. (1981). Inhibition by free radical scavengers and by cyclooxygenase inhibitors of pial arteriolar abnormalities from concussive brain injury in cats. *Circulation Research, 48*(1), 95-103.

White, R., Vallance, P., & Markus, H. (2000). Effect of inhibition of nitric oxide synthase on dynamic cerebral autoregulation in humans. *Clinical Science, 99*(6), 555-560.

Willer, B., & Leddy, J. J. (2006). Management of concussion and post-concussion syndrome. *Current Treatment Options in Neurology, 8*(5), 415-426.

Willie, C., Colino, F., Bailey, D., Tzeng, Y., Binsted, G., Jones, L., . . . Smith, K. (2011). Utility of transcranial Doppler ultrasound for the integrative assessment of cerebrovascular function. *Journal of Neuroscience Methods, 196*(2), 221-237.

Willie, C. K., Tzeng, Y. C., Fisher, J. A., & Ainslie, P. N. (2014). Integrative regulation of human brain blood flow. *The Journal of physiology, 592*(5), 841-859.

Wyatt, J., Cope, M., Delpy, D., Richardson, C., Edwards, A., Wray, S., & Reynolds, E. (1990). Quantitation of cerebral blood volume in human infants by near-infrared spectroscopy. *Journal of applied physiology, 68*(3), 1086-1091.

Xiong, Y., Mahmood, A., & Chopp, M. (2013). Animal models of traumatic brain injury. *Nature Reviews Neuroscience, 14*(2), 128-142.

Yang, G., Chen, G., Ebner, T. J., & Iadecola, C. (1999). Nitric oxide is the predominant mediator of cerebellar hyperemia during somatosensory activation in rats. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 277*(6), R1760-R1770.

Yang, G., Zhang, Y., Ross, M. E., & Iadecola, C. (2003). Attenuation of activity-induced increases in cerebellar blood flow in mice lacking neuronal nitric oxide synthase. *American Journal of Physiology-Heart and Circulatory Physiology, 285*(1), H298-H304.

Yokoyama, K., Kishida, T., & Sugiyama, K. (2004). Stellate ganglion block and regional cerebral blood volume and oxygenation. *Canadian Journal of Anesthesia/Journal canadien d'anesthésie, 51*(5), 515-516.

Zauner, A., & Muizelaar, J. P. (1997). Brain metabolism and cerebral blood flow. *Head Injury. Pathophysiology and Management of Severe Closed Injury. Chapman & Hall Medical, London*, 92.

Zhang, R., Zuckerman, J. H., Iwasaki, K., Wilson, T. E., Crandall, C. G., & Levine, B. D. (2002). Autonomic neural control of dynamic cerebral autoregulation in humans. *Circulation, 106*(14), 1814-1820.

Zonta, M., Angulo, M. C., Gobbo, S., Rosengarten, B., Hossmann, K.-A., Pozzan, T., & Carmignoto, G. (2003). Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. *Nature neuroscience, 6*(1), 43-50.

Appendices

Appendix A: Ethics Approval.

Western Research

Western University Health Science Research Ethics Board
HSREB Full Board Initial Approval Notice

Principal Investigator: Dr. Kevin Shoemaker
Department & Institution: Health Sciences\Kinesiology,Western University

HSREB File Number: 105937
Study Title: Investigating novel treatments for concussion: Impact of compression vest on rehabilitation outcomes.
Sponsor: Children's Health Foundation

HSREB Initial Approval Date: December 22, 2014
HSREB Expiry Date: December 22, 2015

Documents Approved and/or Received for Information:

Document Name	Comments	Version Date
Instruments	Vestibular-Occulor Motor Screening instrument	
Instruments	Anxiety questionnaire	
Other	Study Design Model	2014/10/16
Instruments	Compression Garment Patient Survey	2014/10/16
Advertisement		2014/11/24
Assent	Updated Children Assent	2014/12/16
Revised Western University Protocol	105937_Clean_Revisions_Dec16	2014/12/16
Revised Letter of Information & Consent	LOI for control participants_Clean	2014/12/16
Revised Letter of Information & Consent	LOI for concussed participants_Clean	2014/12/16

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review. If an Updated Approval Notice is required prior to the HSREB Expiry Date, the Principal Investigator is responsible for completing and submitting an HSREB Updated Approval Form in a timely fashion.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice Practices (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Ethics Officer, on behalf of Dr. Joseph Gilbert, HSREB Chair

Ethics Officer to Contact for Further Information

Erika Basile	___ Grace Kelly	___Mina Mekhail	___Vikki Tran

This is an official document. Please retain the original in your files.

Western Research

Western University Health Science Research Ethics Board
HSREB Amendment Approval Notice

Principal Investigator: Dr. Kevin Shoemaker
Department & Institution: Health Sciences\Kinesiology,Western University

Review Type: Full Board
HSREB File Number: 105937
Study Title: Investigating novel treatments for concussion: Impact of compression vest on rehabilitation outcomes.
Sponsor: Children's Health Foundation

HSREB Amendment Approval Date: July 13, 2016
HSREB Expiry Date: December 22, 2016

Documents Approved and/or Received for Information:

Document Name	Comments	Version Date
Data Collection Form/Case Report Form	Sleep Questionnaire (Received 8Jun16)	
Revised Letter of Information & Consent	Control LOI Clean	2016/05/01
Recruitment Items	Concussed Poster Clean	2016/05/01
Recruitment Items	Control Poster Clean	2016/05/01
Revised Assent	Child Assent Clean	2016/05/01
Revised Letter of Information & Consent	Concussed Letter of Information - Clean	2016/05/01
Revised Western University Protocol	Received 8Jun16	

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the amendment to the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice Practices (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Ethics Officer, on behalf of Dr. Joseph Gilbert, HSREB Chair

Ethics Officer: Erika Basile ___ Katelyn Harris ___ Nicole Kaniki ___ Grace Kelly___ Vikki Tran ___ Karen Gopaul ___

Western Research

Western University Health Science Research Ethics Board
HSREB Amendment Approval Notice

Principal Investigator: Dr. Kevin Shoemaker
Department & Institution: Health Sciences\Kinesiology, Western University

Review Type: Full Board
HSREB File Number: 105937
Study Title: Investigating novel treatments for concussion: Impact of compression vest on rehabilitation outcomes.
Sponsor: Children's Health Foundation

HSREB Amendment Approval Date: February 06, 2017
HSREB Expiry Date: December 22, 2017

Documents Approved and/or Received for Information:

Document Name	Comments	Version Date
Revised Letter of Information & Consent	LOI - intervention to 12 weeks	2017/01/20
Advertisement	Poster	2017/01/20
Revised Western University Protocol	Protocol - intervention to 12 weeks	2017/01/20
Letter of Information & Consent	LOI - Control to 12 weeks	2017/01/20
Revised Assent	Assent	2017/01/20
Advertisement	Poster Concussed	2017/01/20

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the amendment to the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice Practices (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Ethics Officer, on behalf of Dr. Joseph Gilbert, HSREB Chair

EO: Erika Basile ___ Nicole Kaniki ___ Grace Kelly ___ Katelyn Harris___ Nicola Morphet ___ Karen Gopaul ___

Western Research

Western University Health Science Research Ethics Board
HSREB Full Board Initial Approval Notice

Principal Investigator: Dr. Kevin Shoemaker
Department & Institution: Health Sciences\Kinesiology, Western University

Review Type: Full Board
HSREB File Number: 107619
Study Title: Cerebrovascular and autonomic function outcomes in adolescents diagnosed with a concussion
Sponsor: Children's Health Foundation

HSREB Initial Approval Date: March 23, 2016
HSREB Expiry Date: March 23, 2017

Documents Approved and/or Received for Information:

Document Name	Comments	Version Date
Data Collection Form/Case Report Form	Generalized Anxiety Disorder Questionnaire (Received 06Jan16)	
Data Collection Form/Case Report Form	Visual Analog Anxiety Scale (Received 06Jan16)	
Data Collection Form/Case Report Form	Sport Concussion Assessment Tool - 3 (Received 06Jan16)	
Data Collection Form/Case Report Form	Tanner Scale of Development (Received 06Jan16)	
Recruitment Items	Email script to be sent to local sport organizations/teams (Control Recruitment) (Received 06Jan16)	
Advertisement	Control_Poster_Clean	2016/02/17
Advertisement	Concussed_Poster_Clean	2016/02/17
Assent	Children Assent_Concussed_Clean	2016/02/12
Assent	Children Assent_Control_Clean	2016/02/12
Western University Protocol	Western_Protocol_Clean (Received 17Feb16)	
Letter of Information & Consent	Control	2016/03/22
Letter of Information & Consent	Concussed	2016/03/22

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice Practices (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Ethics Officer, on behalf of Dr. Marcelo Kremenchutzky, HSREB Vice Chair

Ethics Officer to Contact for Further Information: Erika Basile ___ Nicole Kaniki ___ Grace Kelly ___ Katelyn Harris___ Vikki Tran

This is an official document. Please retain a copy for your files.

Western Research

Western University Health Science Research Ethics Board
HSREB Amendment Approval Notice

Principal Investigator: Dr. Kevin Shoemaker
Department & Institution: Health Sciences\Kinesiology, Western University

Review Type: Full Board
HSREB File Number: 107619
Study Title: Cerebrovascular and autonomic function outcomes in adolescents diagnosed with a concussion
Sponsor: Children's Health Foundation

HSREB Amendment Approval Date: January 30, 2017
HSREB Expiry Date: March 23, 2017

Documents Approved and/or Received for Information:

Document Name	Comments	Version Date
Revised Assent	Assent Concussed	2017/01/20
Revised Letter of Information & Consent	Concussed LOI	2017/01/20
Advertisement	Concussed Poster	2017/01/20
Revised Assent	Control Assent	2017/01/20
Revised Letter of Information & Consent	Control LOI	2017/01/20
Advertisement	Control Poster	2017/01/20
Revised Western University Protocol	Western Protocol	2017/01/20

The Western University Health Science Research Ethics Board (HSREB) has reviewed and approved the amendment to the above named study, as of the HSREB Initial Approval Date noted above.

HSREB approval for this study remains valid until the HSREB Expiry Date noted above, conditional to timely submission and acceptance of HSREB Continuing Ethics Review.

The Western University HSREB operates in compliance with the Tri-Council Policy Statement Ethical Conduct for Research Involving Humans (TCPS2), the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use Guideline for Good Clinical Practice Practices (ICH E6 R1), the Ontario Personal Health Information Protection Act (PHIPA, 2004), Part 4 of the Natural Health Product Regulations, Health Canada Medical Device Regulations and Part C, Division 5, of the Food and Drug Regulations of Health Canada.

Members of the HSREB who are named as Investigators in research studies do not participate in discussions related to, nor vote on such studies when they are presented to the REB.

The HSREB is registered with the U.S. Department of Health & Human Services under the IRB registration number IRB 00000940.

Ethics Officer, on behalf of Dr. Joseph Gilbert, HSREB Chair

EO: Erika Basile ___ Nicole Kaniki ___ Grace Kelly ___ Katelyn Harris___ Nicola Morphet ✓ Karen Gopaul ___

Appendix B: Letter of Information and Consent Forms.

STUDY TITLE: Investigating novel treatments for concussion: Impact of compression vest on rehabilitation outcomes.

The Principal Investigators: Kevin Shoemaker, Ph.D.

Co-Investigators: Dr. Douglas Fraser, MD, PhD, FRCPC
　　　　　　　　Lisa Fischer, BScPT, MD, CCFP
　　　　　　　　Tim Doherty, MD
　　　　　　　　Jeff Holmes, Ph.D.
　　　　　　　　Shannon McGuire, PT
　　　　　　　　Arlene Fleischhauer, R.N.
　　　　　　　　Kolten Abbott, MSc
　　　　　　　　Erin Moir, BA

Funding Source:
The Children's Health Foundation

Introduction:
The pronouns "you" and "your" in the letter should be read as referring to the participant rather than the legal representative who may be signing the consent form. If you are a substitute decision maker (i.e. someone who makes the decision about participation on behalf of a study participant) please remember that "you" refers to the study participant.

You are being asked to voluntarily take part in a research study. We are asking you or your substitute decision maker to give your consent (sign and date) separately to each of the following three procedures. Before you decide, read all the details about the purpose, procedures, risks, benefits, discomforts and precautions of this study. You should take as much time as you need to make your decision. This form may contain words or details that you do not understand. Please ask the study staff to explain anything that you do not understand and make sure that all of your questions are answered. Feel free to talk about this study with anyone you wish. If all of your questions have been answered to your satisfaction, and you decide to take part of this study, you will be asked to sign the consent form and upon your request you will receive a copy of this letter of information and consent form to keep.

Background and Purpose:
The purposes of this research are 1) to determine the impact of wearing a compression vest (a tight fitting vest or shirt) on the symptoms related to your concussion, 2) to see how symptoms of your concussion relate to the control of your heart rate, and 3) to see if physical exercise improves your concussion. To complete the study we will be recruiting approximately 120 concussed patients and 85 non-concussed age-matched, healthy individuals.

Inclusion: To be included in the study you will be 12-40 years of age with a **medical diagnosis of, and being treated for, a concussion** with symptoms that have lasted less than one year. This study will also be completed by 85 individuals with no concussion (healthy, age-matched controls) to quantify age specific normative values. Adolescents (aged 12-18 yrs) and adults (aged 19-40 years) will be studied as separate groups.

Exclusion: You will not be included in the study if you have any of the following concerns:
- Bone or muscle problems that could impact balance or how well you walk,
- Diagnosis of <u>pre-existing</u> heart disease,
- Medications that affect heart or blood vessel control
- Pre-existing brain disorders such as Parkinson's, Multiple sclerosis, Raynaud's, Multiple System Atrophy, metabolic disorders such as diabetes, a history of significant neck injury, disease or surgery, Rheumatoid arthritis, vertebral basilar artery insufficiency, or focal neurologic deficit
- Primary or metastatic bone tumor,
- Severe osteoporosis,
- If you are, or think you might be, pregnant or are breastfeeding
- You are not able to understand English,

Procedures:

Outline of the study:
You will be tested once a week, over a 6 week time period for a total of 6 testing sessions. Testing sessions 1 and 2 will occur before your rehabilitation begins. The 3rd, 4th 5th and 6th test session will occur over 4 weeks of rehabilitation, unless you are asymptomatic and discharged from the clinic. See Figure 1 for a flow diagram of the study.

Testing session 1: Upon diagnosis with a concussion, you will be invited to participate in this study. If you consent to participate you will be randomized to either the compression vest or no compression vest condition and will complete a series of clinical and research tests (balance, anxiety/depression questionnaire, cardiovascular, exercise tolerance and cognitive evaluations).

Testing session 2: You will be required to come back to the clinic 1-2 weeks after testing session #1. The same clinical and research tests conducted during testing session 1, will be conducted throughout testing session 2. If on day #1 you were randomized to the compression vest group you will wear the vest again. If you were randomized to the no vest group, you will not wear a compression vest. If the physician determines that you are asymptomatic then you will exit the study. However, if you are symptomatic and willing to continue with the study, you will begin the 4-week rehabilitation program. For the 4-week rehabilitation program you will be randomized into one of three groups:
1) <u>Exercise + Vest:</u> Exercise three times per week with one session occurring during your physiotherapy clinic visit and the two other sessions on your own at home. You will be instructed about how hard you are to exercise and when to wear the

compression vest. If your symptoms become worse during any exercise session you are to stop that session and rest. You are asked to keep a record of your exercise and any symptoms or emotions you feel. A log book will be provided.

2) <u>Exercise - No Vest:</u> Exercise three times per week with one session occurring during your physiotherapy clinic visit and the two other sessions on your own at home. You will be instructed about how hard you are to exercise. If your symptoms become worse during any exercise session you are to stop that session and rest. You are asked to keep a record of your exercise and any symptoms you feel. A log book will be provided.

3) <u>Rehabilitation Control</u>: This is the current clinical management protocol. You will be provided 4 physiotherapy appointments (one per week) but you will not be completing a prescribed exercise program. You are asked to keep a record of your exercise activities outside of the clinic appointment as well as any symptoms you feel. A logbook will be provided.

Testing Session 3-5: You will come to the clinic for your physiotherapy appointment as well as complete a series of clinical and research tests (balance, anxiety/depression questionnaire, cardiovascular and cognitive evaluations, exercise tolerance test). Testing should take ~60 minutes in addition to your physiotherapy appointment.

Testing Session 6: You will come to the clinic to complete a series of clinical and research tests (balance, anxiety/depression questionnaire, cardiovascular and cognitive evaluations, and exercise tolerance test). Testing should take ~60-80 minutes. You will then exit the study.

*At all testing sessions, you will be asked to give up to 1-15 ml venous blood sample.
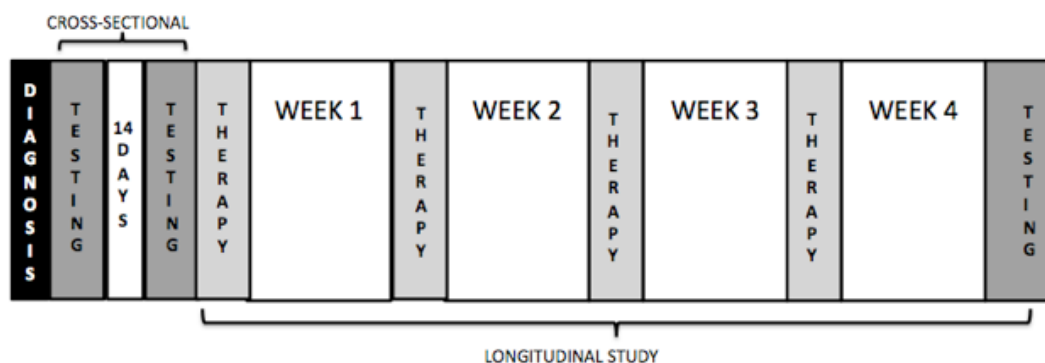
Study Flow Diagram

Detailed Procedures:
NOTE: the tests outlined below will take place at either the Fowler Kennedy Sports Medicine Clinic (adolescent and adult groups) or the Parkwood Hospital outpatient rehabilitation clinic (adults only), depending on where your treatment is being performed.

By signing the consent form, you are giving your permission for the research team to obtain your complete medical history and imaging, and neuropsychological tests from other hospitals, clinics and imaging facilities.

Concussion Questionnaires and Clinical Data: You will be asked to fill out a brief questionnaire about your levels of anxiety or stress.

Exercise Tolerance Test: Briefly, you will complete an endurance exercise protocol, either on a treadmill or cycle ergometer. Initially the workload will be very low, but will progressively increase each ensuing minute until: 1) you become too exhausted to continue 2) your symptoms become too strong to continue 3) maximal speed or incline on the treadmill/cycle ergometer is achieved. Every minute your heart rate (HR), symptoms (visual analog scale 1-10) and rating of perceived exertion (20 point Borg scale) will be evaluated. If the maximum incline is achieved before symptom onset or before you become too fatigued to continue then the speed will be increased by 0.4 mph each minute until you can no longer continue. HR and RPE at the cessation of exercise) will be documented and used to determine your individualized exercise program. The exercise tolerance test will be conducted up to 6 times during your enrollment in this investigation (one exercise tolerance test per visit).

Brain Blood Flow Study: Using a head strap, a small probe that emits ultrasound waves (transcranial Doppler ultrasound) will be held against your head just behind your eye. This probe will assess changes in blood flow to your brain. Finger blood pressure and the electrocardiogram will also be measured. Measures will be made while you sit quietly on a chair and then while you move between the seated and upright positions (unassisted standing and sitting) in two-minute increments (3 minutes seated, 2 minutes quiet standing). This will be repeated up to five times and stopped if symptoms get worse. These studies will be performed in Neurovascular Research Laboratory, Room 3110 Thames Hall (an elevator ride and ~20 metre walk from the Fowler Kennedy Sports Medicine Clinic).

Balance Testing:
Patients will have their balance assessed using one or more of the following tests:
1) Modified Clinical Sensory of Integration and Balance Test: This test involves measuring your balance with your eyes open and closed while standing on either a firm surface or on a foam surface. You may be asked to stand on two feet, on one foot. The tasks involved will take up to 10 minutes to complete.
2) "HUR" balance assessment: This test requires you to maintain your balance while completing a series of maze and star-like patterns on the HUR platform. The tasks involved will take up to 10 minutes to complete.

Vestibular Oculomotor Study: This test is to see how your eyes affect your balance. This is a standard physical therapy test called the "Vestibular Ocular Motor Screen" (VOMS). If you are receiving treatment at Parkwood Hospital, you may also be asked to participate in another standard procedure to improve your vision, called the "Binocular Vision Assessment" (BVA) (approximately 20 minutes)

Neurofunction: In addition to the normal clinical diagnostic questionnaires (e.g., SCAT3), you are asked to complete the Generalized Anxiety Disorder Questionnaire 7 (GAD-7) and the Cognigram. The Cognigram is an online cognitive task, which evaluates different aspects of cognition. The test consists of 4 different playing-card tasks, in which you are instructed to answer as quickly and accurately as possible. Before testing, one of the research assistants will provide you with instructions. The tasks involved will take up to 10 minutes to complete.

Heart Rate and Blood Pressure Monitoring: During your standard treatment your heart rate (the electrocardiogram), finger blood pressure (Finometer device) and breathing patterns (a loose strap around your chest) will be measured. These will be made during 5-10 min of rest and also during each of your therapist's treatments when possible such as during a neck treatment or balance testing, as well as during the sit-and-stand test, and exercise.

Compression Vest: In some cases, the measurements and tests outlined above will performed while you wear a compression vest around your chest. In some cases, this vest will include sensors that can measure your electrocardiogram (from your heart), how you breathe, and how you move. You may be asked to wear this vest at home along with instructions about how to record the data using the small device attached to the vest. You will then return the shirt and data device on your next clinic visit. See Figure 2 below.

Compression Vest © Parkwood Hospital

Exercise Training: You are asked to perform endurance exercise (cycling, running) for 20 minutes at each session. The intensity of this exercise will be determined first by your therapist but you will be able to adjust the intensity as needed to a level just below that which causes your symptoms to increase, or as directed by your therapist. You will perform this exercise once in the clinic at your weekly appointment, and then two more times during the week at home. You will be requested to maintain a brief log book documenting this exercise, additional activities and how you feel (anxiety, fatigue level etc.) throughout each day. You will receive instructions on how to exercise at home.

Sleep Analysis: The Groningen Sleep Quality Scale (GSQS) will be included in your exercise training log book (described above under *exercise training*). While enrolled in the study you will be asked to rate the quality of 2-3 sleeps per week. The GSQS consists of 15 true/false questions and should take no longer than 5 minutes to complete.

Blood Analysis: We would like to take 1-15ml blood sample from you at each of the 6 proposed days of clinical assessment (diagnosis, 2-week, 3-week, 4-week, 5-week and 6-week post diagnosis). Blood samples will be drawn by either physicians at Fowler Kennedy or by members of the research team who certified in phlebotomy. You will be sitting comfortably in a chair with an armrest for the specimen collection. Prior to the blood drawn you will be thoroughly briefed on procedure. Furthermore you will be provided blankets, pillows, food and drink if in the event you react negatively.

Risks/Discomforts/Inconvenience:
Questionnaires: There are no known risks with the questionnaires. In the event of exacerbated post-concussion symptoms, all testing will be stopped immediately and resumed again only after a minimum period of 24 hours of rest until your symptoms return to normal.

Exercise Tolerance Test: The end of the exercise test will be determined by when your symptoms are more than 3 points above baseline symptom scores (symptoms rated on a 0-10 visual analog scale). If they do not get worse, the test will stop when you are too tired to continue. The test may make you tired for a few hours. There is a 1/1000 - 1/10,000 chance of major complications such as heart attack, myocardial ischemia, stroke or death during vigorous exercise. Being older or having a pre-existing heart condition can increase the risk. You will be watched by a medical physician or your physical therapist and the test will be stopped immediately when you report a change in your concussion symptoms or you feel too tired to continue, whichever comes first.

Electrocardiogram: The adhesive on the electrodes used to measure your heart rate may cause a small rash to develop under the electrode. However, this rash should disappear in a day or two.

Blood pressure cuff: There are no known risks of using the finger cuff methods (Finometer) of examining arterial blood pressure. With the finger cuff the fingertip may turn a little blue and feel numb during the prolonged test sessions but this resolves immediately when the cuff is removed. Standard arm cuff blood pressure measures of arterial pressure will also be obtained periodically, a method that has no known risks.

Brain Blood Flow: There are no known risks associated with transcranial Doppler ultrasound. There is a small risk that you may experience a temporary loss of balance. A personal support staff member will minimize this risk.

Balance: These tests are part of normal clinical treatment, and will be performed in the clinical setting providing immediate medical support and knowledgeable testing environment. There is a small risk that you may experience a temporary loss of balance

during the completion of tasks used to assess their balance and gait. This risk is anticipated to be no greater than that which the patients would normally experience performing routine activities over the course of a day. Hand rails and/or a personal support staff member will minimize this risk. Tests will be stopped if concussion-related symptoms are exacerbated.

Cognitive Function Analysis: There are no known risks associated with the Cognigram evaluation. However, if the computer screen exacerbates your symptoms you will be instructed to stop testing immediately.

Blood Analysis: There is risk of infection or cross-contamination when venous blood samples are drawn. Therefore, either the physician, registered nurse of trained phlebotomist, will employ sterile blood handling techniques and take into account all preventive measures to ensure you are not exposed to any additional risks.

Your Rights:
Participation in this study is voluntary. You may refuse to participate, refuse to answer any questions or withdraw from the study at any time with no consequences.

Benefits to Being in the Study:
You may or may not receive any direct benefit from being in this study. If you sustain a concussion during the study period, you will receive direct referral to Fowler-Kennedy Sports Medicine Clinical for assessment. Information learned from this study may help other people with concussions in the future.

Withdrawal:
You can withdraw from any or all parts of this study at any time with no consequences.

Questions:
If you have any questions or concerns about the study, you can contact Dr. Kevin Shoemaker

If you have any questions about your rights as a research participant or have concerns about this study, you may contact Dr. David Hill, Scientific Director, Lawson Health Research Institute, at

Conflict of Interest:
The Children's Health Foundation represents the funding source of this study. The Clinics and Institutions involved in this study all have an interest in completing this study. Their interests should not influence your decision to participate in this study. You should not feel pressured to join this study.

Confidentiality:
If you agree to join this study, your medical information collected during the study will be kept confidential by the study staff and will not be made available unless disclosure is required by law. Following collection, all information collected during this study will be

assigned a number and then entered into a secure electronic database at the institution, along with the subject identification code assigned to each subject. Therefore, the data cannot be linked to you. The data collected will be available to principal investigator and researchers involved in the study, whereas encrypted personal identification (name, date of birth) will be restricted to only to small number of authorized research staff. You will not be named in any reports, publications, or presentations that may come from this study. If you decide to leave the study, the information about you that was collected before you left the study will still be used. No new information will be collected without your permission.

Please be aware that Representatives of the Western University Health Sciences Research Ethics Board and/or Lawson Quality Assurance Education Program may contact you or may require access to your study related records to monitor the conduct of the research.

Compensation:
If you are in the Adolescent age category and treated at Fowler Kennedy Sports Medicine Centre, up to four physiotherapy rehabilitation visits will be provided free of charge. Otherwise, you will receive no compensation for your participation in any parts of this research except for reimbursement of the parking up to a maximum of $50.

Consequences of getting ill or harmed during the study:
If you become ill, injured or harmed as a result of the investigations in this study, you will receive care. The reasonable costs of such care will be covered for any injury, illness or harm that is directly a result of the investigations this study. By signing the consent form, you do not waive your legal rights nor release the investigators, sponsors or involved institutions from their legal and professional responsibilities.

<center>LETTER OF INFORMATION</center>

Study Title: Investigating novel treatments for concussion: Impact of compression vest on rehabilitation outcomes.

Consent

I have read the letter of information, have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction.

I consent to do the following procedures:

                             ___ Concussion questionnaires/survey/clinical examination
                             ___ Exercise Test
                             ___ Exercise Intervention
                             ___ Sleep Analysis
                             ___ Cognigram (Cognitive Evaluation)
                             ___ Brain Blood Flow Test
                             ___ Balance Test
                             ___ Vestibular Oculomotor Study
                             ___ Venous Blood sample

_____   _____   _____

Print Study Participant's Name   Signature   Date

(You will be given a signed copy of this consent form)
Your signature means that you have explained the study to the participant named above. You have answered all questions.

_____   _____   _____

Print Name of Person Obtaining Consent   Signature   Date

Was the participant assisted during the consent process? ☐ **YES** ☐ **NO**

If **YES**, please check the relevant box and complete the signature space below:

☐ The person signing below acted as a translator for the participant during the consent process and attests that the study as set out in this form was accurately translated and has had any questions answered.

_____   _____   _____

Print Name of Translator   Signature   Date

_____   _____

Relationship to Participant   Language

☐ The consent form was read to the participant. The person signing below attests that the study as set out in this form was explained accurately, and has had any questions answered.

_____   _____   _____
Print Name of Witness   Signature   Date

_____
Relationship to Participant

Consent in the event a substitute decision maker is applicable:
This study and its purpose have been explained to me as well as the participant named above. I have been given the chance to ask questions and to discuss the participant's participation in this research database with the study doctor. I have been told that study participation can be ended at any time. As the Substitute Decision Maker for this participant, I hereby give consent for this participant to participate in this research database. I conclude that the participant wishes to participate in this research and has indicated his/her assent to do so. I will give a copy of this signed and dated document to the participant and will be given a copy as well.

_____
Name of Substitute Decision Maker (Print)

_____   _____
Signature of Substitute Decision Maker   Date

_____   _____
Signature of Person Obtaining Consent   Date

LETTER OF INFORMATION

TITLE: Cerebrovascular and autonomic function outcomes in adolescents diagnosed with a concussion

Principal Investigator: Kevin Shoemaker, PhD
Co-Investigators: Douglas Fraser MD, PhD; Lisa Fischer, MD; John McCuaig, MD;
                 Arlene Fleischhauer, RN; Erin Moir, BA; Alexandra Harriss, MSc

Funding Source: The Children's Health Foundation

INTRODUCTION AND PURPOSE

The pronouns "you" and "your" in the letter should be read as referring to the participant rather than the legal representative who may be signing the consent form. If you are a substitute decision maker (ie. someone who makes the decision about participation on behalf of a study participant) please remember that "you" refers to the study participant.

Since you have been diagnosed with a concussion, you are being invited to participate in a research study that will examine the health and function of your heart and brain blood flow. We are particularly interested in how a concussion affects brain blood flow when you move from lying down, to sitting upright and then to standing. The study will consist of up to six testing sessions in our lab. Each of these testing sessions may occur before or after your appointment with a concussion physician at Fowler Kennedy Sport Medicine Clinic (FKSMC). Each laboratory testing session will last anywhere from 1.5 to 2.0 hours. A total of 160 participants will be recruited for this study. Before agreeing to participate, please read this LETTER OF INFORMATION and ask any questions you wish.

PARTICIPANT INCLUSION/EXCLUSION CRITERIA

This study will include a group of healthy 'control' participants as well as a group who has been diagnosed with a concussion, and are still experiencing symptoms from their concussive injury.

Inclusion Criteria: To be included in this investigation you must be between 12-18 years of age and have been diagnosed with a concussion by a physician at Fowler Kennedy Sport Medicine Clinic (FKSMC) or already diagnosed and subsequently referred to FKSMC for further evaluation.

Exclusion Criteria: You will not be included in the study if you have any of the following: pre-existing heart disease, currently taking medications that could affect heart or blood vessel control, significant respiratory disease/illness (such as, but not limited to, COPD, cystic fibrosis), pre-existing neurological disorder (such as, but not limited to Parkinson's, multiple sclerosis, or multiple system atrophy), bone or muscle problems that could impact balance or gait, major psychiatric condition, unable to provide written informed consent, complete questionnaires or health history forms due to language or cognitive difficulties. Lastly, you will not be included in the study if you are, or think you might be, pregnant.

STUDY DESIGN and PROCEDURE

If you agree to participate in this research program, you will be asked to come to the laboratory for up to 6 testing sessions. Testing sessions will be conducted for up to 12 weeks. Your enrollment in the study will end either after 12 weeks of testing or when you are discharged from clinical care by your physician at FKSMC (whichever comes first).

Tests: Each testing session will last between 1.5 and 2.0 hours and will be conducted in the Neurovascular Research Laboratory, Thames Hall Rm 3110 at Western University. Here is a sample of the tests you will be asked to perform on each day (the order of testing may vary depending on schedules):

Sample Laboratory Testing Day – Flow Diagram:

	Arrive at the laboratory
10 min	Complete Questionnaires
5-30 min	Rest Period
5 min	Blood Sample
10 min	Attach Devices for Heart Rate, Blood Pressure, etc.
10 min	Measures While Laying Down
10 min	Hand Grip Protocol
50 min	Sit-Stand Protocol

EXPERIMENTAL MEASURES: Listed below is a description of each experimental measure used in these studies. These are in addition to the measures made by the staff at FKSMC (but may be a repeated measurement). *Note – by signing the consent form, you are giving your permission for the research team to view information from your medical history, which is relevant to this investigation.

Sport Concussion Assessment Tool (SCAT): A standardized paper and pencil questionnaire designed to evaluate your concussion rehabilitation. The questionnaire will take up to 10 minutes to complete and will evaluate of i) your symptoms, ii) how well you can remember or think, iii) your balance, and iv) your muscle coordination.

Anxiety Questionnaire: Two paper and pencil measures of anxiety are included: The GAD-7 evaluates your anxiety over the last week. The Visual Analog Scale evaluates your anxiety at that specific moment in time. Both questionnaires will take up to 5 minutes to complete.

Tanner Scale: For this questionnaire you will circle the images which best reflect your current body status. You will be provided the option to complete this scale either with or without your parents in the room. You are allowed to skip any questions that you do not want to answer.

Blood Sample: After resting quietly for up to 30 minutes, a blood sample will be taken from a vein near your elbow. The total volume of the blood sample will not exceed 6-10 table spoons (40mL). Blood samples will be drawn by either physicians/nurses at Fowler Kennedy or by trained members of the research team. For the blood draw you will be either laying down or sitting comfortably in a chair with an armrest. Blankets, pillows, food and drink will be available in the event you react negatively.

Cardiovascular Monitoring: Throughout the testing procedure your heart rate (standard 3-lead electrocardiogram), blood pressure (a small cuff placed on your finger and a larger cuff around your upper arm) and breathing patterns (an elastic band placed around your chest) will be measured.

End Tidal CO2 Gas Collection: Throughout the testing procedure you will wear a facemask that straps to the back of your head and covers your nose and mouth. This mask will allow us to measure gases in your breath.

Transcranial Ultrasound: Using a headband, a small probe will be held just against your skin just behind your eyes. This probe emits ultrasound waves and is used to measure blood flow to your brain.

Submaximal Hand Grip: Using a plastic hand grip device you will complete up to 5 handgrip contractions at efforts that are no more than 50% of your maximal strength. Each contraction will last no more than 30 sec and you will be able to rest for about 60 seconds between each contraction.

Sit-to-stand test: For this task you will sit quietly in a chair for up to 3 minutes, stand for 2 minutes, and then sit again. This protocol will be repeated 12 times under 4 different conditions (listed below). The 4 conditions will alter your end tidal CO_2 (the concentration of carbon dioxide that you breathe out). Carbon dioxide is produced by your body and affects the blood flow in your brain. The conditions are as follows:
1) Spontaneous normal breathing (~35 mmHg end tidal CO_2)
2) Hypocapnia: You will be breathe a little faster than normal using a metronome. This will reduce the carbon dioxide levels in your exhaled air to about 25 mmHg.
3) Normocapnia: You will continue breathing a little faster than normal (to the metronome). This time a small amount of carbon dioxide will be added to the air you breathe to keep your body levels of carbon dioxide the same as in condition 1 above.

4) Hypercapnia: You will continue breathing a little faster than normal (to the metronome). This time an amount of carbon dioxide will be added to the air you breathe to keep your body levels of carbon dioxide a little higher than normal (~40 mmHg end tidal CO_2)

STUDY BENEFITS

There is the possibility that you will receive no personal benefit from this study. However, your participation may benefit society as information learned from this study may help advance concussion diagnoses and rehabilitation in the future.

STUDY RISKS

Venous Blood Sample: There is a small risk of bruising or infection when collecting blood from your vein. Some people may experience mild pain and discomfort and some may feel nauseous, dizzy or faint when blood is taken. To avoid this, we will be collecting blood from you while you are lying down.

Electrocardiogram: The adhesive on the electrodes used to measure your heart rate may lead to temporary redness of the skin on your chest.

Blood Flow/Pressure Measures: There are no known harmful effects with the measures of blood vessels or blood flow using ultrasound imaging, or blood pressure, as used in this study.

Questionnaires/ health evaluations: There are no risks with the questionnaires

Sit-to-stand test: You may feel a little dizzy or light-headed upon standing. The test will be stopped if you feel unwell.

CO_2 gas mixture: Breathing a slightly higher level of carbon dioxide may give you a small headache and it may make you feel breathless. These feelings vanish quickly when you start breathing room air again.

Hand Grip: There are no known adverse effects of completing repeated 30s submaximal handgrip contractions. However, if symptoms are exacerbated participants will be instructed to stop the procedure immediately

YOUR PARTICIPATION

Participation in this study is **voluntary**. You may refuse to participate, refuse to answer any questions or withdraw from the study at any time with no effect on your future care, academic status, or employment.

The blood specimens will be discarded or destroyed once they have been used for the purposes described in the protocol.

If you are participating in another study at this time, please inform the study coordinator right away to determine if it is appropriate for you to participate in this study.

Whether you agree to participate in this study or not, you will be asked if you consent to having your name and contact information added to a master database of individuals who would be willing to be contacted in the future regarding your interest in other research studies.

Representatives of the Western University Health Sciences Research Ethics Board may contact you or require access to your study-related records to monitor the conduct of the research.

CONFIDENTIALITY
Your research records will be stored for up to 15 years in a secure office at Western University. To further protect your confidentiality, your name will be replaced with a subject ID number on all documents. The master list linking your identity and subject ID number and your contact information will be stored separately in a secure office at Western University. Your contact information will be securely maintained at Western University to allow for setting up follow up visits. If the results of the study are published, your name will not be used and no information that discloses your identity will be released or published. No information that could reveal your identity will be released to anyone. If you decide to leave the study, the information about you that was collected before you left the study will be deleted upon your request.

If we find information we are required by law to disclose, we cannot guarantee confidentiality.

Please be aware that Representatives of the Western University Health Sciences Research Ethics Board and/or Lawson Quality Assurance Education Program may contact you or may require access to your study related records to monitor the conduct of the research.

ALTERNATIVES TO STUDY PARTICIPATION
You may choose not to participate in this study.

REIMBURSEMENT
You will be reimbursed for parking/public transit costs up to $50 for your participation in this investigation ($5/per visit). Additionally, to offset the costs of travel and treatments, you will receive up to four free physiotherapy appointments at FKSMC – UWO location (at no cost to you).

CONTACT PERSONS
If you have any questions about the study please contact:
Concussion Research Staff: Alexandra Harriss
Research Nurse: Arlene Fleischhauer
Principal Investigator: Dr. Kevin Shoemaker

Or send an email to
Please note that email is not considered a secure method of communication and you should not send any personal health information via email.

If you have any questions about your rights as a research participant or the conduct of the study you may contact: Dr. David Hill, Scientific Director, Lawson Health Research Institute at _____. You will receive a copy of the fully signed informed consent document for your records. You do not waive any legal rights by signing the consent.

TITLE: Cerebrovascular and autonomic function outcomes in adolescents diagnosed with a concussion

Principal Investigator: Dr. Kevin Shoemaker
Research Staff: Alexandra Harriss, Erin Moir & Arlene Fleischhauer

CONSENT
I have read the letter of information, have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction.

SIGNATURES

_____   _____

Signature of Participant   Date

_____

Print

_____   _____

Signature of Person Obtaining Informed Consent   Date

_____

Print

Do you consent to be contacted by the investigators for future research?   NO ☐   YES ☐

Signature: _____

Curriculum Vitae

Marcy Erin Moir

EDUCATION

The University of Western Ontario
MSc Kinesiology, Integrative Biosciences, 2017 (expected)
Thesis: Impaired dynamic cerebrovascular autoregulation in adolescent concussion
Advisor: Dr. J. Kevin Shoemaker, PhD

The University of Western Ontario
BA Honors Specialization in Kinesiology, 2013

HONOURS & AWARDS

2016-2017	Western Graduate Research Scholarship	($5,268 CAD)
2016	AAS-Lundbeck Travel Fellowship Award	($1,250 USD)
2015-2016	Western Graduate Research Scholarship	($5,268 CAD)
2006	Western Scholarship of Distinction	($1,500 CAD)

RESEARCH CONTRIBUTIONS

Abstracts in Peer Reviewed Conference Proceedings:

Balestrini, C.S., **Moir, M.E.**, et al. (2017). Sex-dependent cardiovagal dysregulation in adolescent concussion. Abstract accepted. *CASEM-AQMS Sports Medicine Conference*, Mont-Tremblant, QC, Canada. June 7-10, 2017. Poster.

Woehrle, E…**Moir, M.E.**, et al. (2017). Investigation of neural cardiac dysregulation using brief 30% isometric handgrip protocol in adolescents diagnosed with concussion. Abstract accepted. *CASEM-AQMS Sports Medicine Conference*, Mont-Tremblant, QC, Canada. June 7-10, 2017. Poster.

Abbott, K.C…**Moir, M.E.**, et al. (2017). Comparison of self-report symptom endorsement between acutely concussed adolescents and healthy, age-matched controls. Abstract accepted. *CASEM-AQMS Sports Medicine Conference*, Mont-Tremblant, QC, Canada. June 7-10, 2017. Poster.

Moir, M.E., et al. (2017). Impaired dynamic cerebral autoregulation to postural stress following concussive injuries in adolescents. Abstract accepted. *Experimental Biology*, Chicago, IL, USA. April 22-26, 2017. Oral (NCARnation Award Session Finalist) and Poster.

Balestrini, C.S., **Moir, M.E.**, et al. (2017). Autonomic dysregulation in adolescent concussion: Characterization and temporal resolution of neurological outcomes. Abstract accepted. *Experimental Biology*, Chicago, IL, USA. April 22-26, 2017. Oral (NCARnation Award Session Finalist) and Poster.

Moir, M.E., et al. (2016). Cardiac autonomic function impairment in concussed adolescents during a sit-to-stand protocol. *27th International Symposium on the Autonomic Nervous System*, San Diego, CA, USA. November 2-5, 2016. Poster (AAS-Lundbeck Travel Fellowship Award).

Abbott, K.C…**Moir, M.E.**, et al. (2016). Cardiac autonomic function is impaired in adolescents with mild traumatic brain injury. *Experimental Biology*, San Diego, CA, USA. April 2016. Poster.

Abstracts in Non-Peer Reviewed Conference Proceedings:

Moir, M.E. and Balestrini, C.S. (2017). Cardiac autonomic and cerebrovascular dysregulation throughout clinical recovery in adolescent concussion. *Fifth Annual Symposium: Research on the Concussion Spectrum of Disorders*, Toronto, ON, Canada. January 21, 2017. Oral.

Woehrle, E...**Moir, M.E.**, et al. (2017). Impaired heart rate response to brief 30% isometric handgrip in adolescents diagnosed with a concussion. *Fifth Annual Symposium: Research on the Concussion Spectrum of Disorders*, Toronto, ON, Canada. January 21, 2017.

Moir, M.E., et al. (2016). Dynamic cerebral autoregulation is impaired in adolescents following concussion. *Michigan Physiological Society 3rd Annual Meeting*, Detroit, MI, USA. May 12-13, 2016. Poster (Poster Award Finalist).

Moir, M.E., et al. (2016). Dynamic cerebral autoregulation is impaired in adolescents following concussion. *Western Kinesiology Graduate Symposium*, London, ON, Canada. April 26, 2016. Poster.

Moir, M.E., et al. (2015). Cerebral autoregulation impairment in adolescents with concussion. *Saltin International Graduate Course in Clinical and Exercise Physiology*, Toronto, ON, Canada. October 10-14, 2015. Oral.

PRESENTATIONS AND GUEST LECTURES

The University of Western Ontario. Address to the Governor General of Canada. March 8, 2017.

The University of Western Ontario. Kinesiology 4432A, Physiology of Exercise. "Dynamic Cerebral Autoregulation and Concussion". December 1, 2016.

The University of Western Ontario. Kinesiology 2236B, Introduction to Athletic Injuries. "Cerebral Blood Flow Regulation and Concussion". March 31, 2016.

The University of Western Ontario. Kinesiology 4432A, Physiology of Exercise. "Dynamic Cerebrovascular Autoregulation Impairment in Adolescents with Concussion". November 24, 2015.

TEACHING ASSISTANTSHIPS

The University of Western Ontario, School of Kinesiology. Kinesiology 2230B, Introductory Exercise Physiology Laboratory. January-April 2017.

The University of Western Ontario, School of Kinesiology. Kinesiology 4432A, Physiology of Exercise. September-December 2016.

The University of Western Ontario, School of Kinesiology. Kinesiology 2236B, Introduction to Athletic Injuries. January-April 2016.

The University of Western Ontario, School of Kinesiology. Kinesiology 4432A, Physiology of Exercise. September-December 2015.