BARORECEPTOR SENSITIVITY AND HEART RATE VARIABILITY IN SPORT RELATED CONCUSSIONS

by

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Abstract
Sports related concussions (SRC) have become a popular topic and more awareness and interest has risen towards this growing type of brain injury. The cardiovascular aspects of a concussion in particular baroreceptor sensitivity and heart rate variability need to be further investigated. Baroreceptor sensitivity (BRS) and heart rate variability (HRV) may be altered due to a biomechanical force to the brain stem, lesions or axonal stretching that occurs after a concussion. If the brain stem is damaged this may cause a disruption to the cardiovascular centers as they are located in the medulla oblongata. Six concussed athletes (5 males and 1 female) with sport-related concussion (age: 17.5± 2 years, BMI: 24 ± 1 kg/m^2) and 6 healthy control-subjects (5 males and 1 female; age: 20 ± 2 years, BMI: 22 ± 2 kg/m^2) participated by performing two rounds of stand squats at frequencies of 0.1Hz and 0.05Hz for 5 minutes. BRS and HRV are not altered after suffering from a SRC. Although this finding contrasts with the original hypothesis that there would be reduced BRS and abnormal HRV in sports related concussions, the issues of small sample size and marked within subject variability are acknowledged. Another likely possibility explaining the lack of notable differences is that, unlike severe head injury, a SRC is not enough of an injury to damage central control of the cardiovascular centers in the medullar regions of the brain and therefore efferent and afferent signal pathways remain intact and are capable of responding to different stressors maintaining BRS and HRV.
**Preface**

This study was approved by the University of British Columbia Clinical Research Ethics Board (H11-02900). Data was collected at the University of British Columbia (Kelowna, BC), by Dr. Paul van Donkelaar, Dr. Philip Ainslie, Dr. Brad Monteleone help with the design, equipment acquisition and funding for the study. Kurt Smith, Katelyn Marsden, Tanis Burnette and Nicole Strachan were responsible for the overall implementation and execution of the study. Kurt Smith helped with technical assistance and study implementation. Nicole Strachan and Katelyn Marsden coordinated both subject recruitment and data collection. Nicole Strachan was also responsible for study implementation, study promotion, analyzed data, including statistical analyses and was responsible for writing the manuscript.
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Dedication

To the individuals who are able to roll with the punches and adapt to whatever is thrown in front of them.
Chapter 1 Literature Review

1.1 Introduction
Contact sports have always been plagued by risk of injury; ranging from a scuff of the knee to fatal brain trauma. Brain injuries, more commonly known as concussions, have become a large concern for all athletes and have become one of the most common forms of traumatic brain injury (Canadian Medical Association). It is important to emphasize that mild traumatic brain injury (mTBI) is often used interchangeably with sport-related concussion in certain literature in the past (Aubry et al. 2002); however, these terms should be separated since concussion is a subset of traumatic brain injury (McCrory et al. 2013). Sports related concussions (SRC) have become a popular topic in the media following the diagnosis of many high profile athletes (Miller. 2009), bringing more awareness and interest towards this type of brain injury (Doolan et al. 2012). As a result of recent studies and changing attitudes, concussions have now become a major public health concern (Lebrun et al. 2013). They are one of the most common and threatening injuries in contact sports (Benson et al. 2013; Cantu. 1997).

1.2 What is a concussion?
The most recent definition of a concussion is from the International Consensus Conferences on Concussion in Sport and is based on the deliberations at the 4th International Conference on Concussion in Sport held in Zurich, in November of 2012. Here, concussion was defined as a “complex pathophysiological process affecting the brain, induced by traumatic biomechanical forces. Several common features that incorporate clinical, pathologic and biomechanical injury construct that maybe utilized in defining the nature of a concussive head injury” (McCrory et al. 2013). There are four main points that aid in the definition of concussion (Table 1.0).
Table 1.0 Summary of the characteristics that may help define a concussive head injury (McCory et al., 2013)

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

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

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

4. Concussion results in a graded set of clinical symptoms that may or may not involve loss of consciousness. Resolution of the clinical and cognitive symptoms typically follows a sequential course. However, it is important to note that some cases may be prolonged.

1.3 Epidemiology of a sport related concussion

Participation in contact sports is increasing and hence likely contributing to the increased concussion prevalence (Doolan et al. 2012; Langlois et al. 2006). The Canadian Institute for Health Information reports that the number of concussions occurring annually is generally underreported due to the fact that many athletes are concerned that they will miss playing time (McCrea et al. 2004). There is also a lack of concussion recognition reported (Collins et al. 2003). The Center for Disease Control has estimated 1.6-3.8 million sports related brain injuries occur each year in the US alone (Langlois et al. 2006). This estimation is low due to underreporting; however, the Canadian Institute for Health Information reports that the leading cause of brain injury admissions to hospitals in 2003-2004 was due to sporting activity (Canadian Medical Association. 2011). In Alberta there were 63,219 visits to the hospital for sports injuries over a ten-year span and 4,935 (7.8%) of them were head injuries and concussion. Furthermore, 70% of the population that suffered these injuries were under the age of 18 (Harris et al. 2012). The occurrence of concussions has risen 16.5% per year in high school athletes.
(Bakhos et al. 2010), (Lincoln et al. 2011) and costs the US 20% of $56 billion spent annually on traumatic brain injuries (Mihalik et al. 2005). The contact sports for males at the high school, college and amateur level that are most likely to have a high incidence of concussion are ice hockey and football. Males are twice as likely to get a concussion than females. For females at the same level, soccer poses the most risk (Koh et al. 2003; Powell and Barber-Foss. 1999a; Powell and Barber-Foss. 1999b). This large number of injuries emphasizes the importance of ongoing research in this area to keep athletes safe by understanding the underlying physiology as well as mechanisms that can be used clinical to help diagnose and ensure return to play is not undertaken too early. This is vital as returning to play early can increase the risk of a repeated concussion and longer recovery.

1.4 Biomechanics and pathophysiology of sport related concussion

A concussion is believed to occur when angular, or rotational accelerating forces are applied to the brain. This may be due to a direct blow to the head; however direct impact to the head is not required (Ommaya and Gennarelli. 1974). This impact causes shearing forces to be transmitted through brain tissue (Meehan and Bachur. 2009), and can cause a series of short-lived neurochemical, gene expression and neurometabolic changes to occur(Hovda et al. 1995; McIntosh et al. 1996). Looking at the deeper basis of concussion there is an immediate stretching of axons, disruption of neuronal membranes, which are a part of diffuse axonal injury and increased permeability. Neurometabolically there is a cascade that follows a concussion. A summary of this cascade is illustrated in Figure 1.0
Figure 1.0 Lists of metabolic cascade events that occur after initial trauma (Giza and Hovda. 2001)

### 1.5 Symptoms of a sport related concussion

In the past, concussions have been known to be an injury that a player could “shake off” and continue playing in a short period of time. Dating back to the days of Hippocrates concussive like symptoms have been present (McCrory. 2001). The severity of the concussive injury has been misunderstood and it is only now that there is mounting evidence indicating that the physical and behavioral symptoms that result from a concussion are actually very serious (Blumbergs et al. 1994; Blumbergs et al. 1995; Finnoff et al. 2011; McKee et al. 2009)

Concussion symptoms usually present in the form of one or more neurological, behavioral or physical symptoms that can be exacerbated by physical effort. Table 1.1 lists examples of common symptoms that may occur after a concussion. However, the most common symptom reported after a concussion is a headache (Blinman et al. 2009; Collins et al. 2003). Symptoms usually resolve within 2-10 days after injury (Ellemberg et al. 2009); however, this is not the
case for everyone (Khurana and Kaye. 2012) as concussions are highly variable and dependent on the individual.

**Table 1.1 Separation and classification of symptoms that may be present after a concussion**

<table>
<thead>
<tr>
<th>SIGNS AND SYMPTOMS OF A CONCUSSION</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SIGNS OBSERVED BY</strong></td>
</tr>
<tr>
<td>PARENTS OR GUARDIANS</td>
</tr>
<tr>
<td>• Appears dazed or stunned</td>
</tr>
<tr>
<td>• Is confused about events</td>
</tr>
<tr>
<td>• Answers questions slowly</td>
</tr>
<tr>
<td>• Repeats questions</td>
</tr>
<tr>
<td>• Can't recall events prior to the</td>
</tr>
<tr>
<td>hit, bump, or fall</td>
</tr>
<tr>
<td>• Can't recall events after the</td>
</tr>
<tr>
<td>hit, bump, or fall</td>
</tr>
<tr>
<td>• Loses consciousness</td>
</tr>
<tr>
<td>(even briefly)</td>
</tr>
<tr>
<td>• Shows behavior or personality</td>
</tr>
<tr>
<td>changes</td>
</tr>
<tr>
<td>• Forgets class schedule or</td>
</tr>
<tr>
<td>assignments</td>
</tr>
<tr>
<td><strong>SYMPTOMS REPORTED BY YOUR CHILD</strong></td>
</tr>
<tr>
<td>OR TEEN</td>
</tr>
<tr>
<td><strong>Thinking/Remembering:</strong></td>
</tr>
<tr>
<td>• Difficulty thinking clearly</td>
</tr>
<tr>
<td>• Difficulty concentrating or</td>
</tr>
<tr>
<td>remembering</td>
</tr>
<tr>
<td>• Feeling more slowed down</td>
</tr>
<tr>
<td>• Feeling sluggish, hazy, foggy, or</td>
</tr>
<tr>
<td>groggy</td>
</tr>
<tr>
<td><strong>Emotional:</strong></td>
</tr>
<tr>
<td>• Irritable</td>
</tr>
<tr>
<td>• Sad</td>
</tr>
<tr>
<td>• More emotional than usual</td>
</tr>
<tr>
<td>• Nervous</td>
</tr>
<tr>
<td><strong>Physical:</strong></td>
</tr>
<tr>
<td>• Headache or “pressure” in head</td>
</tr>
<tr>
<td>• Nausea or vomiting</td>
</tr>
<tr>
<td>• Balance problems or dizziness</td>
</tr>
<tr>
<td>• Fatigue or feeling tired</td>
</tr>
<tr>
<td>• Blurry or double vision</td>
</tr>
<tr>
<td>• Sensitivity to light or noise</td>
</tr>
<tr>
<td>• Numbness or tingling</td>
</tr>
<tr>
<td>• Does not “feel right”</td>
</tr>
<tr>
<td><strong>Sleep</strong>:</td>
</tr>
<tr>
<td>• Drowsy</td>
</tr>
<tr>
<td>• Sleeps less than usual</td>
</tr>
<tr>
<td>• Sleeps more than usual</td>
</tr>
<tr>
<td>• Has trouble falling asleep</td>
</tr>
</tbody>
</table>

*Only ask about sleep symptoms if the injury occurred on a prior day.

**1.6 Diagnosis and management of a sport related concussion**

Recent advances in neuroimaging have opened the door for concussion research. However, the numerous changes that occur during a concussion are typically undetectable using many structural neuroimaging techniques (i.e. CT, MRI) (McCrory et al. 2009; McCrory et al. 2013) and those that can detect changes (i.e. fMRI and DTI), are not easily accessible or affordable forms of assessment in this very large demographic. Therefore, it is very difficult to properly assess the recovery of the neurophysiological processes affected by this biomechanical trauma.

The demand to further investigate the pathophysiology of concussion is a growing necessity in order to be able to manage, understand, and make safer return to play decisions based on objective criteria. There are different characteristics that aid in the diagnosis of concussion and these usually present themselves in the form of clinical symptoms, physiological signs, cognitive impairment, and neurobehavioral issues (See table 1.2). If one or more of these symptoms are
present along with use of the SCAT 2/3 which is a document specific concussion-related symptoms and severity test (Shehata et al. 2009) then a concussion should be suspected and appropriate measures should follow. The best treatment for a concussion is both physical and cognitive rest within the first 24-48hrs after the initial injury. There are mixed opinions on the length and time for rest after this and more research is needed in this area (McCrorry et al. 2013). First steps should be to return to normal social events and school/work. If the individual can handle these situations symptom free then there is a graded return to play protocol that should be followed (Johnston et al. 2004).

Table 1.2: Diagnosis of a concussion can include one or more of the following clinical areas (McCory et al., 2013)

<table>
<thead>
<tr>
<th>1. Symptoms</th>
<th>Somatic, cognitive, and or emotional</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Physical signs</td>
<td>Loss of consciousness, amnesia, confusion</td>
</tr>
<tr>
<td>3. Behavioral changes</td>
<td>Irritable, grumpy</td>
</tr>
<tr>
<td>4. Cognitive impairment</td>
<td>Slow reaction times</td>
</tr>
<tr>
<td>5. Sleep disturbances</td>
<td>Insomnia or sleeping more than usual</td>
</tr>
</tbody>
</table>

1.7 Long term implications of a sport related concussion

If an athlete returns to play within 7-10 days of their first concussion and does not take the appropriate rest time they put themselves at greater risk for a secondary concussion (Guskiewicz. 2003). With secondary concussion or second impact, which is another blow or force transmitted to the head after an individual has already had an initial concussion, there is longer recovery (Iverson et al. 2004). Multiple concussions has also been linked with Alzhiemers disease (Omalu et al. 2005; Omalu et al. 2006) and preliminary findings suggest there is also a link to Chronic Traumatic Encephalopathy (Cantu. 2007). There has also been a link between SRC with depression (Guskiewicz et al. 2005; Guskiewicz et al. 2007). These correlative findings stress the
importance of understanding the physiology of concussion to ensure that athletes are not at risk for long-term effects.

1.8 The growing need for more research
The need for understanding the pathophysiology of concussion is constantly growing. In particular the need for future investigation into cardiovascular regulatory mechanisms and the potential dysfunction has been reported (Len et al. 2011; McCrory et al. 2009). One aspect that encompasses this area is looking at baroreceptor sensitivity (BRS) and heart rate variability (HRV) after an individual suffers from a sport related concussion. The tentative physiological linkage between concussion with BRS and HRV is a good way to investigate the cardiovascular health and responsiveness of an individual. These aspects are considered next.

1.9 What are baroreceptors and baroreceptor sensitivity?
Baroreceptors are an example of short-term blood pressure regulation and act as a negative feedback loop called the baroreflex (Seeley, R. R., Stephens, T. D., & Tate, P. 2008). The baroreflex operates with both the sympathetic and parasympathetic nervous systems. They help control short-term blood pressure responses throughout daily life to maintain homeostasis. Although they do not operate independently from one and another, there are two types of baroreflex systems which are both necessary to maintain homeostasis. The arterial (change in blood pressure and heart rate and cardiovagal baroreflex mechanisms (change in blood volume/pressure) (Wieling et al. 2007). The first system is the low-pressure system. This includes baroreceptors that are also known as cardiopulmonary stretch receptors, and are found in the wall of the right atrium and ventricles of the heart, and the pulmonary vessels and large systemic veins. Low-pressure baroreceptors regulate blood volume which aids in control of mean arterial pressure. Mean arterial pressure is the average pressure of blood flow that is found in the arteries during a single cardiac cycle.

The second baroreflex system is the high-pressure system. This system is more sensitive to rising
blood pressure than falling pressure, which is known as hysteresis (Eckberg. 1980).

Baroreceptors provide the central nervous system with a continuous stream of information on changes in blood pressure. This change is sensed by the stretch receptors that are located in the carotid sinus and aortic arch from where efferent neuronal activity can be dynamically modulated. These baroreceptors aid in short term blood pressure homeostasis. If arterial blood pressure is too low then an individual can be in a hypotensive state and blood flow to the tissues can be reduced. If blood pressure is too high then an individual can be in a hypertensive state and over time this may cause excessive capillary pressures and damage. Cardiovagal/Arterial baroreceptors modulate cardiac output (via changes in HR and SNA) and peripheral vasoconstriction (via changes in SNA) to maintain normal mean arterial pressure. Baroreceptors located in the carotid sinus transmit action potentials through the glossopharyngeal nerves and baroreceptors in the aortic arch transmit action potentials from the vagus nerve. The two nerves then transmit to the cardioregulatory and vasomotor centers, in particular the nucleus tractus solitarius, which is located in the medulla oblongata. If there is a rise in systemic arterial pressure, an increase of the discharge of vagal cardio inhibitory neurons occurs. Along with this discharge, a decrease in the discharge of sympathetic neurons to the heart and peripheral blood vessels ensues. This discharge results in decreased venous return, decreased peripheral resistance, decreased cardiac contractility, and bradycardia (Kirchheim. 1976; La Rovere et al. 2008). With a decrease in systemic arterial pressure, the deactivation of baroreceptors occurs amongst enhancement of sympathetic activity and vagal inhibition. This results in tachycardia and an increase of cardiac contractility, vascular resistance and venous return. The parasympathetic and sympathetic systems both work together to aid in short term regulation of blood pressure via the baroreflex system. However it should be noted that there are many factors such as respiration, behavioral and environmental modulators that contribute to the functioning of the baroreflex and baroreceptor mechanisms. For instance respiration interacts with the
baroreflex in modulation of heart rate. Inspiration decreases while expiration increases baroreceptor stimulation of vagal motor neurons (Eckberg and Orshan. 1977). Behavioural factors can influence the baroreceptor operating point both in terms of hypotension and hypertension. For example the baroreceptor threshold pressure point at which the baroreceptors begin to discharge can change. In hypertension, this has been thought to occur due to the denervation of the arterial baroreceptors and has been attributed to the augmentation of sympathetic outflow from the cardiac and vasomotor centers. Others have found that the chronic resetting of the baroreceptor operating point can occur due to an increase in pressure accompanied by a decrease in pressure sensitivity of the pressure nerve activity relationship. (Koushanpour. 1991) It is important to note that BRS are one aspect of short-term blood pressure regulation in an interrelated system. (Rowell and O'Leary. 1990; La Rovere,M.T., Pinna, G.D, Mortara, A. 1999; Seeley, R. R., Stephens, T. D., & Tate, P. 2008; Rowell. 1993; Smith. 2011)

1.10 What is heart rate variability (HRV)?
Heart rate variability (HRV) reflects the fluctuation in heart rate and is a useful non-invasive technique to investigate the cardiovascular system (King et al. 1997). It provides a non-invasive means by which to gain insight into the sympathetic and parasympathetic control of heart rate (Huikuri. 1995). The fluctuations that occur in heart rate on a beat-to-beat basis can be distinguished by oscillations in the interval between consecutive and instantaneous intervals. These intervals are dependent on interactions between the parasympathetic and sympathetic efferent impulses to the heart and can further be dissected to examine the interaction between each other (Rapenne et al. 2001). Varying oscillations have been associated with different neural effects. Faster occurring oscillations that are present in high frequency bands (0.15-0.4 Hz) are associated with respiration through respiratory sinus arrhythmia (RSA). This entails that a rhythmical fluctuation in heartbeat intervals will be present by the shortening of the R-R interval on inspiration and a lengthening on expiration. This frequency is tied to parasympathetic
activity. Lung expansion inhibits the vagus nerve shortening of the R-R interval and expiration leads to increase in the activity of the vagus nerve via stimulation of the chemoreceptors or baroreceptors (Yasuma and Hayano. 2004) Slower, longer oscillations are associated with a low frequency band (0.04-0.15 Hz) and are related to sympathetic activity. There are many factors that affect HRV such as respiration, tidal volume, age, and postural position. That being said, there is evidence to suggest that the autonomic neural control of heart rate is open to interpretation. It does not allow heart rate itself to be a precise marker of sympathetic activity, however it is easy to quantify and is sensitive to changes in neural cardiovascular control and heart rate assessment and therefore is valuable tool(Valentini and Parati. 2009)

1.11 How are BRS and HRV measured?
Baroreceptors have both a dynamic and steady state condition. This allows their mechanisms to be measured by mechanical or pharmacological manipulation via sudden BP changes that are sensed by the receptors as well as different fluctuations that occur in arterial pressure. When adding an external stimulus such as compression, it forces a rapid change in arterial pressure. The relationship between the change in pressure and the R-R interval can be investigated in an open loop manner. BRS and HRV can be assessed using either time or frequency analysis. For time analysis, BRS can be estimated from the slope of the regression line between arterial pressure and the changes in R-R interval of heart rate (Parati et al. 1988). The frequency analysis uses spectral analysis of the sequences of the R-R interval and blood pressure. With this approach, each oscillation that is produced from blood pressure provokes a similar oscillation at the same frequency in the R-R interval by the effect of the baroreflex activity (La Rovere et al. 1998). HRV can use both time and frequency analysis as well. With frequency analysis the variability of the R-R interval is calculated using a fast Fournier transformation. The square root of variance (SDNN) (standard deviation of the normal to normal intervals) or the total power of the spectral analysis reflects all the cyclic components that are responsible for variability in the
period of recording (Anonymous 1996b). This encompasses different frequency variations mentioned above such as low and high frequency that would be viewed in a 24 hr. or 5 minute recording to ensure enough oscillations have occurred. A minimum of one minute of an ECG recording is needed to assess the HF components of HRV while 2 are needed to assess LF component. In order to standardize readings across different studies, 5 minutes is required (Anonymous 1996b). Frequency analysis is better for short-term recording and time analysis is better for longer duration recordings. Time analysis takes the R-R interval plotted against time and looks at the standard deviation of the heartbeat through all normal-to-normal beats (SDNN) and is a good measurement to estimate the overall HRV. (Gaetz. 2004)

1.12 Why are BRS and HRV important in sport related concussion?

Baroreceptor sensitivity and HRV may be altered due to a biomechanical force to the brain stem, lesions or axonal stretching that occurs after a concussion. If the brain stem is damaged this may cause a disruption to the cardio regulatory centers as they are located in the medulla oblongata (Giza and Hovda. 2001; Lanfranchi and Somers. 2002; Korpelainen et al. 1996). Nerves leave the central nervous system through the thoracic and lumbar spinal nerves and pass into the sympathetic chain. From here the specific nerves (sympathetic) innervate the vasculature of the internal viscera and the heart. The vasomotor centers that are located in the brain (medulla) transmit these impulses through the spinal cord. The vasomotor center has specific areas, the vasoconstrictor area, the vasodilator area, and the sensory area. The vasoconstrictor area has norepinephrinergic neurons, which stimulate the vasoconstrictor neurons of the sympathetic nervous system. The vasodilator area has fibers from the neurons that project upwards to the vasoconstrictor area and inhibit vasoconstrictor activity. Finally the sensory area receives signals from the vagus and glossopharengeal nerve and output signals from the sensory area to control both the vasomotor and vasoconstrictor areas (Figure 1.2). If these areas are damaged then the
outputs may not be effective in regulating blood pressure and there can be a disconnect between the systems (Korpelainen et al. 1996).

**Figure 1.1 Location and areas where signals are transmitted to regulate blood pressure**

BRS is a vital determinant of neural regulation of the cardiovascular system and has been used to provide clinical and prognostic information in various cardiovascular diseases (La Rovere et al. 2013). Issues with decreased/diminished BRS can lead to the development of arrhythmic fibrillations and increased risk of syncope (Schwartz. 1998). Abnormal BRS has been linked with sudden and non-sudden cardiac death (La Rovere et al. 1998; La Rovere et al. 2001; Mortara et al. 1997). Decreased BRS and or reduced HRV have also been associated with life threatening arrhythmic events (Farrell et al. 1992). HRV has been suggested to be an independent predictor of death (Bigger et al. 1992; Farrell et al. 1991; Farrell et al. 1992). Together, this explains why the understanding of both BRS and HRV in concussion is vital to investigate.
BRS and HRV have not been examined extensively following sport related concussion. There have been some studies that have investigated the role these mechanisms play in mild moderate and severe brain injury. These studies are summarized in appendix A.1 and A.2. Collectively, these studies have reported changes such as a disengaging of BRS and low or abnormal HRV in traumatic brain injury that warrant further investigation. In 1998, Goldstein and coworkers first found evidence for cardiovascular and autonomic disconnection in acute brain injury with complete disconnection occurring during brain death. The limitation of this is that it is in much more severe cases of brain trauma and brain death than a concussion; therefore, one is unable to assume this would be the same in sport related concussion. The degree of cardiovascular disconnection, impaired baroreflex sensitivity and decreased heart-rate variability appears to be related to the severity of the traumatic brain injury and can even become permanent in severe traumatic brain injury (Goldstein et al. 1993; Goldstein et al. 1998). This warrants further investigation in the context of sport related concussion to determine if it is present in less severe cases. Another study by McMahon and colleagues (2010) found that moderate traumatic brain injury was connected with significant modification in BRS. The limitation to this study was that it was done in Wistar rats and not humans and therefore more research is needed in human subjects. Data from another study (Baguley et al. 2006) found that their 16 traumatic brain injury patients had a higher mean heart rate than their controls (89.5 vs 67.6 beats per minute). This higher mean heart rate showed a similar trend when compared to controls in a previous study (King et al., 1997), however their values did not reach statistical significance. Both studies did show a disordered association between heart rate variability and sympathetic/parasympathetic balance as estimated via 24hr ECG recordings. Thus, future studies that investigate the role of BRS and HRV in sport related concussion would help fill in many of the missing gaps in the literature as well as aid to explaining the underlying cardiovascular pathophysiology that occurs after an individual suffers from a sport related concussion.
1.13 Purpose of this study
The purpose of this study is to determine if baroreceptor sensitivity and heart rate variability are influenced by sport related concussion in adolescents and young adults aged 14-25. Further investigation into the area of the cardiovascular regulatory mechanisms is needed to better understand the physiological changes that occur after a sport related concussion and the potential dysfunction that may occur. Understanding this information could provide insight into the regulatory mechanisms that may be altered after a sport related concussion. The tentative physiological linkage between concussion with BRS and HRV is a good way to investigate the cardiovascular health and responsiveness of an individual.

1.14 Hypothesis
Outcome measure 1: Baroreceptor sensitivity will be reduced
Outcome measure number 2: an increased trend of abnormal heart rate variability will be present in subjects who have suffered a sport related concussion
Chapter 2 Methods:

2.1 Experimental design

Both female and male athletes aged 14-25 participating in organized contact sports in the Kelowna, B.C. area, were recruited; Participants’ physical characteristics are described in section 2.5. Each participant was tested at the following time points following being diagnosed with a sport related concussion: Day 3.6 ± 0.8, Day 7.8 ± 0.9, Day 16.2 ± 2.6 and day 33.5 ± 9.5 following injury.

Figure 2.0 A flow diagram of the testing schedule over the course of one month.

Controls matched for age, sex, and level of education and physical activity were tested at the same intervals. The continuation through the experimental protocol for all subjects was dependent on symptom exacerbation and pain tolerance during the battery of tests designed to progressively challenge the subjects. Subjects who acted as a control were classified as a control subject (CS).

2.2 Participant recruitment

Participants were recruited for testing through several local hockey leagues, football programs, and university varsity athletics. All coaches, trainers and physicians were given information. This was done through brochures, flyers and e-mails (please see appendix B.2 as well as local information recruitment (i.e. going to games and talking to potential participants). Those diagnosed with a concussion by a physician or therapist were referred to the laboratory facilities at UBC – Okanagan campus for follow-up testing. Classifying all concussions as a single entity, the consensus panel from Zurich 2008 replaced the simple versus complex terminology of a concussion to a list of modifying factors (McCrory et al. 2009). If these factors present
themselves at the time of injury then the appropriate investigation and management of concussion should be undertaken. Please see appendix B.1 for list. Factors include; age, gender, previous concussions, injury specifics (i.e. prolonged loss of consciousness, amnesia), type of sport or position played and symptoms present. Athletes that have a confirmed concussion diagnosis meet the inclusion criteria (described below), and who agreed to participate were entered into the study. Control subjects who met the inclusion criteria were entered into the concussion study control group. Control subjects completed the same protocol and time period as the concussed subjects.

2.3 Inclusion/ Exclusion criteria
The inclusion criterion for SRC participants is a diagnosis of concussion by a physician or athletic therapist. Inclusion criteria for CS was being matched with age, sex, activity level and education level of a SRC subject participating in the study. In order to control for a variety of modifying factors, SRC athletes were excluded from the study if they presented with amnesia or severe cognitive dysfunction or suffered a previous concussion within 12-months of the current concussion. Control subjects had no previous history of concussion. Exclusion criteria for all participants include lower extremity deficiencies or injury that might hinder normal balance control; a history of neurological (e.g., brain tumor, moderate-severe traumatic brain injury, meningitis) or psychiatric (e.g., bipolar disorder) problems; any cardiovascular disorders or diseases (e.g, high/low blood pressure, abnormal HR).

Five subjects were excluded due to the following: 1→ unable to hold mouth piece due to facial injury, 2→ unable to complete due to parental concern. 3→ unable to complete due to irregular ECG, 4→ unable to complete due to previous concussions within the year, 5→ excluded for taking caffeine pills with an inconsistent dose and time period.
2.4 Participant consent procedure
This study was ethically approved by the University of British Columbia Ethics Board #H11-02900 (Appendix B.9). Participants identified as suffering a concussion were informed of the study upon completion of the initial medical examination. If interested in participating, the attending health care professional asked the injured athlete and parents/guardians for permission to have a member of the research team contact them by phone. If permission was granted, the investigator phoned or e-mailed the prospective participant and parents/guardians to provide further information about the study. Informed written consent was obtained from all participants and parents/legal guardians prior to research participation.

2.5 Participants
Six concussed athletes (5 male, Age: 18 ± 3 years, BMI: 24 ± 1.5 kg/m$^2$ and 1 female, Age: 18, BMI 23 kg/m$^2$) and six control subjects (5 male, Age 20: ± 2, BMI: 22 ± 2 and 1 female, Age: 21, BMI: 22 23 kg/m$^2$) participated in this study. The SRC subjects sustained their injuries from football (n=1), soccer (n=1), hockey (n=2), skiing (n=1) and bike (n=1). Subject characteristics and demographics can be seen in in table 2.0.

Table 2.0 Subject characteristics and demographics

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age</th>
<th>Sport</th>
<th>Height (M)</th>
<th>Weight (Kg)</th>
<th>BMI</th>
<th>Education</th>
<th># Days to return</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRC01</td>
<td>M</td>
<td>14</td>
<td>Football</td>
<td>1.705</td>
<td>70.4</td>
<td>24.217</td>
<td>Grade 9</td>
<td>24</td>
</tr>
<tr>
<td>SRC02</td>
<td>M</td>
<td>18</td>
<td>Soccer</td>
<td>1.8</td>
<td>73</td>
<td>22.531</td>
<td>2nd year University</td>
<td>15</td>
</tr>
<tr>
<td>SRC03</td>
<td>M</td>
<td>19</td>
<td>Hockey</td>
<td>1.78</td>
<td>84</td>
<td>26.512</td>
<td>1st year University</td>
<td>12</td>
</tr>
<tr>
<td>SRC04</td>
<td>M</td>
<td>21</td>
<td>Skiing</td>
<td>1.79</td>
<td>81</td>
<td>25.28</td>
<td>4th Year University</td>
<td>?</td>
</tr>
<tr>
<td>SRC05</td>
<td>M</td>
<td>15</td>
<td>Hockey</td>
<td>1.7</td>
<td>67.6</td>
<td>23.391</td>
<td>Grade 10</td>
<td>6</td>
</tr>
<tr>
<td>SRC06</td>
<td>F</td>
<td>18</td>
<td>Bike</td>
<td>1.6</td>
<td>61</td>
<td>23.821</td>
<td>1st year University</td>
<td>?</td>
</tr>
</tbody>
</table>
2.6 Pre-testing guidelines
Prior to testing, subjects were asked to refrain from consumption of alcohol, caffeine and avoid vigorous exercise 24 hours prior to testing. Participants were also encouraged to maintain proper hydration. Upon arrival informed consent was collected and pre-screening questions were administered. Subsequently, anthropometric measurements were taken and a concussion specific symptom questionnaire was administered (appendix B.8). This document is the specific concussion-related symptoms and severity test (Shehata et al. 2009) and was administered at the beginning of the protocol and directly after each stage of the test to measure symptom exacerbation and physiological stress placed on the subject (McCrory et al. 2009).

2.7 Experimental protocol
To maximize internal validity for each subject, the experimental protocol was conducted by the same personnel, with the same equipment, and completed at the same time of day. Next subjects were instrumented with a 3 lead electrocardiography (ECG) for measurement of the R-R interval and the QRS complex. Beat-to-beat arterial pressure monitoring was taken noninvasively by using a finger pulse blood pressure machine. This machine is known as the Finapres digital photoplethysmograph and has been shown to be a reliable measurement of beat-to-beat changes in blood pressure when matched to other invasive methods of measuring blood pressure directly in the brachial artery (Constant et al. 1999; Imholz et al. 1998). The finapres allows testing baroreflex function and sensitivity easier in populations, such as those who have been recently concussed, as they do not have to undergo invasive intra-arterial catheters (Parati et al. 1989). The finometer provides a reasonable approximation of intra-arterial blood pressure and is based on the volume clamp technique by Penaz in 1973. The finapres’s original method has been improved upon by Wesseling and co-workers (Parati et al. 1989). This method uses an inflatable finger cuff with a built in photoelectric plethysmograph that is capable of distinguishing the
dynamic unloading of the finger arterial walls (Imholz et al. 1998) To ensure the air pressure in the cuff is similar to the arterial blood pressure, a fast pneumatic servo system and dynamic servo set point adjuster confirm that arterial unloading is at zero transmural pressure (unloading refers to the size of the artery being measured when its internal pressure (arterial pressure) is equivalent to the external pressure) (Penaz J. 1973; Wesseling, K.H. de Wit, B. Settles, JJ. Klawer, WH. 1982; Boehmer. 1987). Continuous automatic adjustments of external pressure on the artery help maintain this unloaded condition. This is done through adjustments to intrarterial pressure variations that occur instantaneously. The external pressure then equals internal pressure and is reported with the use of systolic mean and diastolic pressure. The built in sensor in the cuff is capable of measuring artery size roughly every minute and correcting for changes in smooth muscle by contraction or relaxations that are sensed. The inflatable bladder in the cuff is used to apply the external pressure to the artery. Automatic calibration and adjustment of the cuff is done using a high-speed electropneumatic servo control system (Boehmer. 1987). The finger pressure waveform produces beat-to-beat outputs of heartbeats, systolic and diastolic, mean pressure and pulse rates (Imholz et al. 1998). Along with the finger cuff the finapres uses a small box that is attached to the wrist with Velcro that aids in the continuous adjustment of cuff pressure according to changes in the plethysmographic output (basis of the amount of blood passing through or present in the finger) (Imholz et al. 1993; Imholz et al. 1991). Both the cuff and the box on the arm feed back into the main unit that encompasses the air pump, electronics and the computer. The Finapres is a good technique to measure the dynamic response of mean and diastolic arterial pressure changes in both resting conditions and during variable changes in

Figure 2.1 Example photograph of the Finapress machine
posture. The Finapres has been proven to be more superior to the manual or automatic blood pressure cuff (sphygmomanometric measurement). This is because the automatic or manual blood pressure cuffs can lose accuracy when the measurement is not performed at rest (Casadei et al. 1988; Mancia. 1983). One of the main benefits of the Finapress measurement is that it can both record beat-to-beat changes but also changes in blood pressure that occur rapidly. For instance the finapres can decipher blood pressure rises, systemic vasoconstriction and blood pressure falls systemic vasodilation, which implies that alteration in blood pressure gradients and peripheral tone do not impair the finapreses ability to read intra-arterial pressure (Parati et al. 1989).

Finapres measurement was consistently taken from the middle finger of the left hand. The appropriate sizing of cuff was also used to insure the best fit to the individual subjects hand and help eliminate error (Imholz et al. 1990). To calibrate the finapres an intermittent arterial pressure was taken both manually and with an automatic blood pressure machine (SunTech Tango, SunTech Medicals, Morrisville NC, USA). All data was collected via a single commercially available software program (LabChart version 7.1 AD Instruments). Data were converted to a digital signal by a system called Powerlab (Powerlab/8SP ML 795; ADInstruments, Colorado Springs, CO, USA).

2.8 Assessment of outcome measures: Baroreceptor sensitivity using stand squats
Acute blood pressure changes that occur due to postural changes are mainly associated with baroreflex engagement (Scheen and Philips. 2012). The main elements of blood pressure regulation (eg. cardiac- R-R interval, Heart rate, and vascular resistance) are key to maintaining blood pressure homeostasis (Sunagawa et al. 2001). A reproducible and non-invasive way to measure baroreceptor response and function is through stand squats. Stand squats (SS) help to open up the closed loop aspect of the baroreceptor function by allowing specific areas such as blood pressure to be manipulated. Investigation of blood pressure and heart rate changes during
both the changes from standing to squatting and squatting to standing can provide insight into
the performance of the baroreflexes and hemodynamic homeostasis (Zhang et al. 2009; Rossberg
and Penaz. 1988). Evaluation of SS may involve both the arterial (change in blood pressure and
heart rate and cardiovagal baroreflex mechanisms (change in blood volume/pressure) (Wieling et
al. 2007). Originally in (Sharpey-Schafer. 1956) 1956 Sharpey-Schafer discovered that squatting
cause an increase in systemic arterial blood pressure that was accompanied by bradycardia.
This implied that the squatting induced an increase in cardiac output by increasing venous return
suggesting that baroreceptor activity was first primary and that bradycardia was a secondary
response in normal individuals via the baroreceptor and chemoreceptor mechanisms. (Sharpey-
Schafer. 1956). Tachycardia proceeded to follow standing (O'Donnell and McIlroy. 1962;
Marfella et al. 1994). O’Donnell et al 1962 found that as well as bradycardia there was also an
increase in arterial blood pressure and central blood volume in the standing position.
O’Donnell’s idea has changed slightly. Upon standing there is a transient decrease in blood
pressure, which is immediate and temporary. This is due to a difference between arterial inflow
and outflow that is visible by a decline in total peripheral resistance and increase in cardiac
output on standing (Wieling et al. 2007). The decrease in vascular resistance, venous emptying,
mediated vasodilation and cardiopulmonary sympathetic withdrawal plays a crucial role in the
interaction between the initial fall in blood pressure and standing ((Krediet et al. 2007; Rossberg
Stand squats produce marked swings in blood pressure. Upon squatting there is a transient peak
in arterial blood pressure after about 2-3 seconds that is attributed to a sudden increase in cardiac
output as a result of increased venous return from the lower limbs (Krediet et al. 2005). This has
been associated with vagal withdrawal and sympathetic activation(Scheen and Philips. 2012).
Upon standing from a squat there is a reduction in blood pressure that has been attributed to a
reduction in cardiac output and vascular resistance due to a prompt translocation of central blood
volume to the lower limbs (Wieling et al. 2007). The dramatic changes that occur in arterial blood pressure and heart rate from SS can partially be attributed to shifts in central blood volume and peripheral vascular resistance (O'Donnell and McIlroy. 1962; Wieling et al. 2007). The dramatic changes in blood pressure and R-R interval can be seen in the form of oscillations.

![Figure 2.2 Example of change in arterial blood pressure (ABP) and heart rate (HR) during a single squat stand.](image)

These oscillations in blood pressure can be represented at different frequencies. The different frequency ranges are Very low Frequency (VLF) which is 0.02-0.07 Hz (i.e., < 2.4 oscillations per minute. The VLF is much less defined in terms of the system that it evokes the most i.e. parasympathetic/sympathetic control compared to low and high frequency and the existence of a specific physiological process attributable to this frequency is still questioned (Anonymous 1996a)(Heart rate task force). The next frequency range is Low Frequency (LF), which is 0.07-0.2 Hz (2.4 -9 oscillations in blood pressure per minute). Low frequency is under both sympathetic and parasympathetic control; however, the periodic oscillations in blood pressure with certain stresses such as squatting are under mostly sympathetic control (Saul et al. 1990). The last frequency is High Frequency (HF), which is 0.2-0.4 Hz (9-24 oscillations per minute). Higher frequency is related to the effects of respiration and cardiovascular control and is solely
under parasympathetic control. It represents regulation via the cardiac vagal activity and represents heart beat oscillations occurring due to respiratory frequency (Goldstein et al. 1998). For the purpose of this experiment HF was not used, as the effects of respiration were not investigated. The main focus was on VLF and LF as baroreflex function has been shown to be present in the frequency range of 0.05 and 0.1 Hz (Hammer and Saul. 2005; van de Vooren et al. 2007).

Figure 2.3 Changes in arterial blood pressure (ABP) and heart rate (HR) under resting and during stand squats.

Stand and squats can be implemented in a clinical setting with minimal equipment and the movements are also very relevant in daily life. These characteristics make it a useful protocol to execute on athletes who have just been concussed. The intense hemodynamic changes that occur from SS can be analyzed and valuable information about BRS and HR homeostasis can be acquired, especially in patients who may have autonomic failure (Philips and Scheen. 2011).

2.9 Stand Squat protocol
Subjects stood from a seated position for 5 minutes to establish baseline. Once baseline was met subjects performed repeated stand squats at two different frequencies set by a metronome. The first frequency is very low 0.05 Hz (10 second squat-10 second stand) and the second frequency is low 0.10 Hz (5 second squat-5 second stand) for 5 minutes. An example can be seen in figure
2.4. In between each frequency there was a 5-minute recovery. During this test the subjects were informed to squat evenly at the same depth each time to ensure they were consistent across five minutes and squatting at the right frequency. To ensure that squats were done evenly the experimenter did close visual monitoring and gave vocal instruction. Subjects were instructed to breath normally as it was imperative they did not hold their breath or perform the valsalva manoeuvre while standing up, as this may alter results and evoke different physiological responses. To ensure breathing was continuing normally end tidal CO$_2$ and O$_2$ patterns were monitored on Labchart. Finally subjects were instructed to keep their finger from rubbing their shorts or any other area on their body to ensure there was no movement to interfere with the finapres.

The above measurements will help measure the primary outcome measures of this study. The primary measurements are to determine if baroreceptor sensitivity and heart rate variability are influenced by sport related concussion in adolescents and young adults aged 14-25. Further investigation into the area of the cardiovascular regulatory mechanisms is needed to better understand the physiological changes that occur after a sport related concussion and the potential dysfunction that may occur. Understanding this information could provide insight into the regulatory mechanisms that could be altered after a sport related concussion.
2.10 Data analysis
Two different analysis procedures were done: frequency and time domain. Frequency analysis takes steady, stationary, fluctuating time dependent signals and breaks them down into sinusoidal components (i.e. taking the R-R interval and breaking it down into its oscillations).

The assessment of the baroreceptor response through repeated stand-squats was analyzed using a transfer function analysis (TFA). TFA allows for the quantification of the magnitude with which the input signal, mean arterial pressure, is reflected in the output signal (R-R interval). This was done by a cross-spectrum between MAP and R-R interval in determining cardiovagal baroreceptor sensitivity to derive the transfer function gain, phase, and coherence indices. Gain is calculated from the relation of tachycardia and or/ bradycardic responses to decrease and/or increases in systemic arterial pressure (Parati et al. 2000). Gain at a given frequency describes how the amplitude of a sinusoid at that frequency is damped or amplified by the system. Moving from squatting to standing the drop in blood pressure is associated with the tachycardia reflex followed by a rapid return to baseline values. The mirror changes that occur in systolic blood pressure and heart rate during the initial stand squat transition have been shown to be equivalent to those that are observed in pharmacological testing (Scheen and Philips. 2012). Phase refers to whether or not the change (i.e., latency) occurs before or after blood pressure changes and can be reflected in a positive or negative phase. Phase describes how a wave at that frequency is shifted in absolute time. Coherence assesses the statistical significance of spectral estimates of gain and phase frequency response. Each 5-minute recording will be subdivided into five successive windows that overlap. The data within each window was linearly detrended, passed through a Hanning window, and subjected to fast Fourier transform analysis. Detrending is used to properly analyze many time series and is used for a few reasons. It prevents time series from being associated if correlations are not present, and if correlations do exist. Detrending shows a real relationship of functional dependence. Detrending linearly is based on the idea that for a
given window size of an entire signal, the method withdraws linear polynomials from the original signal in order to attain local stationary signals (Horvatic. 04/2011). The Hanning window is beneficial for analyzing sections longer than the time duration of the window and for general-purpose applications. The Hanning curve is in the shape of half of a cycle of a cosine wave. Fast Fournier Transform analysis is the Fourier Transform of a block of time data points and represents the frequency composition of the time signal. Fast Fournier Transform analysis is advantageous because it has a fairly simplistic algorithm, the spectral components can be identified easier independently of preselected frequency bands, and there is easy identification of the spectrum with an automatic calculation of low and high frequency power components. (Anonymous 1996b)

For spontaneous oscillations at rest, spontaneous MAP and velocity spectral powers, and the mean value of transfer function coherence, gain and phase will be calculated in the very low (VLF, 0.02-0.07 Hz) and low (LF, 0.07-0.20 Hz) frequency ranges at which the baroreceptor response is thought to be operant (Zhang et al., 2009). In order to make a comparison between spontaneous and dynamic oscillations induced by repeated squat-stand manoeuvres, TFA of the stand-squats will be carried out in the same frequency domains as used for spontaneous oscillations (i.e., squat-stand manoeuvres at 0.05 will be analyzed in the VLF domain (0.02– 0.07 Hz); and 0.1-Hz manoeuvres will be analyzed in the LF domain (0.07– 0.2 Hz). Heart rate variability is analyzed in a short-term recording as opposed to twenty-four hr. recordings and uses VLF, LF, and HF. Recording under five minutes is a suspicious measurement when trying to determine the power spectral density. Autonomic variations of heart periods may vary upon changes in frequency (Malliani et al. 1991). Measurement of VLF, LF, and HF was made in absolute values of power as well as normalized units, which represent relative value of each power minus the VLF. The LF and HF is used to emphasize the control of both the parasympathetic and sympathetic nervous system.
Time domain analysis was also used to assess both HRV and BRS. A series of 9-20 of instantaneous heart rates and each QRS complex (normal-to-normal interval- all intervals between QRS complexes that are a result of sinus node depolarization) values were calculated and the standard deviation was found. The standard deviation of the normal to normal reflects all the cyclic components responsible for variability in the recording period. For BRS mean arterial pressure was compared to the change in heart rate. This was analyzed by taking the peak squat and nadir stand values and averaging them, the same was done with the maximum and minimum heart rate value. Standard deviation and slope of the linear regression were used to calculate gain (i.e. change in HR for a given change in BP, because of known hysteresis in the human baroreflex (Studinger et al. 2007), the linear regressions were calculated separately for the increases (hypertension) and decreases (hypotension) in mean arterial pressure.

2.11 Statistical analysis
Statistical analysis was performed using SPSS 16.0.2 (SPSS, Chicago, IL). The effects of concussion (concussed vs. healthy controls) or testing day (3, 7, 14, 30 days) on blood pressure variables and heart rate were assessed using mixed model two-way ANOVAs. Relationships between blood pressure and heart rate variables were determined by linear regression. To determine the day-to-day variability, the coefficient of variation and the intra-class correlation coefficient was used of each outcome measure (Atkinson and Nevill, 1998). Post-hoc comparisons of testing day or interaction effects were examined using Bonferroni correction. Data were presented as means ± SD, and a level of P ≤ 0.05 was considered statistically significant.

It is important to note that within variables there was a mix of normally and not normally distributed data (assessed using the Kolmogorov-Smirnov test). Both parametric and non-parametric tests were run on the data set. Similar results were found between tests; however,
when using the Bonferroni correction, any significance seen between or within groups was lost in the non-parametric tests. Therefore, the results are reported from the parametric outputs.

For the non-parametric tests, between group differences were assessed using the Kruskal-Wallis test and between days comparisons were run using the Friedman’s ANOVA. Post-hoc analysis was conducted using the Mann-Whitney test (the independent t-test equivalent) and the Wilcoxon signed-rank test (the dependent t-test equivalent).

Before testing occurred, reliability measurement testing was done using a test re-test method. The test re-test method allowed for a subject to be tested at two separate time points to ensure that measurements were taken appropriately. Reliability testing is crucial as it allows insight into the amount of variation that can occur between subjects in the main outcome measurements such as BP and HR, and gives insight into whether or not these changes or variation is a result of physiological changes or do to tester error (Field 2010).

To regulate the day-to-day variability, two common types of statistical analysis were run on each main outcome measure. The first was the coefficient of variation and the second was the intra-class correlation coefficient.

The coefficient of variation (CV) was used to calculate the distribution of variability within the data set. The CV is calculated by taking the standard deviation divided by the group mean. The results from CV indicate if there is a greater or lesser distribution within the variable. For example when the CV is high, it means that there is a higher distribution within the variable and more variability. When the CV is low there is less of a distribution of the variable and therefore a better sense of reliability. To inquire about the within subject variation between days ANOVA was run to adjust absolute deviations (Field 2010). Results of the coefficient of variation can be viewed in Figure 2.5 and 2.6.
Intra-class correlation coefficient (ICC) was used along with Coefficient of Variations to assess the within subject differences. The ICC uses the analysis of variance and establishes error. The ICC method is beneficial as it is sensitive to systematic bias, which could have been present within the data. Intra-class correlation coefficient is measured using a scale. An ICC greater than 0.9 indicates excellent reproducibility while 0.7-0.8 is very good and less than 0.6 is weak.
Within this study there was a large variation between subjects however this has been attributed to different physiological differences and variations between subjects as opposed to intra rater error (Field 2010). The average ICC’s can be viewed in table 2.1

Table 2.1 Intra-class coefficient of absolute heart rate and mean arterial pressure values at rest and during exercise in a subset control group.

<table>
<thead>
<tr>
<th></th>
<th>ICC</th>
<th>Lower</th>
<th>Upper</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Base BP</td>
<td>0.75</td>
<td>0.359</td>
<td>0.934</td>
<td>12.557</td>
<td>0.05</td>
</tr>
<tr>
<td>Base HR</td>
<td>0.90</td>
<td>0.167</td>
<td>0.986</td>
<td>8.251</td>
<td>0.019</td>
</tr>
<tr>
<td>Max MAP</td>
<td>0.72</td>
<td>0.478</td>
<td>0.903</td>
<td>12.664</td>
<td>0.049</td>
</tr>
<tr>
<td>Max HR</td>
<td>0.85</td>
<td>0.135</td>
<td>0.978</td>
<td>7.649</td>
<td>0.022</td>
</tr>
<tr>
<td>Min MAP</td>
<td>0.74</td>
<td>0.234</td>
<td>0.905</td>
<td>6.578</td>
<td>0.05</td>
</tr>
<tr>
<td>Min HR</td>
<td>0.90</td>
<td>0.371</td>
<td>0.985</td>
<td>9.521</td>
<td>0.014</td>
</tr>
</tbody>
</table>

HR, Heart rate, MAP Mean arterial pressure, ICC Intra-Class Correlation Coefficient. Max referes to the peak in blood pressure and min referes to the nadir values of blood pressure.
Chapter 3 Results

3. 1 Rest

Concussed athletes were tested on Day 4±1, Day 8±1, Day 16±3 and Day 30±6 following their SRC. Controls were tested on Day 3±2, Day 7±1, Day 15±4, and Day 28±2. Subject characteristics are depicted in Table 3.0. The average age of SRC was 17.5±2 years old with an average BMI of 24±1 kg/m². Control subjects (CS) were 20±2 years old and had a BMI of 22±2 kg/m². There was a significant difference for age and BMI between groups (Table 3.0, p < .05). Nevertheless, resting cardiovascular variables (HR, MAP, SBP, DBP) and gas exchange (P\textsubscript{ET}O\textsubscript{2}, P\textsubscript{ET}CO\textsubscript{2}) variables were not significantly different between groups or across any day of testing Figure 3.0

Table 3.0 Comparison of anthropometric and resting data of concussed athletes (SRC) and control subjects (CS).

<table>
<thead>
<tr>
<th></th>
<th>CS Mean ± SD</th>
<th>SRC Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>20 ± 2</td>
<td>17.5 ± 2*</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>182 ± 10</td>
<td>172 ± 8</td>
</tr>
<tr>
<td>Body Mass (kg)</td>
<td>73 ± 10</td>
<td>73 ± 9</td>
</tr>
<tr>
<td>BMI (kg.m\textsuperscript{-2})</td>
<td>22 ± 2</td>
<td>24 ± 1*</td>
</tr>
<tr>
<td><strong>Cardiovascular:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>85 ± 7</td>
<td>86 ± 7</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>118 ± 6</td>
<td>123 ± 6</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>68 ± 9</td>
<td>67 ± 10</td>
</tr>
<tr>
<td>HR (beats · min\textsuperscript{-1})</td>
<td>64 ± 9</td>
<td>66 ± 7</td>
</tr>
<tr>
<td><strong>Gas Exchange:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P\textsubscript{ET}CO\textsubscript{2} (mmHg)</td>
<td>37 ± 4</td>
<td>40 ± 2</td>
</tr>
<tr>
<td>P\textsubscript{ET}O\textsubscript{2} (mmHg)</td>
<td>107±7</td>
<td>105±4</td>
</tr>
</tbody>
</table>

There was no significant difference in resting cardiovascular, or gas-exchange variables across days, the resting averages reported here are the average across days. BMI Body Mass Index, MAP Mean Arterial Pressure, SBP Systolic Blood Pressure, DBP Diastolic Blood Pressure, P\textsubscript{ET}CO\textsubscript{2} End-tidal carbon dioxide, P\textsubscript{ET}O\textsubscript{2} End-tidal oxygen. Significance between groups * p<.05. Values are mean ± (SD)
Figure 3.0 Resting averages of cardiovascular and gas-exchange variables ($P_{ETCO_2}$, $P_{ETO_2}$, HR, MAP, SBP and DBP) across the four testing days in concussed athletes (black dots) and controls subjects (white dots) Error Bars represent the mean ± SD.
3.2 Analysis in the time domain

The absolute change in variables from stand squats is summarized in Table 3.1. Figure 3.1 and Figure 3.2 Illustrate the absolute maximum and minimum changes of BP and HR from baseline across the four different days of testing in both 0.1Hz as well as 0.05Hz. There was no significant difference after running a nonparametric test other than an interaction between day and group in peak MAP 0.1Hz (f 3.574) and p<0.040 and HR min 0.05Hz (f 4.43) and p<0.024; however, this difference was not significant when compared to controls. Further t-tests were run in both 0.1 Hz and 0.05Hz. On Day 7 for the difference from max MAP and baseline 0.1 Hz was a statistical significance of p <0.05 (t score= 2.183). This was similar for peak MAP on Day 7 p<0.020 t-score 2.757 and min MAP and baseline 0.1Hz on Day 30 p<0.037 t-score -2.409 Table 3.1.
Figure 3.1 Changes Graphs reflect changes from baseline during stand squats at 0.1Hz. Mean arterial pressure (MAP) and heart rate (HR), Maximum (Max), Minimum (Min) from Baseline. Results show no significant difference between groups.
Figure 3.2 Graphs reflect changes from baseline during stand squats at 0.05Hz. Mean arterial pressure (MAP) and heart rate (HR), Maximum (Max), Minimum (Min) from Baseline. Results show no significant difference between groups.
3.3 Analysis in the frequency domain

During squatting, arterial pressure increased acutely and then fell slightly and stabilized at a level above baseline. Mean values of increase in SBP on average across all four visits for SRC were 22 mmHg in 0.05Hz and 20 mmHg in 0.01 Hz and for CS were 20 mmHg in 0.05Hz and 18 mmHg in 0.01 Hz. There were no between-group differences in these responses. In response to the changes in blood pressure, heart rate decreased on average by 15 beats/min in 0.05Hz and 10 beats/min in 0.01Hz in SRC. In CS, heart rate also decreased on average by 16 beats/min in 0.05Hz and 13 beats/min in 0.01Hz. During standing SBP on average fell by 24 mmHg in 0.05Hz and 20 in 0.1Hz in SRC and in CS it fell by 26 mmHg in 0.05Hz and 21 in 0.1Hz in. When pressure fell, heart rate in SRC increased on average by 30 beats per minutes in 0.05Hz in and 26 beats/min in 0.1Hz. In CS heart rate increased by 34 beats/min in 0.05Hz and 27 beats/min in 0.1Hz. There was no difference in the ratio of maximal changes in R-R interval divided by maximal changes in SBP indicating that BRS was not different in SRC or CS and there were no difference in HRV (see Table 3.1, Figure 3.8 and 3.10.).
Table 3.1 Cardiovascular variability in mean arterial pressure results from time domain analysis in both SRC and CS.

<table>
<thead>
<tr>
<th>Visit</th>
<th>SRC Baseline (mmHg)</th>
<th>SRC Max MAP (mmHg)</th>
<th>SRC Min MAP (mmHg)</th>
<th>SRC Diff MAP Max &amp; Base (mmHg)</th>
<th>SRC Diff MAP Min &amp; Base (mmHg)</th>
<th>SRC Diff MAP Max &amp; Min (mmHg)</th>
<th>CS Baseline (mmHg)</th>
<th>CS Max MAP (mmHg)</th>
<th>CS Min MAP (mmHg)</th>
<th>CS Diff MAP Max &amp; Base (mmHg)</th>
<th>CS Diff MAP Min &amp; Base (mmHg)</th>
<th>CS Diff MAP Max &amp; Min (mmHg)</th>
</tr>
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<tbody>
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</tr>
<tr>
<td>0.05Hz</td>
<td>85±11</td>
<td>88±9</td>
<td>107±16</td>
<td>115±12</td>
<td>58±16</td>
<td>69±17</td>
<td>22±19</td>
<td>28±10</td>
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<td>-27±14</td>
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<td>0.1Hz</td>
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<td>107±14</td>
<td>114±7</td>
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<td>-14±12</td>
</tr>
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<td>Visit 2</td>
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<tr>
<td>0.05Hz</td>
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<td>66±10</td>
<td>22±24</td>
<td>17±7</td>
<td>-21±18</td>
<td>-21±12</td>
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<td>106±8</td>
<td>104±14</td>
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<td>-26±11</td>
<td>-12±8</td>
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<td>32±4</td>
</tr>
</tbody>
</table>

Mean arterial pressure (MAP) Maximum (MAX), Minimum (MIN), Baseline (Base). * Indicated significant difference (p<0.05) between days. There was no significant difference between groups in any of the cardiovascular variables during the time domain analysis in MAP or HR. There were however significant differences between days for SRC however they were not significant compared to controls. Values are mean (SD)
Table 3.2 Cardiovascular variability in heart rate results from time domain analysis in both SRC and CS.

<table>
<thead>
<tr>
<th>Visit</th>
<th>HR Baseline (BPM)</th>
<th>Max HR (BPM)</th>
<th>Min HR (BPM)</th>
<th>Difference HR Max &amp; Base (BPM)</th>
<th>Difference HR Min &amp; Base (BPM)</th>
<th>Difference HR Max &amp; HR Min (BPM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SRC</td>
<td>CS</td>
<td>SRC</td>
<td>CS</td>
<td>SRC</td>
<td>CS</td>
</tr>
<tr>
<td>0.05Hz</td>
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<td></td>
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<tr>
<td>Visit 1</td>
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<tr>
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<td>111±9</td>
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</tr>
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<td>100±6</td>
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<tr>
<td>Visit 4</td>
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<td>99±7</td>
<td>98±9</td>
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<td>59±10</td>
</tr>
</tbody>
</table>

Heart rate (HR), Maximum (MAX), Minimum (MIN), Baseline (Base). * Indicated significant difference (p<0.05) between days. There was no significant difference between groups in any of the cardiovascular variables during the time domain analysis in MAP or HR. There were however significant differences between days for SRC however they were not significant compared to controls. Values are mean (SD)
During active stand squats, marked oscillations in arterial pressure and heart rate were produced at the frequencies of 0.05 Hz and 0.1 Hz. When compared to CS induced R-R variability was equivalent to that of SRC, consistent with the similar values in transfer function gain between SBP- R to R interval. The SRC had an average gain of 7.2 ms/mmHg over the four visits and CS had a gain of 7.5 ms/mmHg in 0.1Hz. In 0.05Hz SRC had an average gain of 7.2 ms/mmHg over the course of the four visits and CS had a gain of 8.6 ms/mmHg (Figure 3.3 & 3.4). These values are consistent with normal BRS gain values. There was a change in gain between Day 3 and Day 14 for SRC. On Day 3 mean gain was 6.7 ms/mmHg and was reduced on Day 7 to 5.8 ms/mmHg suggesting that on Day 7 SRC subjects had a reduced BRS. Upon Day 14, however, gain values returned to 6 ms/mmHg and then on Day 30 were up to 7ms/mmHg suggesting that BRS had returned to more sensitive values than on Day 7. Although there were changes in gain values in SRC these were not significant when compared to CS. Nor was there a significant difference in RRI values in SRC indicative of HR changes (Figure 3.8). There was a significant difference in non-parametric testing for phase in SRC 0.1Hz between Day 7 and Day 30 p<0.028 that could indicate the change in HR reacting faster after blood pressure change over the course of the visits Phase curves are illustrated in Figure 3.5 &3.6. Table 3.2 represents a summary of cardiovascular variability during active stand squats in SRC and CS. For separation by visit and Hz, and related individual responses, please see all of appendix C.

Symptom monitoring throughout testing and the change in symptoms can be seen in table 3.4 and 3.5.
Peak value of coherence was between 0.81 to 0.99 during squat-stand maneuvers under all conditions (Figure 3.7). Gain and phase at the peak value of coherence were similar to the mean values averaged over each of the corresponding frequency range.

<table>
<thead>
<tr>
<th>Frequency (Hz)</th>
<th>SRC</th>
<th>CS</th>
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<th>SRC</th>
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<th>SRC</th>
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<th>SRC</th>
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<tbody>
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<td><strong>Day 3</strong></td>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>0.05 Hz</td>
<td>0.047 ± 0.005</td>
<td>0.049 ± 0.003</td>
<td>0.047 ± 0.005</td>
<td>0.049 ± 0.003</td>
<td>0.047 ± 0.005</td>
<td>0.049 ± 0.003</td>
<td>0.047 ± 0.005</td>
<td>0.049 ± 0.003</td>
<td>0.047 ± 0.005</td>
<td>0.049 ± 0.003</td>
<td>0.047 ± 0.005</td>
<td>0.049 ± 0.003</td>
</tr>
<tr>
<td>0.1 Hz</td>
<td>0.094 ± 0.007</td>
<td>0.096 ± 0.009</td>
<td>0.094 ± 0.007</td>
<td>0.096 ± 0.009</td>
<td>0.094 ± 0.007</td>
<td>0.096 ± 0.009</td>
<td>0.094 ± 0.007</td>
<td>0.096 ± 0.009</td>
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<tr>
<td>0.05 Hz</td>
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<td>0.051 ± 0.004</td>
<td>0.048 ± 0.004</td>
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<td>0.048 ± 0.004</td>
<td>0.051 ± 0.004</td>
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</tr>
<tr>
<td>0.1 Hz</td>
<td>0.098 ± 0.006</td>
<td>0.100 ± 0.004</td>
<td>0.098 ± 0.006</td>
<td>0.100 ± 0.004</td>
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Figure 3.3. Graphs A, B, C represent the changes in gain over the course of four visits (V) from stand squats at 0.1Hz. A: Change in SRC & CS. B: Change in Gain CS only. C: Change in Gain SRC only.

There was no significant difference between groups.
Figure 3.4 Representing Driven gain data from stand squats at 0.1Hz in SRC and CS. Gain is an indicator of the cardio-vagal baroreflex sensitivity. Visit 1 (V1) Visit 2 (V2) Visit 3 (V3) Visit 4 (V4).
Figure 3.5 Graphs A, B, C represent the driven changes in phase over the course of four visits (V) from stand squats at 0.1Hz. A: Change in SRC & CS. B: Change in phase CS only. C: Change in phase SRC only. There was no significant difference between groups.
Figure 3.6 Representing Driven phase data from stand squats at 0.1Hz in SRC and CS. Phase is an indicator of whether or not the change (i.e., latency) occurs before or after blood pressure changes and can be reflected in a negative phase. Phase describes how a wave at that frequency is shifted in absolute time. Visit 1 (V1) Visit 2 (V2) Visit 3 (V3) Visit 4 (V4). There were no significant differences between groups for Phase.
Figure 3.7 Graphs A, B, C represent the driven changes in coherence over the course of four visits from stand squats at 0.1Hz. A: Change in SRC & CS. B: Change in phase CS only. C: Change in phase SRC only.

There was no significant difference between groups.
Figure 3.8 Power spectra of the R-R value from stand squat maneuvers in SRC and CS at 0.01Hz. Mean RRI Power spiked at 0.1Hz, which was the driven frequency (low frequency) for stand squats and then the second spike represents respiration at a higher frequency ranges (respiratory frequency range and is indicative of parasympathetic activity). No significant difference in RRI power between groups.
Figure 3.9 Indicates the power spectra of the systolic blood pressure (SBP) value from stand squat maneuvers in SRC and CS at 0.01Hz. Mean SBP Power was driven at the 0.1Hz (low) frequency, which is indicator of baroreceptor reflex activity. No significant difference in SBP power between groups.
Figure 3.10 Mean RRI Power Driven graph from stand squats at 0.1Hz SRC and CS. No significant difference in RRI power between groups.
Table 3.4 SCAT 2 numbers of symptoms (S) present PRE and POST testing in SRC and percent change between subjects across four visits

<table>
<thead>
<tr>
<th></th>
<th>Day 3</th>
<th></th>
<th>Day 7</th>
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<th>Day 14</th>
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<tr>
<td></td>
<td>Pre #S</td>
<td>Post #S</td>
<td>Difference between Pre and Post % Change</td>
<td>Pre #S</td>
<td>Post #S</td>
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<td>SRC01</td>
<td>17</td>
<td>19</td>
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<tr>
<td>SRC02</td>
<td>2</td>
<td>3</td>
<td>50.0%</td>
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<td>5</td>
<td>66.7%</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>SRC03</td>
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<td>6</td>
<td>0.0%</td>
<td>5</td>
<td>2</td>
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<tr>
<td>SRC04</td>
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<tr>
<td>SRC05</td>
<td>9</td>
<td>11</td>
<td>22.2%</td>
<td>5</td>
<td>9</td>
<td>80.0%</td>
<td>4.5</td>
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<tr>
<td>SRC06</td>
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<td>10</td>
<td>66.7%</td>
<td>5</td>
<td>10</td>
<td>100.0%</td>
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Table 3.5 SCAT 2 Symptom Severity (SS) PRE and POST testing in SRC between subjects across four visits

<table>
<thead>
<tr>
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<th>Day 7</th>
<th>Day 14</th>
<th>Day 30</th>
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<tbody>
<tr>
<td></td>
<td>Pre #</td>
<td>Post #</td>
<td>SS</td>
<td>Difference between Pre and Post % Change</td>
</tr>
<tr>
<td>SRC01</td>
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<tr>
<td>SRC02</td>
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<td>8</td>
<td>166.7%</td>
<td>4</td>
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<tr>
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<td>6</td>
<td>0.0%</td>
<td>6</td>
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<tr>
<td>SRC04</td>
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<td>60.5</td>
<td>14.2%</td>
<td>17</td>
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<tr>
<td>SRC05</td>
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<td>19</td>
<td>-9.5%</td>
<td>8</td>
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<tr>
<td>SRC06</td>
<td>12</td>
<td>20</td>
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Chapter 4 Discussion

4.1 Main findings

The main findings of this study indicate that BRS and HRV are not altered after suffering from a SRC. Although this finding contrasts with the original hypothesis that there would be reduced BRS and abnormal HRV in sports related concussions the issues of small sample size and marked within subject variability are acknowledged. Another likely possibility explaining the lack of notable differences is that, unlike severe head injury, a SRC is not enough of an injury to damage central control of the cardiovascular centers in the medullar regions of the brain and therefore efferent and afferent signal pathways remain intact and are capable of responding to different stressors maintaining BRS and HRV.

Data did show a slight change in BRS gain, which is an indicator of the cardio-vagal baroreflex (the higher the gain the more sensitive the cardio-vagal baroreflex), between Day 3 and Day 14 for SRC. On Day 3 mean gain was 6.7ms/mmHg and was reduced on Day 7 to 5.8ms/mmHg suggesting that on Day 7 SRC subjects had a reduced BRS. Upon Day 14, however, gain values returned to 6ms/mmHg and then on Day 30 were up to 7ms/mmHg suggesting that BRS had returned to more sensitive values than on Day 7. Although there were small changes in gain values in SRC these were not significant when compared to CS or related to pathology. Nor was there a significant difference in RRI values in SRC indicative of HR changes. Finally, the changes were directional and not physiological i.e., BRS was getting worse when the athletes were getting better and returning to play. Therefore, it is concluded that this change in gain in SRC subjects is most likely an artifact related to an type one error (false positive) and due to the large intra-subject variation in gain between SRC and CS and hence lack of between group difference. Otherwise BRS values seem to be consistent with normal BRS in healthy individuals (Parati et al. 1988) and HRV is not altered. Methodological aspects that are important for the interpretation of these conclusions are also considered.
4.2 Comparison to current literature

Resting variables such as HR, MAP and spontaneous HVR between SRC and CS were not different. Such findings are similar to a study by Gall et al. 2004 who found that there were no differences in HRV between concussed athletes and their controls at rest (Gall et al. 2004). This comparison is important as it indicates that there were no major changes in BRS and HRV; if there were, MAP and HR would be expected to differ between groups; therefore, changes in sensitivity are most likely quite subtle. Consistent with these findings, McMahon et al 2011, found that in rats, only moderate traumatic brain injury produced a significant difference in BRS compared to controls whereas mild brain injury did not (McMahon et al. 2011).

Though BRS and HRV were unaltered in this experiment, other studies have found that reduced BRS and HRV (indicative of higher sympathetic nerve activity and lower parasympathetic nerve activity) is more common in severe cases of brain injury and brain death (Korpelainen et al. 1996; Goldstein et al. 1998). This severity has been accredited to central brain regions involved in the regulation of the cardiovascular system that may be affected by brain injury, in particular the medulla, and the medial region of the nucleus tractus solitaries. In severe cases of brain injury medullary brainstem infarction is the reason for altered BRS and HRV (Goldstein et al. 1998; Korpelainen et al. 1996). Damage to the brainstem may cause a disruption of the cardiovascular centers, which are located in the medulla that can alter the vasoconstrictor area, the vasodilator area, and the sensory area. The vasoconstrictor area has norepinephrinergic neurons, which stimulate the vasoconstrictor neurons of the sympathetic nervous system. The vasodilator area has fibers from the neurons that project upwards to the vasoconstrictor area and inhibit vasoconstrictor activity. Finally the sensory area receives signals from the vagus and glossopharengeal nerve and output signals from the sensory area to control both the vasomotor and vasoconstrictor area. If these areas are damaged then the outputs may not be effective in regulating blood pressure or heart rate as the efferent and afferent signals cannot be processed.
properly (Giza and Hovda. 2001; Hovda et al. 1995; Lanfranchi and Somers. 2002; Korpelainen et al. 1996). This type of injury severity may not be present after an individual has suffered a SRC. In this case, the regulatory mechanisms at which many of these signals are centrally processed in the medulla remain intact, and therefore could be one explanation why BRS and HRV were unaltered in this experiment.

A study by Biswas et al in 2000, looked at HRV in critically ill children who had been admitted into the intensive care unit after suffering from traumatic brain injury. Again these individuals were in a more severe state, some even succumbing to brain death by the end of the study. As expected, the study reported alterations in HRV and autonomic dysfunction in subjects who had increased cerebral perfusion pressure (Biswas et al. 2000). Subjects from the current experiment averaged a return to play date after the second visit and are therefore not on the same severity scale which may be why no changes in HRV were seen. The Biswas study was also in children with a mean age of 7.5 yrs. This age group is much younger than the population in the current study and may be another reason why similar findings did not occur, as the way children and adults react to brain injury may be different (McCroty et al. 2004). Baguley et al (2006) also examined HRV following severe traumatic brain injury. This study showed that there was prolonged uncoupling of heart rate and HRV parameters compared to controls. The only issue with this experiment is that it was in subjects who were diagnosed with dysautonomia, which is characterized by paroxysmal changes in blood pressure, heart rate, respiratory rate, or sweating (Baguley et al. 2006). Again these subjects are in a more severe state at rest than any of our concussed subjects were through the course of their visit and are therefore not comparable.

The main findings of our study contrast with a study by Hilz et al. who found there was reduced cardiovagal modulation and BRS was present in individuals who suffered from a mild traumatic brain injury. This finding maybe attributed to the time frame (>5-43 months after mTBI) in which participants were recruited for their study and/or the larger sample size. Many changes
could have occurred between the initial injury and the time of testing. Also if these individuals were still suffering from symptoms 5-43 months after their injury they would be on the more severe end of the population. The greatest manifestations of symptoms following a concussion are seen in the first two weeks following the injury (Ellemberg et al. 2009). In the protocol for the current experiment, the subjects were tested within 72 hours of being concussed. Along with timing differences Hilz et al. 2010 used a standing method, which involved subjects being seated for at least two minutes and then standing. This would elicit a similar BRS response to squat stands, although it would not be as reproducible. Their sample size however was much larger (n=20 concussed, 20 controls) which would have increased their potential effect size.

There was no significant difference between the 5 males and 1 female within this study. This is consistent with findings by Fisher, Cook and Guasti. Their studies indicated that there are no sex differences between young men and young females on arterial baroreceptor function (Fisher et al. 2012; Cooke et al. 2002; Guasti et al. 1999).

In summary, this experiment showed normal BRS values and unaltered HRV in SRC when compared to controls. Changes in BRS and HRV have been reported to be more common in situations where severe brain/brainstem injury has occurred or even brain death. Variation between subjects, different testing times in other experiments in the literature and the population that these experiments were executed in may explain the between studies differences.

4.3 Arterial pressure and heart rate during squat stands and the involvement of leg muscles

Increases in arterial pressure during squats are mainly due to increases in cardiac output. Upon squatting leg muscles contract and arteries and veins in the legs compress as the heart moves closer to the level of the feet. This net effect removed the force of gravity on circulation permitting cardiac output to increase due to the increase in central blood volume and stroke volume (O'Donnell and McIlroy. 1962; Sharpey-Schafer. 1956; Krediet et al. 2005; Hanson et al.
1995). The changes in BP and HR were consistent to those of O’Donnell 1962, Wieling 2007, and Zhang 2009 who showed similar decreases in HR and increases in arterial pressure. This reduction in HR has been credited to the arterial baroreflex mechanisms and not the cardiopulmonary (Sharpey-Schafer. 1956; Zhang et al. 2009). The cardiopulmonary reflex has been associated more with changes in central blood volume and pressure and is thought to have minimal influence on the regulation of heart rate (Sharpey-Schafer. 1956; Zhang et al. 2009). That being said, the role of the cardiopulmonary baroreflex mechanisms cannot be completely ruled out and an integrated interaction between both the cardiopulmonary and arterial baroreceptors are active during changes in body position in day-to-day life (Zhang et al. 2009). The likelihood of an interaction between the activation of the muscle mechanoreflex or central command associated with muscle contraction in the legs has been purposed to influence pressure during stand squats (Rossberg and Penaz. 1988; Wieling et al. 2007). However, Zhang et al. 2009 provided evidence that during active stand squats the muscle reflex/central command does not alter the arterial/cardiac baroreflex function (Zhang et al. 2009).

4.4 Rational for frequencies and stand squat maneuvers
Repeated stand squat maneuvers at the frequencies of 0.05Hz and 0.1Hz produced large and coherent oscillations in arterial pressure and R-R interval. As mentioned (see methods: 2.8) stand squats are a reliable way to produce hemodynamic changes (Claassen et al. 2009; Zhang et al. 2009). Stand squats are a spontaneous non-invasive measurement technique that is clinically useful, easily reproduced, and allows for the avoidance of invasive pharmacological injections that can place a subject at risk for carotid stenosis rupture and transient hypo and hypertension (Fadel et al. 2003; Zollei et al. 2003). Other BRS measurement techniques such as the valsalva maneuver, are highly dependent on the subjects cooperation, not always an easy maneuver to perform, and do not always guarantee that significant blood pressure changes are occurring in certain diseased populations (Zollei et al. 2003). The modified Oxford technique provides little
control over the time course of the BP perturbation either between or within individuals across repeated tests and therefore would not be as beneficial in repetitive days as the stand squats would (Horsman et al. 2013). Although there is no gold standard for BRS measurement, stand squats are easy for the subject to perform as it is a common movement in daily life and there are no invasive methods to cause further discomfort in this population (Laude et al. 2004). The large and coherent oscillation in BP produced by repeated stand squats at two different frequencies VLF and LF, provided insight into cardiovascular regulation between BP and HR as well as the parasympathetic and sympathetic systems. The mirror changes in systolic BP and HR that appear from pharmacological testing using vasodilator/vasopressor agents during the Valsalva maneuver mimic those occurring during the initial squat stand transition (Scheen and Philips. 2012).

4.5 Assessment of baroreflex function using transfer function analysis
Transfer function analysis has been used to assess spontaneous changes in arterial pressure and R-R interval to gain insight into baroreflex function. Transfer function gain (higher baroreflex sensitivity) was higher, however not significantly higher, in 0.05Hz (7.2 ms/mmHg SRC/8.6 ms/mmHg CS) as opposed to 0.1Hz (7.2 ms/mmHg SRC/7.5 ms/mmHg CS) and there was a reduction in phase. This is consistent with the low pass filtering properties of the baroreflex function (Saul et al. 1991). When there is a decline in frequency, phase usually falls as well reflecting the low pass filter and the description of the arterial response to vagal perturbations. The fall in phase indicates that there is an increasing vagal excitation complemented by a decrease in arterial rate (Berger et al. 1989). Peak value of coherence was 0.81 to 0.99 at both frequencies supporting the validity of using the transfer function method (Zhang et al. 2009). This is important as the frequency range of coherent response is related to the spectral brand of input excitation (Berger et al. 1989). It should be noted that the magnitude of spontaneous changes that occur in BP and R-R interval might be variable among individuals and not just an
attribution of the baroreflex (Cooley et al. 1998). This is important to note as the sample size in this experiment is small and this large variation could explain some of the differences that were seen within SRC groups. Although these differences within SRC were not significant compared to control subjects it should be recognized that there are many lifestyle factors that can also influence HRV, such as environment or body temperature, which can cause decreases in HRV with environmental stress (Anonymous 1996b). Chronic activity level can cause HR to change and become lower over time and age can cause HRV to decrease (Anonymous 1996). Therefore it is important to note that there is fluctuation between subjects and when there is a sample size of six it is even more important to try and control for these things by having a good baseline and control subject matches.

4.6 Study limitations
The sample size of this experiment was small and therefore findings should be viewed as preliminary only. Participants were recruited from numerous teams and organizations within the Okanagan area. Some of these organizations included the UBCO Heat varsity athletes, Okanagan Hockey Academy, Pursuit of Excellence Hockey Academy, The Okanagan Sun football Club and various high school sports teams in the area to name a few. However due to scheduling issues, a lack of communication and commitment from parents and subjects, and a newly established concussion research center, participation in this study was a major issue. The original sample size was planned to be 15-20 subjects as this is the common number in most published literature in this area (Papaioannou et al. 2008; Goldstein et al. 1998; Hilz et al. 2011; Conci et al. 2001; Korpelainen et al. 1996; Baguley et al. 2006). In order to measure the effect of concussion on BRS and HRV a sample size of 40 or more individuals would be needed to see an effect size of 0.80 (i.e. calculated from BP and HR data using Cohen’s D; Field 2010). The consequences of a small sample size can be a wide confidence interval and a greater degree of variation. By using a power of 0.80 or greater the tester can be confident that their sample is
statistically powerful. Unfortunately due to the small sample size of this experiment the study was highly underpowered and therefore results should be viewed as preliminary. Though this study was underpowered, it does not mean that no effect was present (Altman and Bland 1995). The best recommendation is to follow up this study with a larger study to verify results.

There were strict pre-testing guidelines such as the avoidance of alcohol, caffeine or exercise as well as inclusion exclusion criteria such as ECG abnormalities or injuries that would cause one to be unable to exercise, that caused rescheduling or exclusion of participants. The timing of testing was also an issue as each visit was done at the same time of day over the course of all for visits to keep reliable. This played a role in scheduling conflicts once subjects started to return to their normal schedule of work/school and sport after injury and can help explain the variation of days between subjects.

There were missing data due to symptom exacerbation or a subject’s inability to make a visit and therefore ANOVA’s could not be run with such a small sample size (Field. 2009). Therefore non-parametric testing was run, as all of the data was not normally distributed. A non-parametric test does not rely on the restrictive assumptions of parametric tests and does not assume that the sampling distribution is normally distributed. Non-parametric testing makes few assumptions and is beneficial as it is a conservative method that is good for smaller sample sizes and missing data (Field. 2009). The Wilcoxon rank-sum test and the Mann-Whitney test were run as the equivalent non-parametric t-tests. As a complimentary approach, parametric analysis was also run and there were no differences between them.

4.7 Perspectives, significance and contribution to science
Squatting can be used as an active postural test capable of detecting baroreflex hemodynamic alteration. Measuring BP and HR during the transition from standing to squatting and squatting to standing and is a good measuring tool in populations who may not be able to have invasive measures performed on them.
The significance of altered baroreceptor sensitivity has substantial implications. This area of research at the cardiovascular and autonomic level will help provide insight to the physiological mechanisms that are affected after an individual suffers a concussion. Overall this experiment has established a proof of concept and feasibility of conducting these studies in SRC populations and laid the groundwork for a larger study to occur to help explain the cardiovascular implication of a concussion, which is an area that is in drastic need of investigation.

4.8 Future studies
Future investigation into cardiovascular autonomic function is needed, a larger sample size would help reduce some of the variability between subjects and create a better picture of what is happening in the SRC population. Along with a larger sample size, implementing a study that involved the selective measurement of low pressure, cardiopulmonary, and high pressure baroreceptors would help gain insight into the inner workings of the baroreflex mechanism in the concussed population. Accompanied by investigating the different receptors the increasing need for research on concussions and youth has been voiced (McCrory et al. 2004). Therefore a study that included both a young and older population would be very beneficial. Few studies exist that include adolescents and/or that look at the effect of concussive injuries on adolescents (McCrory et al. 2004; McCrory et al. 2013; Moser et al. 2007). Conducting such a study would provide valuable information on whether there are any differences between younger and older athletes who have been concussed. There is also evidence that if an adolescent shows greater symptoms after an injury they may have sustained a greater impact to the head than an adult with the same symptoms. Adolescents experience more serious acute and long-term symptoms than adults (Purcell and Carson. 2008; Moser et al. 2005; McCrory et al. 2004); therefore, adolescent physiology and the ability to respond to mechanical stressors maybe different than adults (McCrory et al. 2013). Research in this area is vital because we cannot translate the information that we have on adults to adolescents. Another interesting direction would be to examine the
severity of injury vs. functional outcome of HRV and BRS i.e. those who lost consciousness vs. those who did not.

**4.9 Conclusion**

Sport related concussions research is needed as there is a large population effected by this type of concussion. Many questions around the cardiovascular implications after a concussion still need to be answered and more research in this area is needed. There have been studies in different forms of brain injury however it is still unknown if these findings are transferable to the sport related concussion population. Cardiovascular measurement such as BRS and HRV are a beneficial way to assess autonomic function and can provide insight into cardiovascular health. This experiment did not show any impairment in BRS or alterations in HRV however, these findings are likely reflecting of the mild nature of SRC and/or the relatively small sample size. More research in this area is needed.
Bibliography


Appendices

Appendix A

Appendix A. 1 Summary of current research in traumatic brain injury and baroreceptor and cardiovascular irregularities.

<table>
<thead>
<tr>
<th>PAPER</th>
<th>What they did</th>
<th>Type of BRS measurement</th>
<th>Result</th>
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<tbody>
<tr>
<td>Effect of Acute Traumatic Brain Injury on Baroreflex Function (McMahon et al. 2011)</td>
<td>Brain injury was induced using the lateral fluid percussion brain injury model producing mild and moderate TBI, on 8 male Wistar rats. Control animals underwent identical surgical procedures but no applied cortical pressure.</td>
<td>Arterial baroreflex was assessed by determining the relationship between R-R interval and systolic blood pressure using the modified phenylephrine pressor test adapted for the rat. The arterial baroreflex was tested before (Tcon), post-TBI, at 10 min (T10), and 30 min (T30)</td>
<td>Acute TBI of moderate severity is associated with an early significant modification in arterial BRS. The clinical implications of this observation require further investigation.</td>
</tr>
<tr>
<td>Investigation of heart rate and blood pressure variability, baroreflex sensitivity, and approximate</td>
<td>20 brain injured patients due to multiple causes and treated in the intensive care unit were used, with HR and blood pressure recorded from monitors and analyzed on a daily basis.</td>
<td>Power spectral analysis estimating low frequencies (LF: 0.04-0.15 Hz), high frequencies (HF: 0.15-0.4 Hz), and their ratio were calculated to the approximate entropy, transfer function (TF), and approximate entropy (APEn)</td>
<td>In acute brain injury patients, low baroreflex sensitivity, and sustained decrease in LF/HF of HR signals are linked with a high mortality rate</td>
</tr>
<tr>
<td><strong>Uncoupling of the autonomic and cardiovascular systems in acute brain injury</strong> (Goldstein et al. 1998)</td>
<td>Studied 24 patients with a diagnosis of acute brain injury. Hypothesized that acute brain injury results in decreased heart rate (HR) variability and baroreflex sensitivity indicative of uncoupling of the autonomic and cardiovascular systems and that the degree of uncoupling should be proportional to the degree of neurological injury.</td>
<td>Used HR and blood pressure power spectral analysis to measure neuroautonomic regulation of HR and BP and the transfer function magnitude between BP and HR as a measure of baroreflex modulation. Severity of neurological injury and outcome are inversely associated with HR and BP variability and 2) there is direct evidence for cardiovascular and autonomic uncoupling in acute brain injury with complete uncoupling during brain death.</td>
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<td><strong>Frequency Analysis Unveils Cardiac Autonomic Dysfunction after Mild Traumatic Brain Injury</strong></td>
<td>Tested whether baroreflex challenge unveils abnormal responses of heart rate, blood pressure (BP), and sympathetic and parasympathetic responses to standing in patients with a history of mTBI.</td>
<td>Active standing up From 2-min intervals at rest and the initial 60-sec intervals after standing up, mean values and SD of all bio-signals were calculated. While supine, mTBI patients had reduced cardiovagal modulation and BRS. Upon standing, their BRS was still reduced, and patients did not withdraw parasympathetic or augment sympathetic</td>
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<td>(Hilz et al. 2011)</td>
<td>Impaired autonomic modulation probably contributes to cardiovascular irregularities post-mTBI.</td>
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<td>Blood pressure and heart rate variability and baroreflex sensitivity before and after brain death (Conci et al. 2001)</td>
<td>Eleven patients (six men; five women) aged between 21 and 66 years (mean age 49 years) neurological intensive care unit were enrolled in the study. Admitted for head injury, subarachnoid haemorrhage, cerebral haemorrhage, and cerebral tumors. All patients were in a coma, with a Glasgow coma scale of 3 to 4. Continuous monitoring of the baroreflex function without any intervention on the patient, estimated the baroreflex sensitivity on the heart (BRS) from the spontaneous fluctuations of SB. Simultaneously estimated BRS by two independent and complementary methods to obtain a more comprehensive evaluation of the baroreflex function: the sequence technique, which focuses on the baroreflex response to pressure transients, and the calculation of the so called “alpha index”, which reflects Baroreflex function was relatively normal up to a few hours before brain death then changed with the onset of brain death. All the changes found are likely to reflect the cessation of activity of the cardiovascular brain stem centers. These findings indicate that dynamic assessment of baroreflex sensitivity may be useful to complement the diagnosis of brain stem death.</td>
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the baroreflex response to rhythmic oscillations.
Appendix A.2 Summary table of current papers investigating HRV, concussion and brain trauma

<table>
<thead>
<tr>
<th>Paper</th>
<th>What they did</th>
<th>How they measured it</th>
<th>Outcome</th>
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<tr>
<td>Heart Rate Variability of Recently Concussed Athletes at Rest and Exercise (Gall et al. 2004)</td>
<td>Assess the neuroautonomic cardiovascular regulation in recently concussed Athletes at rest and in response to low-moderate steady-state exercise, using heart rate variability (HRV).</td>
<td>5-min ECG sample was taken at rest and then again a 5-min ECG sample was taken for 4 to 9 of the low-moderate intensity steady state exercise bout was used to assess HRV during exercise.</td>
<td>No difference at rest was detected between the concussed athletes and their matched controls in any of the HRV variables measured. However, across both exercise tests, the concussed group demonstrated a significant decrease in the mean RR interval, and low- and high-frequency power ($P &lt; 0.05$) in relation to their matched controls.</td>
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<tr>
<td>Heart rate variability after acute traumatic brain injury in children (Biswas et al. 2000)</td>
<td>Evaluate heart rate variability (HRV) by power spectral analysis of heart rate and its relationship to intracranial pressure (ICP), cerebral perfusion pressure (CPP), and outcomes in children with acute traumatic head injury.</td>
<td>The normalized total power from 0.04 to 0.15 Hz was used to quantify low-frequency HRV and from 0.15 to 0.40 Hz to quantify high-frequency HRV. The power spectral data from the 5-min samples were averaged over each hour of data collection, and an</td>
<td>Suggest that an ICP of $&gt;30$ mm Hg or a CPP of $&lt;40$ mm Hg may be associated with marked autonomic dysfunction and poor outcome. We speculate that HRV power spectral analysis may be a useful adjunct in determining the severity of neurologic insult and the prognosis for recovery in children.</td>
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<tr>
<td>Heart-rate variability in chronic traumatic brain injury (King et al. 1997)</td>
<td>Determine whether abnormal HRV is present in patients with traumatic brain injury (TBI) during the post-acute recovery phase.</td>
<td>24-h ambulatory ECG monitoring in seven TBI patients and in seven controls</td>
<td>Abnormalities in both time and frequency domains of HRV are present in TBI during the post-acute recovery phase.</td>
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<tr>
<td>Abnormal heart rate variability reflecting autonomic dysfunction in brainstem infarction (Korpelainen et al. 1996)</td>
<td>Brainstem infarctions frequently cause disturbances of cardiovascular and other autonomic functions, but the pathophysiologic mechanisms of these prognostically unfavorable complications are not well-known and plan to investigate</td>
<td>Analyzed the power spectrum of heart rate variability in 15 consecutive patients with brainstem infarction and in 15 age- and sex-matched healthy control subjects. The components of the power spectrum were measured from 24-hour electrocardiogram in the acute phase and at</td>
<td>Measured components of heart rate variability, i.e. total power (p&lt;0.01), very-low-frequency power (p&lt;0.001), low-frequency power (p&lt;0.01), and high-frequency power (p&lt;0.05), were significantly lower in the patients with medullary brainstem infarction than in the control subjects in the</td>
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1 month and 6 months after the infarction. By 6 months, these abnormalities had been reversed. On the contrary, heart rate variability in pontine brainstem infarct patients did not differ significantly from that in the control subjects.

| Dysautonomia and heart rate variability following severe traumatic brain injury (Baguley et al. 2006) | Investigate disconnection theories postulated as the cause of dysautonomia following severe traumatic brain injury (TBI) through analysis of heart rate variability (HRV) | Data were collected on age-matched subjects with and without dysautonomia (eight subjects in each group) and 16 non-injured controls. Data included injury details, continuous electrocardiograph recordings and rehabilitation outcome. | Dysautonomic subjects revealed prolonged uncoupling of heart rate and HRV parameters compared to non-dysautonomic subjects and controls. These findings represent direct pathophysiological evidence supporting the disconnection theory postulated to produce dysautonomia following TBI. |
| Analysis of heart-rate variability: a noninvasive predictor of death and poor outcome in patients with severe head injury | Measured HRV in patients with severe head injury to assess its potential as a monitoring tool. | Analysis of HRV was prospectively done on all intensive care unit patients. Concurrent data on intracranial pressure (ICP) and cerebral perfusion pressure (CPP) were collected. | Low HRV was associated with increased mortality and decreased rate of discharge to home. Abnormal HRV was associated with episodes of increased ICP and decreased CPP. |
**Heart rate variability (HRV) of patients with traumatic brain injury (TBI) during the post-insult sub-acute period**

(EW.ERRO R - Unable to find reference:59 7)

Evaluate heart rate variability (HRV) of patients with traumatic brain injury (TBI).

HRV was assessed in 20 patients with TBI during the sub-acute period post-injury (the first test was performed at a mean time post-insult of 38 days) and a matched control. The patients were examined twice, 1 month apart. The assessment included HRV (both in time and frequency domains), GCS, length of coma, brain CT, FIM and FAM.

Significant difference was found between patients and controls concerning HRV total power, i.e. frequencies between 0.01–0.6 Hz (high frequency p 1⁄4 0.003, low frequency p 1⁄4 0.013, total power p 1⁄4 0.034) and for standard deviation of RR interval p 1⁄4 0.011. HRV changes were related more to the timing of the evaluation than to the severity of the brain damage.
Appendix B.1 Diagnosis of a concussion can include one or more of the following clinical areas (McCory et al., 2013)

1. **Symptoms** ➔ Somatic, cognitive, and or emotional
2. **Physical signs** ➔ Loss of consciousness, amnesia, confusion
3. **Behavioral changes** ➔ Irritable, grumpy
4. **Cognitive impairment** ➔ Slow reaction times
5. **Sleep disturbances** ➔ Insomnia or sleeping more than usual

Appendix B.2 Concussion Brochure
Emerging Evidence

Concussion's symptoms can also impair mental functions associated with memory loss, rather than determining a player's ability to return to play. There is emerging evidence that a player's ability to return from a concussive injury may be more delayed than previously thought, even after current methods of assessment deem them 'healthy' and ready to return to play.

The SCRI is located at UBC School of Health and Exercise Sciences in conjunction with UBC HealthCare, Medicine and Dentistry. This project is funded by the Canadian Institute for Health Research, and The Canadian Academy of Sports and Exercise Medicine.

Team up with the Integrative Sports Concussion Research Group to help prescribe a safe return to play.

Integrative Research
Symptoms associated with mild TBI are caused by damaged brain function. This potential impairment in brain function may manifest in just one of many tasks. The SCRI will be examining.

Our study is designed to assess memory and cognitive function in young athletes. Our aim is to provide a new benchmark in assessing return to play for athletes using both measures of performance - mental and physical - to assess health.

We are recruiting both male and female athletes, between the ages of 14 and 25 years old, who play a contact sport. Three groups of subjects will be separated into:

- Recently concussed athletes (recruited within three days of testing)
- Previously concussed athletes who are currently asymptomatic
- Healthy age-matched controls who have never received a concussion.

Here's what will happen

Subjects will be asked to visit our laboratory at UBC's Okanagan campus. At this session, the participant will undergo a test battery of tests in one month, each session will be about two hours in duration. Upon arrival, the subject will take part in a performance test battery, which includes SCAT2 (Sport Concussion Assessment Tool), balance, cognitive, and reaction time. After every test, subjects will maintain neuromotor reactions and balance. The entire process will also include a question to assess brain function, which will be performed mental tasks.
Participant Information Sheet

Integrative Sports Concussion Research at UBC Okanagan

Your participation is completely voluntary, and if you decide to participate, you may still choose to withdraw from the study at any time. Before you decide, it is important for you to understand what the research involves.

We are recruiting male and female athletes between the ages of 14-25yrs playing contact sport, who are:

1) Recently Concussed Athletes (within the first 3-days of injury)

2) Healthy Non-Concussed Athletes

3) Athletes will be tested four times for approximately 1.5hrs per session over one month.

DAY 1-3       DAY 7       DAY 14       DAY 30

Contact n-strachan@hotmail.com if you have further questions.

The Protocol (1.5hrs)

SCAT2 Questionnaire to quantify symptoms

Brain Blood Flow Use ultrasound on the neck to measure blood flow going to the brain at rest

Brain Reactivity Use changes in breathing pattern and gases to measure how the brain reacts to CO₂

Stand and Squats Subjects will cycle through stand-and-squats for two 5-minute sessions

Exercise and Brain Blood Flow Subject exercises at a low and moderate intensity for 5-minutes to assess responsiveness of brain blood flow.

Athletes will only progress through the protocol as their health and symptoms allow.

This study is non-invasive and is completely experimental and will have no bearing on the athletes return to play.
1) Prior to the **FIRST SESSION**, subjects will be emailed **TWO** forms (Informed Consent Form and Pre-Screen Questionnaire) that must be read and completed prior to testing. Please make sure both the parents/guardians and subjects have read and understood the consent form. If there are any questions or concerns, please do not hesitate to contact us.

2) Prior to **ALL SESSIONS**, **subjects must refrain from caffeine, exercise, and alcohol for 24hrs before testing** and please refrain from eating 3hrs prior to testing.

Sincerely,
Nicole Strachan and Katelyn Marsden

**UBC Okanagan’s Address:**
3333 University Way
Kelowna, B.C.
V1Y 1Y7
Email: n-strachan@hotmail.com
Number: 250-575-7151
Website: www.iscr.ca
INFORMED CONSENT FORM – CONCUSSION PARTICIPANTS

Title of Project: Cerebral Blood Flow Response to Exercise following Concussion

Principal Investigator: Dr Paul van Donkelaar, PhD
         UBC Okanagan, School of Health and Exercise Sciences
         Health and Science Centre
         Work: 250-807-8980

Co-investigators: Dr Brad Monteleone, MD, PhD
                  Dr. Philip Ainslie, PhD
                  Mr Kurt J Smith, PhD Candidate
                  Ms. Katelyn R. Marsden, MSc Candidate
                  Ms. Nicole Strachan, MSc Candidate

Institution: School of Health and Exercise Sciences
            University of British Columbia, Okanagan Campus

You are being invited to participate in this research study because you have recently received a concussion. Please take time to read this document carefully and to discuss it with the investigator, your family, your doctor, or others before you decide to participate in this study.

Participants: Your participation is completely voluntary. You have the right to refuse to participate in this study. If you decide to participate, you will be required to sign the consent from at the end of this document. Also, if you do decide to participate, you may still choose to withdraw from the study at any time without any negative consequences to the medical care, education, or other services to which you are being entitled or are presently receiving.

Before you decide, it is important for you to understand what the research involves. This document will provide you with all the necessary information regarding why the research is being done, what will happen to you during the study and the possible benefits, risks and discomforts.

Who is conducting the study: You are being invited to take part in a study within the School of Health and Exercise Sciences in the Faculty of Health and Social Development within the University of British Columbia Okanagan. This study will be conducted and overseen by Dr. Phil Ainslie, Dr. Brad Monteleone, Dr. Paul van Donkelaar, Mr Kurt Smith, and Ms. Katelyn Marsden.

The purpose of this study: The purpose of this study is to understand how the brain responds to exercise and how this response is affected by a concussion. To do so, we need to compare subjects with concussion to healthy people.

Who can participate in this study? You can participate in this study as a concussed subject if you are 1) between the ages of 14-25 years of age, and 2) have recently (within the first 3 days) received a concussion. You must be able to speak and read English fluently.
**Who should NOT participate in this study?** You will not be able to participate in this study if you 1) are over 25, 2) have a history of multiple concussions within the past six months, 3) have a previous history of cardiorespiratory/cerebrovascular/neurological illness or events, 4) are taking any medication that might alter your blood pressure or brain blood flow, 5) have a body mass index above 30 kg/m², 6) if you are pregnant, or 7) are a smoker.

**What does this study involve?** You will visit the lab on 4 separate occasions each lasting about 2 hours and spaced 1-2 weeks apart. During each session, we will 1) measure your height and weight, 2) ask you questions about what you ate and drank, how much physical activity you have had in the last 12 hours, and whether you have any concussion symptoms, 3) Immediately following, you will complete a concussion symptom evaluation questionnaire (SCAT2) and remain seated for a 5-minute baseline. 4) Afterwards you will be asked to breathe in 5% carbon dioxide for 4 minutes and then immediately after resting to regaining resting values, you will be asked to breathe as fast and as hard as you can for 4 minutes. You will be seated and at no risk of falling, however, there will always be a member of the research team monitoring you closely. 6) Following 5 minutes of resting, you will be asked to perform stand and squats while being spotted for two 5 minute sessions (session 1: a cycle of stand for 10 seconds, squat for 10 seconds; and session 2; a cycle of stand for 5 seconds, squat for 5 seconds) separated by a break. Once you have successfully recovered from the stand and squats, 7) Finally, you will be placed upright on a stationary bicycle to regain resting values before commencing the exercise protocol. On the completion of resting measurements will lead into the start of a continuous 16-minute bout of progressive incremental exercise. In particular, you will cycle for 5 minutes at 30% of your estimated heart rate reserve (HRR) provided you remain symptom free; will attempt to exercise at 70% of you estimated HRR for another 5 minutes. On the completion of the final Stroop test you will be cool-down by reducing the workload on the bike. There will always be a spotter near the bike in-case of an emergency or if you feel faint.

All tests and data collection will take place in Laboratory Room 108, Health Sciences Centre, at the University of British Columbia – Okanagan Campus.
Potential risks involved with your participation? : There are some risks that we have to highlight involving your participation in this study. Female participants in this study should not be pregnant. Participation while pregnant may result in potential harm to your fetus.

Exercise: Exercise in concussion patients has been known to result in the exacerbation of concussion symptoms (e.g.; headache, dizziness, nausea, and fatigue). A sports medicine specialist experienced in treating concussions will be on hand throughout each experimental session. The protocol will be stopped at the first sign of concussion symptom onset or at the subject's request. We will use a specialized bike to ensure your safety while exercising. However, if you feel any discomfort while exercising (i.e.; chest pain, shortness of breath, dizziness or nausea) stop exercising and inform us immediately. The protocol will be terminated for your safety.

Ultrasound: Ultrasound of blood flow in your brain is a non-invasive and painless technique in this experiment. It poses no risk.

Vascular assessments: The inflation of a blood pressure cuff around your arm can be painful and may give an unpleasant sensation in the lower arm, similar to pins and needles. This sensation disappears when the cuff is deflated. Additionally, exercising during cuff inflation can also heighten sensation to a slight burn, also your hand may feel heavy, but again these sensations are lessened when exercise is stopped and disappear on cuff deflation.

Standing up and squat stand protocol: Upon standing out of bed or during the squat stand protocol, you may experience symptoms associated with fainting (light-headedness, dizziness) or may possible faint. These symptoms should subside however if they progress and you feel you may faint you will be returned to a lying position. We will be continuously
monitoring your blood pressure and brain blood flow, from these measurements we will be able to identify if you are at risk of fainting, and prevent this from occurring.

**Assessment of cerebral reactivity (an index of the blood flow reserve to the brain):** There are no risks associated with the mild changes in carbon dioxide; however you may experience minor headache or dizziness during increases in carbon dioxide. You will be closely monitored throughout the protocol, however, and in our experience of conducting greater than 2000 of these tests there have been no ill effects reported.

A physician will be on-call during all experimental sessions should any complications arise. In the unlikely event of any complication, such as cardiac arrest, emergency medical response will be immediately alerted. Dr. Brad Monteleone will supervise the stress testing. An investigator certified to perform cardiopulmonary resuscitation and in the use of an automated external defibrillator will be present at every testing session and will follow standard emergency protocols. However, complications are very unlikely given the rigorous screening you will first undertake prior to admission to the study.

**What are the benefits for participating?** You will not benefit personally from participation in this study. Your participation in this experiment will contribute to the understanding of how concussions affect the brain during exercise and tasks demanding high levels of concentration, with the hope of developing a method to quantifying the safe return of athletes post concussion to sport.

**What are my responsibilities?** Since prior exercise, alcohol and caffeine intake all affect the ability to regulate brain blood flow and blood pressure you will also be asked to refrain from alcohol and caffeine consumption and vigorous exercise 24 hours prior to experimental days. Also, you will be asked to eat a light meal 3 hours prior to the experimental sessions.

**What happens if I decide to withdraw my consent?** Your participation in this research is entirely voluntary. You may withdraw from this study at any time and you do not have to provide any reasons for your withdrawal, if you do not wish to do so. If you decide to enter the study and to withdraw at any time in the future, there will be no penalty or loss of benefits to which you are otherwise entitled, and your future medical care will not be affected. The study doctor(s)/investigators may decide to discontinue the study at any time, or withdraw you from the study at any time, if they feel that it is in your best interests. If you choose to enter the study and then decide to withdraw at a later time, all data collected about you during your enrolment in the study will be retained for analysis. By law, this data cannot be destroyed.

**Confidentiality:** Your confidentiality will be respected.

However, research records and health or other source records identifying you may be inspected in the presence of the Investigator or his or her designate, by representatives of Health Canada, and the UBC Research Ethics Board for the purpose of monitoring the research. No information or records that disclose your identity will be published without your consent, nor will any information or records that disclose your identity be removed or released without your consent unless required by law.
You will be assigned a unique study number as a subject in this study. Only this number will be used on any research-related information collected about you during the course of this study, so that your identity [i.e. your name or any other information that could identify you] as a subject in this study will be kept confidential. Information that contains your identity will remain only with the Principal Investigator and/or designate. The list that matches your name to the unique study number that is used on your research-related information will not be removed or released without your consent unless required by law. Your rights to privacy are legally protected by federal and provincial laws that require safeguards to insure that your privacy is respected and also give you the right of access to the information about you that has been provided to the sponsor and, if need be, an opportunity to correct any errors in this information. Further details about these laws are available on request to your study doctor.

Please note that you may ask questions at any time. We will be glad to discuss your results with you when they have become available and we welcome your comments and suggestions. If you have any concerns about your rights as a research subject and/or your experiences while participating in this study, contact the Research Subject Information Line in the University of British Columbia Office Research Services' at 604-822-8598.

A trained research assistant will be available on every occasion to explain the procedure and answer any questions.

**What happens if something goes wrong during this study?** Signing this consent form in no way limits your legal rights against the sponsor, investigators, or anyone else. Any adverse event that should arise will be logged in an investigators laboratory book, and will be followed through to ensure your safety and well being. The researchers of this study will be freely available if you would like to discuss any problems or concern that may arise. Following the completion of the project you will be provided with a feedback sheet explaining the outcome and any substantive findings.

**Can I be asked to leave the study?** In a rare event that a medical emergency occurs during this study period, you will be automatically withdrawn from the study to ensure your safety and well-being.

**After this study is completed?** Results of this project may be published and presented at national and internal conferences. Any data presented will not be linked to any specific participant, as your data will be assigned a personal identification number to ensure anonymity in the raw data collected, analysis and documentation of results. You will be provided with a feedback sheet explaining the outcome and any substantive findings.
**Contact Information**: Please feel free to contact us at any time with questions and concerns you may have about participating in this research study.

Dr. Phil Ainslie  
Room 118, Health Science Centre  
University of British Columbia  
Email: Philip.ainslie@ubc.ca  
bmontele@telus.net  
Work: 250-807-8980

Dr. Bradley Monteleone  
Room #104, 1634 Harvey Avenue  
Kelowna  
Email:  
Work: 250-860-4122

Dr. Paul van Donkelaar  
Room 360C, ARTS Building  
University of British Columbia  
Email: paul.vandokelaar@ubc.ca  
Work: 250-807-8858

Kurt Smith  
Room 180, ARTS Building  
University of British Columbia  
Email: kurt.smith@ubc.ca  
Cell: 250-863-8528 (24 hr Contact)

Katelyn Marsden  
Room 180, ARTS Building  
University of British Columbia  
Email: kit_marsden@hotmail.com  
Cell: 250-928-0273 (24hr Contact)

Nicole Strachan  
Room 180, ARTS Building  
University of British Columbia  
Email: n-strachan@hotmail.com  
Cell: 250-575-7151

If you wish to contact an independent person regarding any aspect of your participation in this study please contact:  
Dr Gordon Binsted  
Dean, School of Health and Exercise Sciences  
Faculty of Health and Social Development  
University of British Columbia  
3333 University Way, Kelowna, B.C.  
V1V 1V7
Consent Form for Participants

In signing this form you are consenting to participate in this research project. I have received a satisfactory explanation of what the study entails, and I have read and understand the purpose and procedures of this study as described in the participant information sheet, and I voluntarily agree to participate. I understand that at any time during the investigation I will be free to withdraw without jeopardizing any medical management, employment or educational opportunities. I have received all pages of the consent form and understand the contents of these pages, the proposed procedures and possible risks. I have had the opportunity to ask questions and have received satisfactory answers to all inquiries regarding this study. I will receive a signed and dated copy of the consent form.

I know that:

1) My participation in the project is entirely voluntary, and I am free to withdraw from this study at any time without any disadvantage

2) The data on which the results of the project depend upon will be retained in secure storage for 5 years, after which they will be destroyed

3) I will be required to complete a pre-screen and a familiarization of the lab

4) I understand that any personal information collected during the study remain anonymous and confidential.
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<tr>
<th>Signature of Subject</th>
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<tr>
<td>Signature of Parent/Guardian</td>
<td>Printed name</td>
<td>Date</td>
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<tr>
<td>Principal Investigator or/ designated representative</td>
<td>Printed name</td>
<td>Date</td>
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</tbody>
</table>
INFORMED CONSENT FORM – CONTROL PARTICIPANTS

Title of Project: Cerebral Autoregulatory Response to Exercise following Concussion

Principal Investigator: Dr Paul van Donkelaar, PhD
UBC Okanagan, School of Health and Exercise Sciences
Health and Science Centre
Work: 250-807-8858

Co-investigators: Dr Brad Monteleone, MD, PhD
Dr. Philip Ainslie, PhD
Mr Kurt J Smith, PhD Candidate
Ms. Katelyn R. Marsden, MSc Candidate
Ms. Nicole Strachan, MSc Candidate
Ms. Tanis Burnett, MSc Candidate

Institution: School of Health and Exercise Sciences
University of British Columbia, Okanagan Campus

You are being invited to participate in this research study because you are a healthy individual. Please take time to read this document carefully and to discuss it with the investigator, your family, your doctor, or others before you decide to participate in this study.

Participants: Your participation is completely voluntary. You have the right to refuse to participate in this study. If you decide to participate, you will be required to sign the consent form at the end of this document. Also, if you do decide to participate, you may still choose to withdraw from the study at any time without any negative consequences to the medical care, education, or other services to which you are being entitled or are presently receiving.

Before you decide, it is important for you to understand what the research involves. This document will provide you with all the necessary information regarding why the research is being done, what will happen to you during the study and the possible benefits, risks and discomforts.

Who is conducting the study: You are being invited to take part in a study within the School of Health and Exercise Sciences in the Faculty of Health and Social Development within the University of British Columbia Okanagan. This study will be conducted and overseen by Dr. Phil Ainslie, Dr. Brad Monteleone, Dr. Paul van Donkelaar, Mr Kurt Smith, Ms. Nicole Strachan and Ms. Katelyn Marsden.

The purpose of this study: The purpose of this study is to understand how the brain responds to exercise and how this response is affected by a concussion. To do so, we need to compare subjects with concussion to healthy people.

Who can participate in this study?: You can participate in this study as a healthy control subject if you are 1) between the ages of 11-25 years of age, and 2) have never suffered a concussion or have had an concussion but have been asymptomatic for at least one year. You must be able to speak and read English fluently.

Who should NOT participate in this study? You will not be able to participate in this study if you 1) are over 25, 2) have obtained a concussion in the past twelve months, 3)
have a previous history of cardiorespiratory/ cerebrovascular/neurological illness or events, 4) are taking any medication that might alter your blood pressure or brain blood flow, 5) have a body mass index above 30 kg/m², 6) if you are pregnant, or 7) are a smoker.

**What does this study involve?** You will visit the lab or we will test you at a community-based facility on 4 separate occasions each lasting about 2 hours and spaced 1-2 weeks apart. During each session, we will 1) measure your height and weight, 2) ask you questions about what you ate and drank, how much physical activity you have had in the last 12 hours, and whether you have any concussion symptoms, 3) Immediately following, you will complete a concussion symptom evaluation questionnaire (SCAT2) and perform two cognitive tasks (Task Switching and The Stroop test) on the computer before being set-up with all the equipment. After equipment set-up, you will perform a second round of the Task Switching and The Stroop test on the computer, 4) Visual stimulation protocol while seated. You will be asked to close your eyes for 2 minutes, and then open your eyes reading an article from a computer screen for another 2 minutes. This is immediately followed by 5 cycles of; eyes closed (20-seconds), and open while reading the same article (40-seconds). 5) After a break and while remaining at rest and seated, you will be asked to breathe in 5% carbon dioxide for 4 minutes and then immediately after resting to regaining resting values, you will be asked to breathe as fast and as hard as you can for 4 minutes. You will be seated and at no risk of falling, however, there will always be a member of the research team monitoring you closely 6) Following 5 minutes of resting, you will be ask to perform stand and squats while being spotted for two 5 minute sessions (session 1: a cycle of stand for 10 seconds, squat for 10 seconds; and session 2; a cycle of stand for 5 seconds, squat for 5 seconds) separated by a break. Once you have successfully recovered from the stand and squats, 7) You will lay on your back while we place your lower body into a chamber which seals around your waist, for 10 minutes, followed by another 10 minutes of pressure changes caused by sucking air out of the sealed chamber. You will have full verbal communication with the researchers, so if discomfort does become a concern the test will be terminated 8) Once again after regaining resting values, you will be asked to stand perfectly still with feet shoulder width apart on a 2x2 foot platform, that stand approximately 6 inches off the floor for 5 minutes while a computer software program analyzes postural changes. 9) Finally, you will be placed upright on a stationary bicycle to regain resting values before commencing the exercise protocol. On the completion of resting measurements will lead into the start of a continuous 16-minute bout of progressive incremental exercise. In particular, you will cycle for 5 minutes at 30% of your estimated heart rate reserve (HRR), thereafter you will perform the Stroop test while continuing to exercise at 30% of your HRR and provided you remain symptom free; will attempt to exercise at 70% of you estimated HRR for another 5 minutes. Thereafter you will perform your last Stroop test while continuing to exercise at 70% HRR. On the completion of the final Stroop test you will be cool-down by reducing the workload on the bike. There will always be a spotter near the bike in-case of an emergency or if you feel faint. On the final testing session, subjects who have remained asymptomatic throughout all the protocols may be asked to partake in a running gait analysis on a specially designed treadmill.

All tests and data collection will take place either in ARTS 180, at the University of British Columbia – Okanagan Campus or at a community-based facility at which you practice/compete.
Potential risks involved with your participation? : There are some risks that we have to highlight involving your participation in this study. Female participants in this study should not be pregnant. Participation while pregnant may result in potential harm to your fetus.

Exercise: Exercise in healthy patients can raise your heart rate, and increase breathing frequency. We will use a specialized bike to ensure your safety while exercising. However, if you feel any discomfort while exercising (i.e. chest pain, shortness of breath, dizziness or nausea) stop exercising and inform us immediately. The protocol will be terminated for your safety.
Ultrasound: Ultrasound of blood flow in your brain is a non-invasive and painless technique in this experiment. It poses no risk.

Vascular assessments: The inflation of a blood pressure cuff around your arm can be painful and may give an unpleasant sensation in the lower arm, similar to pins and needles. This sensation disappears when the cuff is deflated. Additionally exercising during cuff inflation can also heighten sensation to a slight burn, also your hand may feel heavy, but again these sensations are lessened when exercise is stopped and disappear on cuff deflation.

Standing up and squat stand protocol: Upon standing out of bed or during the squat stand protocol, you may experience symptoms associated with fainting (light-headedness, dizziness). These symptoms should subside however if they progress and you feel you may faint you will be returned to a lying position. We will be continuously monitoring your blood pressure and brain blood flow, from these measurements we will be able to identify if you are at risk of fainting, and prevent this from occurring.

Lower Body Negative Pressure: This technique is specifically designed to rhythmically lower your blood pressure and return it to its normal pressure in specific steps using a specially designed device. This device simulates a similar change in blood pressure as the squat stand technique, only it allows for precise control of blood pressure fluctuations. The unit consists of a large clear glass box on a padded bed that will alter the pressure on your lower body in a manner that resembles a vacuum sucking the air out of room. This device is safe and has been used in many studies designed to induce syncope, or loss of consciousness, however at no point will we be inducing syncope in you, and will stop should you begin to feel faint or request a stop in the protocol.

Assessment of cerebral reactivity (an index of the blood flow reserve to the brain): There are no risks associated with the mild changes in carbon dioxide; however you may experience minor headache or dizziness during increases in carbon dioxide. You will be closely monitored throughout the protocol, however, and in our experience of conducting greater than 2000 of these tests there have been no ill effects reported.

A physician will be on-call during all experimental sessions should any complications arise. In the unlikely event of any complication, such as cardiac arrest, emergency medical response will be immediately alerted. Dr. Brad Monteleone will supervise the stress testing. An investigator certified to perform cardiopulmonary resuscitation and in the use of an automated external defibrillator will be present at every testing session and will follow standard emergency protocols. However, complications are very unlikely given the rigorous screening you will first undertake prior to admission to the study.

What are the benefits for participating? You will not benefit personally from participation in this study. Your participation in this experiment will contribute to the understanding of how concussions affect the brain during exercise and tasks demanding high levels of concentration, with the hope of developing a method to quantifying the safe return of athletes post concussion to sport.

What are my responsibilities? Since prior exercise, alcohol and caffeine intake all affect the ability to regulate brain blood flow and blood pressure you will also be asked to refrain from alcohol and caffeine consumption and vigorous exercise 24 hours prior to experimental days. Also, you will be asked to eat a light meal 3 hours prior to the experimental sessions.

What happens if I decide to withdraw my consent? Your participation in this research
is entirely voluntary. You may withdraw from this study at any time and you do not have to provide any reasons for your withdrawal, if you do not wish to do so. If you decide to enter the study and to withdraw at any time in the future, there will be no penalty or loss of benefits to which you are otherwise entitled, and your future medical care will not be affected. The study doctor(s)/investigators may decide to discontinue the study at any time, or withdraw you from the study at any time, if they feel that it is in your best interest. If you choose to enter the study and then decide to withdraw at a later time, all data collected about you during your enrolment in the study will be retained for analysis. By law, this data cannot be destroyed.

**Confidentiality:** Your confidentiality will be respected. However, research records and health or other source records identifying you may be inspected in the presence of the Investigator or his or her designate, by representatives of Health Canada, and the UBC Research Ethics Board for the purpose of monitoring the research. No information or records that disclose your identity will be published without your consent, nor will any information or records that disclose your identity be removed or released without your consent unless required by law.

You will be assigned a unique study number as a subject in this study. Only this number will be used on any research-related information collected about you during the course of this study, so that your identity [i.e. your name or any other information that could identify you] as a subject in this study will be kept confidential. Information that contains your identity will remain only with the Principal Investigator and/or designate. The list that matches your name to the unique study number that is used on your research-related information will not be removed or released without your consent unless required by law. Your rights to privacy are legally protected by federal and provincial laws that require safeguards to insure that your privacy is respected and also give you the right of access to the information about you that has been provided to the sponsor and, if need be, an opportunity to correct any errors in this information. Further details about these laws are available on request to your study doctor.

Please note that you may ask questions at any time. We will be glad to discuss your results with you when they have become available and we welcome your comments and suggestions. If you have any concerns about your rights as a research subject and/or your experiences while participating in this study, contact the Research Subject Information Line in the University of British Columbia Office Research Services' at 604-822-8598.

A trained research assistant will be available on every occasion to explain the procedure and answer any questions.

**What happens if something goes wrong during this study?** Signing this consent form in no way limits your legal rights against the sponsor, investigators, or anyone else. Any adverse event that should arise will be logged in an investigators laboratory book, and will be followed through to ensure your safety and well being. The researchers of this study will be freely available if you would like to discuss any problems or concern that may arise. Following the completion of the project you will be provided with a feedback sheet explaining the outcome and any substantive findings.

**Can I be asked to leave the study?** In a rare event that a medical emergency occurs during this study period, you will be automatically withdrawn from the study to ensure your safety and well-being.

**After this study is completed?** Results of this project may be published and presented at national and internal conferences. Any data presented will not be linked to any specific participant, as your data will be assigned a personal identification number to ensure
anonymity in the raw data collected, analysis and documentation of results. You will be provided with a feedback sheet explaining the outcome and any substantive findings.

**Contact Information:** Please feel free to contact us at any time with questions and concerns you may have about participating in this research study.

Dr. Phil Ainslie  
Room 118, Health Science Centre  
University of British Columbia  
Email: Philip.ainslie@ubc.ca  
Work: 250-807-8980  

Dr. Bradley Monteleone  
#104, 1634 Harvey Avenue  
Kelowna  

Dr. Paul van Donkelaar  
Room 360C, ARTS Building  
University of British Columbia  
Email: paul.vandokelaar@ubc.ca  
Work: 250-807-8858  

Kurt Smith  
Room 180, ARTS Building  
University of British Columbia  
Email: kurt.smith@ubc.ca  
Cell: 250-863-8528 (24hr Contact)  

Nicole Strachan  
Room 180, ARTS Building  
University of British Columbia  
Email: n-strachan@hotmail.com  
Cell: 250-575-7151 (24hr Contact)  

Katelyn Marsden  
Room 180, ARTS Building  
University of British Columbia  
Email: kit_marsden@hotmail.com  
Cell: 250-928-0273 (24hr Contact)  

If you wish to contact an independent person regarding any aspect of your participation in this study please contact:  
Dr. Gordon Binsted  
Dean, School of Health and Exercise Sciences  
Faculty of Health and Social Development  
University of British Columbia  
3333 University Way, Kelowna, B.C.  
V1V 1V7  
Office: 250-807-9642  

**Consent Form for Participants**

In signing this form you are consenting to participate in this research project. I have received a satisfactory explanation of what the study entails, and I have read and understand the purpose and procedures of this study as described in the participant information sheet, and I voluntarily agree to participate. I understand that at any time during the investigation I will be free to withdraw without jeopardizing any medical management, employment or educational opportunities. I have received all pages of the consent form and understand the contents of these pages, the proposed procedures and possible risks. I have had the opportunity to ask questions and have received satisfactory answers to all inquiries regarding this study. I will receive a signed and dated copy of the consent form.
I know that:

1) My participation in the project is entirely voluntary, and I am free to withdraw from this study at any time without any disadvantage.

2) The data on which the results of the project depend upon will be retained in secure storage for 5 years, after which they will be destroyed.

3) I will be required to complete a pre-screen and a familiarization of the lab.

4) I understand that any personal information collected during the study will remain anonymous and confidential.
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<tr>
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<th>Date</th>
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<tr>
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<tr>
<td>Signature of Parent/Guardian</td>
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<tr>
<td>Principal Investigator or/</td>
<td></td>
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<tr>
<td>designated representative</td>
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</table>
Appendix B.6  Pre-Screen Questions

Title of Project: Cerebral Autoregulatory Response to Exercise following Concussion

Subject Identification Code (i.e. S101): _______________________

Age: __________________

Sex:  M ☐  F ☐

Weight: __________________ lbs ☐ kg ☐

Height: __________________

Education Level: _______________________

Personal Medical History Assessment (please ☑ and/or give relevant detail)

Have you ever been diagnosed with or experienced any of the following:

1) Mild traumatic brain injury or concussion?

   Yes ☐  No ☐
If Yes:

How many concussions have you had? _______________

How did the concussion(s) occur (i.e. blow to the head during hockey game)?

___________________________________________________________________

___________________________________________________________________

Was your concussion classified as Mild, Moderate, or Severe? _______________

What were the symptoms you exhibited? ________________________________

How long ago did the concussion(s) occur? ____________________________

How long have you been asymptomatic (no obvious symptoms)? ____________

What kind of treatment or care did you receive post-concussion?

___________________________________________________________________

___________________________________________________________________

2) Epilepsy or any other neurological disorder that requires medication (ADD or ADHD)?

Yes ☐  No ☐

3) Heart Conditions (pacemaker, arrhythmia, angina, coronary heart disease, bradycardia)?

Yes ☐  No ☐

4) High Blood Cholesterol?
5) High or Low Blood Pressure?
Yes ☐ No ☐

6) Any Metabolic Disorders (e.g. diabetes, irritable bowel syndrome, anorexia, gastrointestinal disorder or history of impairments of gag reflex)?
Yes ☐ No ☐

7) Have you have had a stroke?
Yes ☐ No ☐

8) Do you ever lose consciousness (fainting or black outs)?
Yes ☐ No ☐

9) Do you have any respiratory diseases/problems (e.g. asthma, cold, pulmonary vascular disease, emphysema)?
Yes ☐ No ☐

10) Do you have and muscular skeletal disease (e.g. osteoporosis, arthritis)?
Yes ☐ No ☐

11) Recent lower body or joint issues that limit your ability to perform exercise (hip, knee, or ankle)
12) Do you Smoke?
Yes ☐ No ☐

13) Do you have any allergies to any medication (e.g. liquids or tables)?
Yes ☐ No ☐

14) If females, could you be pregnant?
Yes ☐ No ☐

15) Are you currently on any medication (e.g. antihistamines, cold mixtures)?
Yes ☐ No ☐

16) What sports are you involved in?
________________________________________

17) How many days are you physically active (>30mins/session)? ____________
Thank you for your time and co-operation!

All information provided on this questionnaire will be kept confidential.

Feel free to ask any question regarding this questionnaire or the research project.

Researcher Only:

Manual Blood Pressure Check

Systolic: __________

Diastolic: __________

Participant has no significant current or past medical history of clinical significance

Yes ☐  No ☐

Participant given signed informed consent (witnessed)

Yes ☐  No ☐
Appendix B.7 Example of recruitment email

School of Health and Exercise Sciences
University of British Columbia
Health and Science Centre
3333 University Way
Kelowna, BC Canada V1V 1V7

RE: Integrative Sports Concussion Research Group

Dear [Name],

The School of Health and Exercise Sciences, located in the new Health Sciences Centre on the UBC-Okanagan campus is conducting an experiment that is aimed at investigating the progression of post concussion symptoms on cognitive functioning and cerebrovascular functioning before, during, and after exercise. This is a novel research area that has the potential to generate positive health and economic benefits to all individuals engaged in contact sports, or active people who have suffered a concussion. In addition, further information on the clinical implications and potential screening ability for people who have had a concussion will be attractive to all family and sports physicians across Canada who are currently limited in their ability to objectively diagnose concussion and prescribe a safe and valid return to sport or physical activity.

We are looking to recruit healthy volunteers between the ages of 14-25 years of age as well as similar aged athletes who have recently suffered a concussion. If you would like to participate in this study, please contact any member of research team listed at the end of this email, and please read the accompanying participant information sheet for further information on the investigation and the involvement that is required. Participation in the
study is voluntary and you would have the right to withdraw at any point, with no explanation.

If you would still like to participate in this investigation after reading the informed consent, we will ask that you come to the laboratory to discuss the project and the next steps of action. If you have any questions regarding the research study or the procedures involved, please do not hesitate to contact us. We will be happy to answer any questions. If you do not wish to take part in this study, thank you for your time.

Yours sincerely,

Nicole Strachan

Dr. Bradley Monteleone  
#104, 1634 Harvey Avenue  
Kelowna, B.C.  
Email: bmontele@telus.net  
Work: 250-860-4122

Dr. Paul van Donkelaar  
Room 113 Health Science Centre  
University of British Columbia  
Email: paul.vandonkelaar@ubc.ca  
Work: 250-807-8858  
Kurt Smith  
Room 108 Health Science Centre  
University of British Columbia  
Email: kurt.smith@ubc.ca  
Cell: 250-863-8528 (24hr Contact)

Nicole Strachan  
Room 108 Health Science Centre  
University of British Columbia  
Email: n-strachan@hotmail.com  
Cell: (250)575-7151 (24hr Contact)
Katelyn Marsden  
Room 108 Health Science Centre  
University of British Columbia  

Email: kit_marsden@hotmail.com  
Cell: 250-928-0273 (24hr Contact)
Appendix. B.8 SCAT 2
### H11-02900  Concussions and Brain Blood Flow during Exercise  (Version 4.0)

**Principal Investigator:** Paul van Donkelaar  

#### 1. Principal Investigator & Study Team - Human Ethics Application

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Employer.Name</th>
<th>Email</th>
</tr>
</thead>
<tbody>
<tr>
<td>van Donkelaar</td>
<td>Paul</td>
<td>UBC Health and Exercise Sciences</td>
<td><a href="mailto:paul.vandonkelaar@ubc.ca">paul.vandonkelaar@ubc.ca</a></td>
</tr>
</tbody>
</table>

**Enter Principal Investigator Primary Department and also the primary location of the PI's Institution:**  

Health and Exercise Sciences, UBCO

**1.2. Primary Contact Provide the name of ONE primary contact person in addition to the PI who will receive ALL correspondence, certificates**

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strachan</td>
<td>Nicole Cecelia</td>
<td>Graduate Student</td>
</tr>
</tbody>
</table>
of approval and notifications from the REB for this study. This primary contact will have online access to read, amend, and track the application.

1.3. Co-Investigators List all the Co-Investigators of the study. These members WILL have online access which will allow them to read, amend and track the application. These members will be listed on the certificate of approval (except BC Cancer Agency Research Ethics Board certificates). If this research application is for a graduate degree, enter the graduate student's name in this section.

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Institution/Department</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burnett</td>
<td>Tanis</td>
<td>UBC/UBCO Health &amp; Social Development/UBCO Health and Exercise Sciences</td>
<td>Graduate Student</td>
</tr>
<tr>
<td>Marsden</td>
<td>Katelyn</td>
<td>UBC/UBCO Health &amp; Social Development/UBCO Health and Exercise Sciences</td>
<td>Graduate Student</td>
</tr>
<tr>
<td>Strachan</td>
<td>Nicole</td>
<td>UBC/UBCO Health &amp; Social Development</td>
<td>Graduate Student</td>
</tr>
<tr>
<td>Monteleone</td>
<td>Brad</td>
<td>UBC/Medicine, Faculty of/Family Practice</td>
<td>Clinical Instructor</td>
</tr>
<tr>
<td>Smith</td>
<td>Kurt</td>
<td>UBC/UBCO Health &amp; Social Development</td>
<td>Graduate Student</td>
</tr>
<tr>
<td>van Donkelaar</td>
<td>Paul</td>
<td>UBC/UBCO Health &amp; Social Development/UBCO Health and Exercise Sciences</td>
<td>Professor</td>
</tr>
</tbody>
</table>

1.4. Additional Study Team Members - Online Access
List the additional study team members who WILL have online access to read, amend, and track the application but WILL NOT be listed on the
1.5. Additional Study Team Members - No Online Access
Click Add to list study team members who WILL NOT have online access to the application and will NOT be listed on the certificate of approval.

<table>
<thead>
<tr>
<th>Last Name</th>
<th>First Name</th>
<th>Institution / Department</th>
<th>Rank / Job</th>
<th>Email Address</th>
</tr>
</thead>
</table>

Tri Council Policy Statement2 (TCPS2) Tutorial
All undergraduate and graduate students and medical residents are required to complete the TCPS2 Tutorial (CORE) before submission. This tutorial provides an essential orientation to Canadian human research ethics guidelines. The Principal Investigator and all Co-Investigators must be familiar with the TCPS2. Indicate completion of the TCPS2 (CORE) tutorial below: 1.6.A. All Undergraduate/Graduate Students:

Yes

1.6.B. All Medical Residents: N/A (no medical residents participating in this study)

Comments:
<table>
<thead>
<tr>
<th>1.7. Project Title Enter the title of this research study as it will appear on the certificate. If applicable, include the protocol number in brackets at the end of the title. If this is a class-based project, see guidance on the right.</th>
<th>Using cerebrovascular biomarkers to better diagnose adolescent sports concussion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.8. Project Nickname Enter a nickname for this study. What would you like this study to be known as to the Principal Investigator and study team?</td>
<td>Concussions and Brain Blood Flow during Exercise</td>
</tr>
<tr>
<td>2 Study Dates and Funding Information - Human Ethics Application [View Form]</td>
<td></td>
</tr>
<tr>
<td>You plan to start collecting data immediately after obtaining ethics and any other required approvals (the start date on the ethics certificate will reflect the approval date),</td>
<td>no</td>
</tr>
<tr>
<td>You plan to start data collection at a later date i.e., 2 months or more after approvals are obtained. Click the calendar icon below to select the dates (Internet Explorer) or enter the dates manually using the format</td>
<td>November 23, 2012</td>
</tr>
<tr>
<td>yyyy-mm-dd. Estimated start date:</td>
<td></td>
</tr>
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<td>---------------------------------</td>
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</tr>
<tr>
<td>2.1. B. Estimated end date:</td>
<td>November 22, 2013</td>
</tr>
<tr>
<td>2.2.A. Types of Funds Please select the applicable box(es) below to indicate the type(s) of funding you are receiving to conduct this research. You must then complete section 2.3 and/or section 2.4 for the name of the source of the funds to be listed on the certificate of approval.</td>
<td>Grant</td>
</tr>
<tr>
<td>2.2.B. For Industry Sponsored studies, please provide a sponsor contact.</td>
<td></td>
</tr>
<tr>
<td>2.3. Research Funding Application/Award Associated with the Study that was Submitted to the UBC Office of Research Services Please click Add to identify the research funding application/award associated with this study. Selecting Add will list the sources of all research funding applications that have been submitted by the PI (and the person completing this application if different from UBC</td>
<td></td>
</tr>
<tr>
<td>UBC Number</td>
<td>Title</td>
</tr>
<tr>
<td>F12-04140</td>
<td>Using cerebrovascular biomarkers to better diagnose adolescent sports concussion</td>
</tr>
<tr>
<td>F12-01963</td>
<td>Executive function deficits following concussion in adolescents</td>
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</table>
the PI). If the research funding application/award associated with this study is not listed below, please enter those details in question 2.4.

<table>
<thead>
<tr>
<th>UBC Number</th>
<th>Title</th>
<th>Sponsor</th>
</tr>
</thead>
</table>

2.4. Research Funding Application/Award Associated with the Study not listed in question 2.3. Please click Add to enter the details for the research funding application/award associated with this study that is not listed in question 2.3. When you press Add you can do a search for your funding award by doing a search in the Sponsor box - over 7000 options are listed.

2.5.A. Is this a DHHS grant? (To view a list of DHHS funding agencies click on add in 2.5.B below)

<table>
<thead>
<tr>
<th>DHHS Sponsor List:</th>
<th>Order:</th>
<th>Active:</th>
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<tbody>
<tr>
<td>no</td>
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</table>

2.5.B. If yes, please select the appropriate DHHS funding agency from the selection box, and attach the grant to box 9.8. of the application.

Attach DHHS Grant Application for each sponsor listed above
2.6. Conflict of Interest Do any of the following statements apply to the Principal Investigator, Co-Investigators and/or their partners/immediate family members? Receive personal benefits in connection with this study over and above the direct cost of conducting this study. For example, being paid by the funder for consulting. (Reminder: receiving a finders fee for each participant enrolled is not allowed). Have a non-financial relationship with the sponsor (such as unpaid consultant, advisor, board member or other non-financial interest). Have direct financial involvement with the sponsor (source of funds) via ownership of stock, stock options, or membership on a Board. Hold patent rights or intellectual property rights linked in any way to this study or its sponsor (source of funds).

<table>
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<tr>
<th>Question</th>
<th>Answer</th>
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<td>no</td>
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4. Study Type - Human Ethics Application [View Form]
<table>
<thead>
<tr>
<th>4.1. UBC Research Ethics Board</th>
<th>Indicate which UBC Research Ethics Board you are applying to and the type of study you are applying for:</th>
<th>UBC Clinical Research Ethics Board</th>
</tr>
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<tbody>
<tr>
<td>N/A</td>
<td>no</td>
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<tr>
<td>4.2.A. Institutions and Sites for Study</td>
<td>Institution Site</td>
<td>UBC Okanagan</td>
</tr>
<tr>
<td>4.2.B. Please enter any other locations where the research will be conducted under this Research Ethics Approval (e.g., private physician's office, community centre, school, classroom, participant's home, in the field - provide details).</td>
<td>Lifemark Health Sports Medicine Centre, Kelowna, B.C. Community-based facilities (e.g., hockey arenas, schools, football facilities) in the Kelowna, B.C. region.</td>
<td></td>
</tr>
<tr>
<td><em><em>4</em> Clinical Study Review Type</em>*</td>
<td>[View Form]</td>
<td></td>
</tr>
<tr>
<td>4.3. Relationship with other proposals 4.3.A. If this proposal is closely linked to any other proposal previously/simultaneously submitted, enter the Research Ethics Board number of that proposal.</td>
<td></td>
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<tr>
<td>4.3.B. If applicable, please describe the relationship between this proposal and</td>
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<td>the previously/simultaneously submitted proposal listed above.</td>
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<tr>
<td><strong>4.3.C.</strong> Have you received any information or are you aware of any rejection of this study by any Research Ethics Board? If yes, please provide known details and attach any available relevant documentation in question 9.7.</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>4.4. Level of Risk After reviewing the minimal risk guidance notes and the criteria for minimal risk, does this study qualify for minimal risk review? Note that all studies which do not fall into the minimal risk category will undergo full board review.</strong></td>
<td>yes</td>
<td></td>
</tr>
<tr>
<td><strong>Peer Review</strong> If this research proposal has received any independent scientific/methodological peer review, please include the names of committees or individuals involved in the review. State whether the peer review process is</td>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>
ongoing or completed. All above minimal risk studies generally require a peer review. 4.5.A. External peer review details:

4.5.B. Internal (UBC or hospital) peer review details: None

4.5.C. If this research proposal has NOT received any independent scientific/methodological peer review, explain why no review has taken place. It is a new project hence first submission to CREB

4.6. Harmonized review of multi-jurisdictional studies
Please read and review the guidance note on the right prior to completing this question. Is this study a multi-jurisdictional study that will also require REB review/approval at one or more of the following institutions with which UBC has a collaborative review agreement? (See the guidance to the right for details about the harmonized process.) Simon Fraser University University of Alberta University of no
<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.7.A Creation of a Research Database, Registry or Tissue Bank</td>
<td>no</td>
</tr>
<tr>
<td>4.7.B Is the purpose of this application exclusively to obtain approval</td>
<td></td>
</tr>
<tr>
<td>for the creation of a research database, registry or tissue bank?</td>
<td></td>
</tr>
<tr>
<td>[Note if the creation of the database or registry or tissue repository</td>
<td></td>
</tr>
<tr>
<td>is part of a bigger project also included in this application, you must</td>
<td></td>
</tr>
<tr>
<td>answer no below.]</td>
<td></td>
</tr>
<tr>
<td>Clinical Chart Review 4.8.A. Is this an application for research using</td>
<td>no</td>
</tr>
<tr>
<td>the review of clinical charts?</td>
<td></td>
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<tr>
<td>4.8.B. Insert the date range of the charts to be included in this</td>
<td></td>
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<tr>
<td>research.</td>
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<tr>
<td>Question</td>
<td>Answer</td>
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<td>------------------------------------------------------------------------</td>
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</tr>
<tr>
<td>4.8.C. Is this a retrospective chart review where the only source of data will be medical charts/records that are currently in existence? (i.e., will pre-date the date of your ethics approval?)</td>
<td></td>
</tr>
<tr>
<td>4.8.D. Is this a retrospective chart review study that will involve the collection of NO personally identifiable information of any sort?</td>
<td></td>
</tr>
<tr>
<td>4.8.E. Is this a retrospective chart review study for which you are requesting a waiver of consent</td>
<td></td>
</tr>
</tbody>
</table>

5. Summary of Study and Recruitment - Human Ethics Application for Clinical Study [View Form]

5.1. Study Summary 5.1.A
Provide a short summary of the project written in lay language suitable for non-scientific REB members. DO NOT exceed 100 words and do not cut and paste directly from the study protocol.

Concussions are a common injury in contact sports and pose a serious threat to long-term neurological and mental health. The primary screening protocol for return to sport is cognitive functioning and exercise tests, which often results in a return of concussion symptoms. Advanced imaging techniques have failed to provide an accurate and identifiable cause. To date, no study has linked concussion symptom exacerbation with cerebral auto-regulation, despite known instances of cerebral auto regulation impairment during exercise. It is our belief that in looking at cerebral auto regulation, we may be able to identify an objective physiological tool in diagnosing concussions.

5.1.B Summarize the Purpose: There is no direct evidence to support the putative
| **Research Proposal:** | relationship between dysfunctional CA and exercise-induced symptom exacerbation in concussion. The studies proposed in this application will be the first to directly measure the physiological correlates of this phenomenon and to quantify how these correlates are related to the return of symptoms and associated executive function deficits.  

**Hypothesis:** The proposed study lead us to suspect that in subjects suffering from a concussion or mild traumatic brain injury will have an impaired cerebral auto regulation compared to age and activity matched control subjects. Furthermore, with the common use of exercise as a concussion diagnostic tool, it is believed that the common return of concussion symptoms a further impairment in executive functioning, cerebral blood flow regulation compared to control data will follow.  

**Justification:** Mild traumatic brain injury or concussion is defined as a complex pathophysiological process affecting the brain, induced by traumatic biomechanical forces. Following a brain injury event there is a rapid onset of one or more graded neurological impairments that may or may not involve loss of consciousness and that usually resolve spontaneously over the course of a few days to weeks. The underlying cause of these impairments is due to neuropathological changes that appear to be both physiological and anatomical in nature. Moreover, the microstructural changes associated with physiological and anatomical damage accompanying concussion injuries are not always detected on static brain images using expensive imaging techniques (e.g. fMRI, CT, PET). Given the subtle nature of the deficits following a concussion, it is quite often difficult to determine when a patient has recovered. |
sufficiently to return to normal activities. Therefore, to quantify and identify if cerebrovascular functioning as indicated by dynamic CA and exercise cerebral perfusion, might be an acceptable method of determining return to exercise and sport readiness in the treatment of concussion. Provided that CA and executive function are appropriate outcome measures, they may provide an invaluable objective tool in assessing concussion at all levels of contact sport. The second control group, the asymptomatic and previously concussed subjects group will be used to explore the long term effects of concussions on CA and executive functioning.

Objective: To directly monitor CA processes at rest and during exercise in concussed individuals and correlate the resulting changes with the level of symptom exacerbation and executive dysfunction.

Research Method: The protocol will be a longitudinal design, covering a period of 1 month, with four consecutive testing sessions. Our experimental group will be athletes from a variety of contact sports who have recently suffered a concussion, and our two separate control groups will be age, gender, education and activity matches with our experimental group and have either have never suffered a concussion or have suffered a concussion in the past and are no longer symptomatic. Subjects will be tested 4 times over the course of 1 month. The concussion group will be tested within 3 days of experiencing a concussion, 1 week, 2 weeks, and 1 month after the injury. The control groups will be tested at similar intervals, approximately within the same time line. On the final testing session, subjects who have remained asymptomatic throughout all the protocols, may be asked to...
partake in a running gait analysis on a specially designed treadmill.

Statistical Analysis: The aim of this study is to generate the data needed to identify physiological and cognitive markers associated with post concussion syndrome. In order to generate and design a study with adequate power we estimated the required sample size based on previous data (Leddy et al. 2007, NeurRehabilitation; Aries et al., 2010, Stroke; van Donkelaar et al., 2005, Brain Injuries) from our laboratories in both healthy and disease populations, including concussed subjects. Power calculations are based on the desired endpoint to detect a change in cerebrovascular and executive function. Therefore, assuming our published standard deviations for these measures, a sample size of 20-30 subjects in each group would provide >90% probability that the study will detect a group difference at a two sided 5% significance level. However, accounting for potential dropouts (~10%), we will recruit 30 subjects per group for a total sample size of 60. All variables will be explored for parity with a normal distribution. At this time no sex difference measurements are planned, as it will be difficult to plan for a specific number of male and female concussed subjects. An amendment may be done if we feel that we will have an equal representation by each of the sexes. Relationships and differences between variables will be evaluated using multiple regression analysis or linear mixed model analysis. Alpha value will be set a priori at P=0.05. All data will be analyzed using SPSS 16.0.2 (SPSS, Chicago, IL).

5.2. Inclusion Criteria

**Inclusion Criteria. Describe the participants being recruited and separated into one of three groups;**

1) normal healthy individuals (n=30) who have never suffered from a concussion,
2) asymptomatic
**selected for this study, and list the criteria for their inclusion. For research involving human pluripotent stem cells, provide a detailed description of the stem cells being used in the research.**

| athletes (n=30) with a prior history of concussions (1 or more), and 3) athletes who have recently (<3days) suffered a concussion (n=30). Subjects will be carefully screened during each visit to the laboratory (e.g., cardio vascular disease history, spirometry, carotid screening, and exercise stress test). Participants recruited will be young individuals and between the ages of 11-25. All individuals will have a body mass index (BMI) <30 kg/m2. |

**5.3. Exclusion Criteria**

Exclusion Criteria. Describe which potential participants will be excluded from participation, and list the criteria for their exclusion.

| Exclusion criteria will include any history or signs of history, cardio-respiratory or cerebrovascular disease (specifically ischemic or other heart disease, carotid or other vascular disease, previous transient ischemic attack or stroke, previous head injury complicated by long term neurological sequelae, epilepsy), lower body injury limiting exercise performance, or who are smokers or pregnant. Subjects will have BMIs <30 kg/m2. All participants who cannot speak or read English will be excluded. |

**5.4. Recruitment**

Provide a detailed description of the method of recruitment. For example, describe who will contact prospective participants and by what means this will be done. Ensure that any letters of initial contact or other recruitment materials are attached to this submission on Page 9.

| Availability for the opportunity to volunteer in this study will be displayed on local community and university noticeboards, along with verbal invitation within the School of Health and Social Development at the University of British Columbia Okanagan campus. An email detailing the study will also be emailed among the university community, and local sports teams organizations. Additionally, Dr Monteleone, via his sports medicine clinic and involvement with local athlete clubs, will have access to a number of concussed subjects, who would satisfy the study inclusion. Subject recruitment will be completed using the informed consent application (page 9), as well as, the recruitment email sent out to the local sports teams and organizations (Kelowna Suns Football team, Kelowna’s Pursuit of Excellence program). |
| 5.5. *Recruitment of Normal/Control Participants*  
*Describe how prospective normal/control participants will be identified, contacted, and recruited, if the method differs from the above.* | No undue influence will be exercised in the recruitment of subjects, which will emphasize their volitional involvement. Upon recruitments participants will be given a unique subject number for identification purpose. Contact information will be collected by the principle investigator for scheduling purposes only (i.e. if a test needed to be cancelled/rescheduled). |
|---|---|
| 5.6. *Use of Records*  
If existing records (e.g. health records, clinical lists or other records/databases) will be used to IDENTIFY potential participants, please describe how permission to access this information, and to collect and use this information will be obtained. | Same as above. Additionally, through Dr Monteleone's extensive involvement in the athletic community and personal contacts, he will be able to provide prospective normal/control subjects for our study. However, Dr. Monteleone will not be recruiting prospective normal/control subjects from his clinical practice. |
| 5.7. *Summary of Procedures* | Experimental Procedures – Prior to and after each testing session, each subject will complete the SCAT2 symptom evaluation questionnaire to document specific symptoms and their severity. Dr Monteleone has extensive experience of assessing and using the SCAT2 symptom evaluation questionnaires. |
Each testing session will be approximately two hours in duration. The subjects, upon arrival will have all anthropometric measurements taken (height, weight, BMI), as well as, a brief interview to confirm that pre protocol steps were followed (e.g. adequate hydration, no consumption of alcohol and caffeine within 12 hours, no strenuous physical activity). Immediately following the anthropometrics, the subjects will complete a concussion symptom evaluation questionnaire (SCAT2) and perform two neurocognitive tasks (Task Switching and The Stroop test) before the subject is set-up with all the instrumentation (listed above). After set-up, subjects will be asked to perform a second round of the Task Switching and The Stroop test (described later on).

Task Switching Protocol - Executive function will be assessed prior to the bout of exercise using a task-switching protocol. During the protocol, subjects will be comfortably seated in front of a computer monitor on which visual targets consisting of a circle (subtending ~1 degree of visual angle) appearing either at the top or the bottom of a vertically oriented rectangle (subtending ~5 x 2 degrees of visual angle) will be presented. Throughout the different components of the protocol either compatible or incompatible responses to the position of the circle will be required: compatible responses to the circle appearing at the top and bottom require the participant to press the “7” and “1” key, -respectively on the numeral keypads on the right hand side of a standard computer keyboard. By contrast, incompatible responses require the opposite mapping. We will assess performance in two different conditions (each with six blocks of 48 trials). In the single-task condition participants will perform either the
compatible or incompatible versions of the protocol alone. This condition serves as a baseline for the general control demands of the task-switching condition. The task-switching condition requires switching between the two tasks in alternating runs of two trials. Participants will perform the alternating sequence without any external guidance (however, with feedback in case of errors that allows participants to realign themselves with the appropriate sequence. The interval between the response to the previous trial and the next stimulus will be 100 ms. Data collection will take approximately 15 minutes. The dependent variables for the task switching protocol include the reaction time and response accuracy. Reaction time is defined as the time period from the presentation of the visual stimulus to the pressing of the key by the participant. Response accuracy is defined as the percentage of trials within a condition in which the participant pressed the correct key. The “global cost” of switching will be computed by calculating the relative change in reaction time during repeat trials in switch blocks compared to average single task reaction times. The “switch cost” will be computed by calculating the relative change in reaction time during switch trials compared to repeat trials within the switch blocks.

Neurovascular Coupling: Neurovascular coupling will be measured using a Visual Stimulation protocol while seated. The subject will be asked to close their eyes for 2 minutes, then open their eyes read an emotionally neutral article from a computer screen for another 2 minutes. This is immediately followed by 5 cycles of; eyes closed (20-seconds), and open while reading same document (40-seconds).
Cerebral Reactivity: Assessment of cerebrovascular reactivity in both hypercapnic and hypocapnic ranges will be carried out only at rest in an upright and seated position. The hypercapnic range will consist of 4 mins of an inspired fraction of CO2 5% with the intention of raising PETCO2 up 10mmhg. After a break to allow the subject to regain baseline values, the subject will be asked to stimulate the hypocapnic range, which will consist of 4 mins of volitional hyperventilation with the intention of reducing PETCO2 approximately 10mmHg. Safe ranges for human experimentation for hypocapnia should be 20mmHg, similarly marked subject discomfort is apparent with administration of 8% CO2 (15mmHg PaCO2 above rest); thus changes in PaCO2 under this range is recommended for safety and subject related comfort. However, mild changes in CO2 can be followed by minor headache or dizziness during increases in CO2. Subjects will be closely monitored throughout the protocol, however, and in our experience of conducting greater than 2000 of these tests there have been no ill effects reported. The tight controlling of the CO2 and O2 levels used in the technique will be performed using a closed circuit re-breathing system (Respiract, Thornhil Research, Toronto). In normal healthy humans PETCO2 and PETO2 provide accurate estimates of PaCO2 and PaO2. The use of this device has been approved by a previous CREB ethics board (H11-01105).

Dynamic CA: Assessment of dynamic CA is accomplished by combining beat-by-beat cerebral blood flow measurements (transcranial Doppler ultrasound) and blood pressure (finger photoplethysmography) recordings using spectral transfer function techniques. Impairment in dynamic CA is strongly linked to adverse clinical outcomes.
measures we propose for dynamic CA are Stand-Squat and Lower Body Negative Pressure.

Squat-stand technique: The dynamic CA protocol will incorporate what is referred to as the squat and stand technique. Following a 5 min resting baseline subjects are asked to stand while being spotted at all times by two individuals. Once subjects are comfortable and a new steady state standing baseline is achieved, subjects will begin the repeated squat-stand maneuvers, attempting to mimic the lead researchers movements and a metronome as best as possible. The subjects will perform two randomized sets the squat-stand technique with five minutes of resting between each set. The two different sets will differ by the frequency rate the subject is moving from the squat position to the standing position. The two frequencies are a 0.05 Hz (10 second squat-10 second stand) or a 0.10 Hz (5 second squat-5 second stand) for 5 minutes. Careful attention is paid to the depth of the squat obtained, as it is imperative to avoid a valsalva maneuver, which is known to increase brain blood flow, which may influence the results.

Lower Body Negative Pressure: The second method for determining dynamic CA is by using a lower body negative pressure chamber. This chamber allows for the loading and unloading of a supine subject’s heart by methods of negative pressure oscillations. The typical protocol used is a 10 minute resting period, followed by 10 minute pressure fluctuation ranging from 0 mmHg to -40 mmHg. The above techniques for dynamic CA have been approved or are in review by the CREB ethics board (H09-02682, H11-01105).
Force Platform and Centre of Pressure - Subjects will be asked to stand perfectly still with feet shoulder width apart on a 2x2 foot platform, that stand approximately 6 inches off the floor for 5 minutes while a computer software program analyzes postural changes. These postural changes will allow for the computer to perform a series of calculations and return a value of the subject’s centre of pressure. This value will be used as an index of balance, and is safe.

Exercise Protocol - Once subjects finish the stand and squat protocol, subjects will be seated on a specially designed upright bicycle to regain baseline values, before commencing the exercise protocol. Completion of baseline measurements will lead into the start of a 16-minute bout of progressive incremental exercise. In particular, subjects will cycle for 8-minutes at 30% estimated heart rate reserve and provided they remain symptom free; will be ramped up (by adding watts) and attempt to exercise at 70% of estimated heart rate reserve for the final 8-minutes. At each given HRR (30% and 70%), once the subject reaches a steady state for at least 5 minutes, they will then perform the Stroop test while continuing to exercise.

Stroop Task Protocol - During the bout of exercise, the Stroop task will be administered at during the final 2 minutes of each exercise stage. This will allow us to monitor the putative effects of disrupted CA during exercise on a simple probe of executive function. In the Stroop task, a 20-item list of color names are presented either in compatible (e.g. “blue” written in blue ink) or incompatible (e.g. “blue” written in red ink) configurations. The participant is required to read through the
list of names as quickly and accurately as possible. The time required to complete the list and the accuracy of the responses are the dependent variables of interest. At each testing period within the exercise bout the incompatible or compatible list will be presented in a counterbalanced order.

Symptomology will be recorded throughout exercise, and ended if symptoms increase to performance impairing effects. Exercise will finish following a 5-minute warm-down with subjects resting for 10 minutes while remaining seated on the bike before completing a final SCAT2 form. All tests will be terminated at the onset of any returning concussion symptoms (e.g. headache, dizziness, nausea, and fatigue) or on yours (the subjects) request without any discourse or repercussions.

For a time course and order of each protocol please see the attached protocol (pg. 9) for a depiction of the experimental design.

6. Participant Information and Consent Process - Human Ethics Application for Clinical Study [View Form]

6.1. Time to Participate How much time will a participant be asked to dedicate to the project beyond that needed for normal care? 4 visits of 2.5 hours over a one month period.

6.2. Time to Participate – Normal/Control Participants If applicable, how much time will a normal/control volunteer be asked to dedicate to the project? Same as above
<table>
<thead>
<tr>
<th>6.3. Risks/Harms Describe what is known about the risks (harms) of the proposed research.</th>
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<tbody>
<tr>
<td>Exercised induced exacerbation of concussion symptoms (ie; headache, nausea, dizziness) are possibilities. At all times a physician will be present when testing concussion patients who are expected to present symptoms during testing. The presentation of these symptoms without subsiding will be the primary determinant for testing cessation. Upon standing from a resting stage, or during the squat stand protocol, the individuals may experience symptoms associated with fainting or may possible faint. These movements are incorporated into everyday life and have previously used in other studies that have gained ethical approval. Subject will be made aware of these symptoms and if the experience any or they progress rapidly then they will be instructed to inform us and individuals will be returned to the laying position. Also visual recordings of blood pressure will be available, this will allow blood pressure to be monitored, a drop in blood pressure below 80mmHg for more than 10s this is an indication that fainting may occur, if any individuals experience this, they will be rapidly returned to the laying down position. This has been employed in previous studies. There are no major risks associated with the mild changes in PaCO2, however participants may experience light headedness or dizziness during the inspiration of the gas mixture, in which case the gas mixture will be removed and subjects inspire normal room air. Patients will be closely monitored throughout the protocol, however, and in our experience of conducing &gt;2000 of these tests there have been no ill effects reported.</td>
</tr>
<tr>
<td>6.4. Benefits Describe any potential benefits to the participant that could arise</td>
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<tr>
<td>There are no benefits to participants of this study.</td>
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</table>
from his or her participation in the proposed research.

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<tr>
<th>6.5. Reimbursement</th>
<th>Describe any reimbursement for expenses (e.g. meals, parking, medications) or payments/incentives/gifts-in-kind (e.g. honoraria, gifts, prizes, credits) to be offered to the participants. Provide full details of the amounts, payment schedules, and value of gifts-in-kind.</th>
<th>Subjects will not be reimbursed for this study.</th>
</tr>
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<tbody>
<tr>
<td>6.6. Obtaining Consent</td>
<td>Specify who will explain the consent form and consent participants. Include details of where the consent will be obtained and under what circumstances.</td>
<td>Subjects will be free to contact any of the investigators if they wish to discuss the procedures of the experiment following notification either by recruitment, flyers or word of mouth. A meeting will be arrange between the intending volunteer and the investigator, where the study will be presented and experimental procedure and equipment will be explained in detail. After which subjects will decide if they would like to take part in the study. It the participant wants to take part in the study, verbal and written consent will be obtained by one of the investigators, at the research lab on the UBCO campus or at the community-based facility.</td>
</tr>
<tr>
<td>6.7.A. Waiver/Alteration of Consent</td>
<td>If you are asking for a waiver or an alteration of the requirement for participant informed consent, please justify the waiver or alteration and explain how</td>
<td>Not applicable</td>
</tr>
</tbody>
</table>
the study meets all the criteria on the right. Please address each criterion on the right individually.

6.7.B. Waiver of Consent in Individual Medical Emergencies If you are asking for a waiver or an alteration of the requirement for participant informed consent in individual medical emergencies, please justify the waiver or alteration and explain how the study meets all the criteria on the right. Please address each criterion on the right individually.

6.8. Time to Consent How long after being provided with detailed information/consent form about the study will the participant have to decide whether or not to participate? Provide your rationale for the amount of time given.

Subjects will have as long as they need to decide whether they do or do not want to participate.

6.9. Capacity to Consent Will every participant have the capacity to give fully informed consent on his/her own behalf? Please click

Will the Details of If not, If not, will If Yes, participant the nature who he/she be explain t have the of the will able to how assent capacity incapacity consent give assent will be
| Select to complete the question and view further details. | to give fully informed consent? on behalf? to sought. |
|---|---|---|
| No Teenage children aged 14 to 18 will be asked to participate. Parent or Guardian yes Under CREB guidelines the informed consent will be readable by all participants 14 years or older and individual will be able to provide assent alongside parent or guardian signatures. |

6.10. Renewal of Consent
Describe any situation in which the renewal of consent for this research might be appropriate, and how this
would take place.

<table>
<thead>
<tr>
<th>6.11. Provisions for Consent</th>
<th>Not Applicable</th>
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<tbody>
<tr>
<td>What provisions are planned for participants, or those consenting on a participant's behalf, to have special assistance, if needed, during the consent process (e.g. consent forms in Braille, or in languages other than English).</td>
<td></td>
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<tr>
<th>6.12. Restrictions on Disclosure</th>
<th>Not Applicable</th>
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<tbody>
<tr>
<td>Describe any restrictions regarding the disclosure of information to research participants (during or at the end of the study) that the sponsor has placed on investigators, including those related to the publication of results. Also, indicate any plans for communicating study results to participants.</td>
<td></td>
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<tr>
<th>7. Number of Participants and Drugs - Human Ethics Application For Clinical Study</th>
<th>[View Form]</th>
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<tr>
<td>7.1. Multi-Centre Studies</td>
<td></td>
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<tr>
<td>7.1.A. Is this a multi-centre study (involves centres outside of those applied for under this Approval?)</td>
<td>no</td>
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If known, please list the other sites below:

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<thead>
<tr>
<th>7.1.B. Is this study being submitted for ethical approval to any other BC or Canadian Research Ethics Board?</th>
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<tr>
<td>Description: No</td>
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</table>

If yes, please provide the name of the REB(s) and if available, contact information:

<table>
<thead>
<tr>
<th>7.2. Number of Participants</th>
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<tbody>
<tr>
<td>7.2.A. How many participants (including controls) will be enrolled in the entire study? (i.e. the entire study, world-wide)</td>
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<td>90</td>
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<tr>
<th>7.2.B. How many participants (including controls) will be enrolled at institutions covered by this Research Ethics Approval? (i.e. only at the institutions covered by this approval)</th>
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<td>90</td>
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<tr>
<th>Of these, how many are controls?</th>
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<td>60</td>
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<tr>
<th>7.3. Drug approvals Enter the generic name of any investigational drug(s) not yet approved or any marketed drug(s) used</th>
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<tbody>
<tr>
<td>Not Applicable</td>
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<td>Section</td>
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<td>outside of its approved indication.</td>
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<tr>
<td>7.4. Marketed Drugs Enter the name of any marketed drug(s) used within its approved indication.</td>
</tr>
<tr>
<td>7.5. Natural Health Products Enter the name of any Natural Health Products used:</td>
</tr>
<tr>
<td>7.6. Experimental Drugs and Devices Enter the name of any new investigational devices, or marketed devices used in experimental mode, that will be used outside of their approved indication.</td>
</tr>
<tr>
<td>7.7. PERs Enter the name of any positron-emitting radiopharmaceuticals (PERs).</td>
</tr>
<tr>
<td>7.8. Health Canada Regulatory Approvals 7.8.A. Health Canada Regulatory Approvals Is this study a clinical trial or investigational test requiring Health Canada regulatory approval (If this study does not require Health Canada approval, skip to 7.10)</td>
</tr>
<tr>
<td>Question</td>
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<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>7.8.B. If Yes, check all that apply from the list below.</td>
</tr>
<tr>
<td>7.8.C. Name the sponsor/institution/investigator responsible for filing a Clinical Trial Application (CTA) or Investigational Testing Authorization (ITA) with Health Canada or Other.</td>
</tr>
<tr>
<td>7.9. Details of Health Canada Regulatory Approvals If regulatory approval from a Health Canada directorate is required for this study, your certificate of ethical approval will not be released until the regulatory approval certificate, approval date and control number are received by REB administration. Click Add to enter the name of the regulatory agency, the date of the application (if pending) or the date of the approval, and the control number and the date of approval, for either the initial application or subsequent amendments. A</td>
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</table>
| 7.10. Stem Cell Research | 7.10. Stem Cell Research  
Does this research fall within the categories of pluripotent stem cell research that need to be submitted to the CIHR Stem Cell Oversight Committee (SCOC)? | no |
| Health Canada NOL Control Number | Health Canada NOL Control Number  
Date of Approval | |
| 7.11. Registration for Publication of Clinical Trials | 7.11. Registration for Publication of Clinical Trials  
7.11.A. Does this clinical study fall within the definition stated on the right (in the guidelines)? | no |
| 7.11.B. If Yes, click Add to enter the following information. (Please note that registration by UBC ORS administration requires the prior ethical approval of the study. In that case, registration information should be added when it becomes available.) | Has it been registered?  
Indicate the Authorized Registry used:  
Enter your Clinical Trial unique identifier: | |
7.12.A. Is there a requirement for this | no |
research to comply with United States regulations for research ethics?

| 7.12.B. If yes, please indicate whether or not FDA (Investigational New Drug) number (drug studies) or an FDA Investigational Device Exception (IDE) is required for the research and provide documentation from the Sponsor or the FDA verifying the IND/IDE number, or explaining the study exemption status, in Question 9.1.C. |

<table>
<thead>
<tr>
<th>8. Data Monitoring- Human Ethics Application For Clinical Study [View Form]</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.1. Unblinding in an Emergency Describe the provisions made to break the code of a double-blind study in an emergency situation, and indicate who has the code. This is not a Double blinded study</td>
</tr>
<tr>
<td>8.2. Data Monitoring Procedures Describe data monitoring procedures while research is ongoing. Include details of planned interim analyses, Data and Safety Monitoring Board, or other There are no planned interim analysis. Data will only be analyzed on completion of the study.</td>
</tr>
<tr>
<td>8.3. Study Stoppage</td>
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<tr>
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<tr>
<td></td>
</tr>
<tr>
<td>8.4. Personal Identifiers</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>8.4.B. Will any personal health information or personal identifiers be collected?</td>
</tr>
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<td></td>
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<td></td>
</tr>
<tr>
<td>8.5. Data Access and Storage</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Section</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>stage of processing and analysis, and indicate whether a current list of the names of study personnel (including co-investigators) and their delegated tasks will be maintained in the study file. If a list will not be maintained, please explain.</td>
</tr>
<tr>
<td>8.5.B. Describe how the data will be stored (e.g., computerized files, hard copy, video-recording, audio recording, personal electronic device, other).</td>
</tr>
<tr>
<td>8.5.C. Describe the safeguards in place to protect the confidentiality and security of the data.</td>
</tr>
<tr>
<td>8.5.D. If any data or images are to be kept on the Web, what precautions have you taken to prevent it from being copied?</td>
</tr>
<tr>
<td>8.6. Disposition of Study Data 8.6.A. Describe what will happen to the data at the end of the study (including how long the study data will be retained, when and how</td>
</tr>
<tr>
<td><strong>the data will be destroyed),</strong> and what plans there are for future use of the data, including who will have access to the data in the future and for that purpose. If this study involves the creation of a research database or registry for the purpose of future research, please refer to the Guidance note linked on the right and provide the requisite information.</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td><strong>8.6.B. If applicable, describe what will happen to the study samples at the end of the study, including how long the study samples will be retained and where, when and how the samples will be destroyed, and what plans there are for future use of the samples, including who will have access to the data in the future and for what purpose.</strong></td>
</tr>
<tr>
<td><strong>8.7. Data Transfer to Other Institutions Will data be sent outside of the Institution where it is being collected?:</strong></td>
</tr>
<tr>
<td>If yes, please describe the</td>
</tr>
</tbody>
</table>
8.8. Data Transfer to Institution
Will the researchers be receiving data from other sites?: no

If yes, please describe the type of data that will be received, who it will be received from, where it will be received from, and how the data will be received.

8.9. Data Linkage
8.9.A. Will the data be linked to any other data source (including a biorepository)? no

8.9.B. Identify the data set, how the linkage will occur, and explain how confidentiality regarding the shared information will be preserved.

9. Documentation - Human Ethics Application for Clinical Study [View Form]

9.1.A. Protocol Examples of types of protocols are listed on the right. Click Add to enter the required information and attach the

<table>
<thead>
<tr>
<th>Name</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
</table>
| Cerebral blood flow and concussions during exercise | 3       | November 15, 2012 | [View]
### 9.1.B. Health Canada regulatory approval (receipt will be acknowledged)

<table>
<thead>
<tr>
<th>Name</th>
<th>Version</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Informed Consent</td>
<td>4</td>
<td>November 15, 2012</td>
</tr>
<tr>
<td>Concussion Informed Consent</td>
<td>2</td>
<td>December 6, 2011</td>
</tr>
<tr>
<td>Controls Informed Consent</td>
<td>4</td>
<td>October 28, 2011</td>
</tr>
<tr>
<td>Concussion Informed Consent</td>
<td>3</td>
<td>May 14, 2012</td>
</tr>
<tr>
<td>Controls Informed Consent</td>
<td>3</td>
<td>May 14, 2012</td>
</tr>
<tr>
<td>Control Informed Consent</td>
<td>5</td>
<td>May 4, 2013</td>
</tr>
</tbody>
</table>

### 9.1.C. FDA IND or IDE letters (receipt will be acknowledged)

<table>
<thead>
<tr>
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<td>May 4, 2013</td>
</tr>
</tbody>
</table>

### 9.2. Consent Forms

Examples of types of consent forms are listed on the right. Click Add to enter the required information and attach the forms.
<table>
<thead>
<tr>
<th>Name</th>
<th>Version</th>
<th>Date</th>
<th>Views</th>
</tr>
</thead>
<tbody>
<tr>
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<td>4</td>
<td>November 15, 2012</td>
<td>[View]</td>
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<tr>
<td>Concussion Informed Consent</td>
<td>5</td>
<td>May 4, 2013</td>
<td>[View]</td>
</tr>
<tr>
<td>Control Informed Consent</td>
<td>2</td>
<td>December 6, 2011</td>
<td>[View]</td>
</tr>
</tbody>
</table>

9.3. Assent Forms Examples
of types of assent forms are listed on the right. Click Add to enter the required information and attach the forms.

<table>
<thead>
<tr>
<th>Name</th>
<th>Version</th>
<th>Date</th>
<th>Views</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concussion Assent</td>
<td>1</td>
<td>May 4, 2013</td>
<td>[View]</td>
</tr>
<tr>
<td>Control Assent</td>
<td>1</td>
<td>May 4, 2013</td>
<td>[View]</td>
</tr>
</tbody>
</table>

9.4. Investigator Brochures/Product Monographs
Please click Add to enter the required information and attach the documents.

<table>
<thead>
<tr>
<th>Name</th>
<th>Version</th>
<th>Date</th>
<th>Views</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concussion Cerebral Blood Flow Recruitment Email</td>
<td>4</td>
<td>May 4, 2013</td>
<td>[View]</td>
</tr>
<tr>
<td>Concussion Cerebral Blood Flow Recruitment Email</td>
<td>3</td>
<td>November 15, 2012</td>
<td>[View]</td>
</tr>
<tr>
<td>Concussion Cerebral Blood Flow Recruitment Email</td>
<td>2</td>
<td>December 6, 2011</td>
<td>[View]</td>
</tr>
<tr>
<td>Concussion Cerebral Blood Flow Recruitment Email</td>
<td></td>
<td>October 28, 2011</td>
<td>[View]</td>
</tr>
</tbody>
</table>

9.5. Advertisement to Recruit Participants Examples are listed on the right. Click Add to enter the required information and attach the documents.
<table>
<thead>
<tr>
<th>9.6. Questionnaire, Questionnaire Cover Letter, Tests, Interview Scripts, etc. Please click Add to enter the required information and attach the documents.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
</tr>
<tr>
<td>Concussion cerebral blood flow pre screen questionnaire</td>
</tr>
<tr>
<td>Concussion cerebral blood flow pre screen questionnaire</td>
</tr>
<tr>
<td>Concussion cerebral blood flow pre screen questionnaire</td>
</tr>
<tr>
<td>Concussion cerebral blood flow pre screen questionnaire</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>9.7. Letter of Initial Contact Please click Add to enter the required information and attach the forms.</th>
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<tbody>
<tr>
<td>Name</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>9.8. Other Documents 9.8.A. Other documents: Examples of other types of documents are listed on the right. Click Add to enter the required information and attach the documents.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>9.8.B. If a Web site is part of this study, enter the URL below. Since URL's may change over time or become non-existent, you must also attach a copy of the documentation contained on the web site to this section or</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>provide an explanation.</strong></td>
</tr>
<tr>
<td>-----------------------------</td>
</tr>
<tr>
<td>10. Fee for Service - Human Ethics Application for Clinical Study [View Form]</td>
</tr>
</tbody>
</table>

**Please indicate which of the following methods of payment will be used for this application.**

<table>
<thead>
<tr>
<th>N/A (Not funded by an Industry For-Profit Sponsors)</th>
</tr>
</thead>
</table>

**Enter information stating when the fee will be sent:**

<table>
<thead>
<tr>
<th>12. Save Application - Human Ethics Application [View Form]</th>
</tr>
</thead>
</table>
Appendix C.1.1 Mean gain

Appendix C.1.1 Mean Gain 0.05Hz on the left 0.1Hz on the right of SRC and CS together and separated.
Appendix C.1.2 Mean Gain Separated by each visit SRC and CS
Appendix C. 1.2 0.05Hz on the left 0.1Hz on the right of SRC and CS Separated by each visit.
Appendix C.1.3 Driven graphs Gain0.05Hz

Appendix C.1.3 Driven graphs Gain0.05Hz all groups together and then separated by CS and SRC
Appendix C.1.4 Driven graphs Gain By visit 0.05Hz

Appendix C.1.4 Driven Graphs Gain 0.05Hz. Separated by SRC and CS by visit.
Appendix C.1.5 Driven graphs Gain 0.1Hz

Appendix C.1.5 Driven graphs Gain0.1Hz all groups together and then separated by CS and SRC
Appendix C.1.6 Driven graphs Gain By visit 0.05Hz

Appendix C.1.6 Driven Graphs Gain 0.1Hz. Separated by SRC and CS by visit.
Appendix C.2.1 Mean Phase

Appendix C.2.1 Mean Phase 0.05Hz on the left 0.1Hz on the right of SRC and CS together and separated
Appendix C.2.2 Mean Phase Separated by each visit SRC and CS
Appendix C.2.2. Mean Phase 0.05Hz on the left 0.1Hz on the right of SRC and CS Separated by each visit.
Appendix C.2.3 Driven graphs Phase 0.05Hz

Phase

Phase

Phase

Appendix C.2.3 Driven graphs Phase 0.05Hz all groups together and then separated by CS and SRC
Appendix C.2.4 Driven graphs Phase by visit 0.05Hz

Appendix C2.4 Driven Graphs Phase 0.05Hz. Separated by SRC and CS by visit.
Appendix C.2.5 Driven graphs Phase 0.1Hz

Appendix C.2.5 Driven graphs Phase 0.1Hz all groups together and then separated by CS and SRC
Appendix C.2.6 Driven graphs Phase By visit 0.1Hz

Appendix C.2.6 Driven Graphs Phase 0.1Hz. Separated by SRC and CS by visit
Appendix C.3.1 Mean Coherence

Appendix C.3.1 Mean Coherence 0.05Hz on the left 0.1Hz on the right of SRC and CS together and separated
Appendix C.3.2 Mean Coherence Separated by each visit SRC and CS
Appendix C.3.2 Mean Coherence 0.05Hz on the left 0.1Hz on the right of SRC and CS Separated by each visit.
Appendix C.3.3 Driven graphs Coherence 0.05Hz

Appendix C.3.3 Driven graphs Coherence 0.05Hz all groups together and then separated by CS and SRC
Appendix C.3.4 Driven graphs Coherence by visit 0.05Hz

Appendix C.3.4 Driven Graphs Coherence 0.1Hz. Separated by SRC and CS by visit
Appendix C.4.1 RRI Power

Appendix C.4.1 RRI Power 0.05Hz on the left 0.1Hz on the right of SRC and CS together and separated
Appendix C.4.2 Mean RRI Power Separated by each visit SRC and CS
Appendix C.4.2 RRI Power 0.05Hz on the left 0.1Hz on the right of SRC and CS Separated by each visit.
Appendix C.4.3 Driven graphs RRI power 0.05Hz all groups together and then separated by CS and SRC
Appendix C.4.4 Driven graphs RRI Power by visit 0.05Hz

Appendix C.4.4 Driven Graphs RRI 0.05Hz. Separated by SRC and CS by visit
Appendix C.4.5 Driven graphs RRI power 0.1Hz

Appendix C.4.5 Driven graphs RRI power 0.1Hz all groups together and then separated by CS and SRC
Appendix C.4.6 Driven Graphs RRI Power by visit 0.1Hz

Appendix C.4.6 Driven Graphs RRI Power 0.1Hz. Separated by SRC and CS by visit
Appendix C.5.1 SBP Power

Appendix C.5.1 SBP Power 0.05Hz on the left 0.1Hz on the right of SRC and CS together and separated
Appendix C.5.2 Mean SBP Power Separated by each visit SRC and CS
Appendix C.5.2 SBP Power 0.05Hz on the left 0.1Hz on the right of SRC and CS Separated by each visit
Appendix C.5.3 Driven graphs SBP power 0.05Hz

Appendix C.5.3 Driven graphs SBP power 0.05Hz all groups together and then separated by CS and SRC
Appendix C.5.4 Driven graphs SBP Power by visit 0.05Hz

Appendix C.5.4 Driven Graphs SBP Power 0.05Hz. Separated by SRC and CS by visit
Appendix C.5.5 Driven graphs SBP power 0.1Hz

Appendix C.5.5 Driven graphs SBP power 0.1Hz all groups together and then separated by CS and SRC.