MEASURING S100B IN CONCUSSED ATHLETES FOLLOWING A MAXIMAL AEROBIC FITNESS TEST

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ABSTRACT

An objective biological test to measure concussion may provide further

insight into a player's decision to return-to-play. Protein S100B is a calcium-

binding protein that has been studied as a sensitive biomarker of central nervous

system injury after mild head trauma and as a useful predictor of clinical outcome

after brain injury. Both head trauma and exercise influence disruption of the

blood-brain barrier, which causes the release of S100B into the peripheral

circulation. Serum samples were taken from post-concussed and non-concussed

collegiate athletes before and immediately after participation in a maximal aerobic

fitness test (VO_2 max) that quantified each individual's capacity for aerobic ATP

synthesis and determined how well each individual sustained high-intensity

exercise. Serum samples were also taken from a control group of collegiate

students. Samples were analyzed using an ELISA procedure for the detection of

S100B in serum. Aerobic fitness did not differ between post-concussed and non-

concussed groups (mean VO_2 max 52.0 ± 4.7 ml/kg/min and 50.7 ± 6.3 ml/kg/min

respectively) and VO_2 max scores were consistent with the aerobic fitness of

collegiate athletes in other published literature. No change in S100B from pre to

post exercise was evident and S100B did not differ between post-concussed and

non-concussed athletes. Although a VO_2 max is indicative of competition, the

exercise protocol used in this study was not enough of a stimulus to induce

detectable levels of S100B into serum as measured by an ELISA immunoassay.

Future research should further explore exercise protocols of varying intensities

and duration, and consequently, the effects of S100B after these exercises.

Future research should also explore the effects of S100B in concussed collegiate

athletes immediately after injury in order to assess the direct effects that injury

has on serum levels of S100B and contribute to the ever-growing body of

literature in this area of research.

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Chapter I: Introduction

This chapter provides an overview of the rationale for the investigation of the effect of exercise on concentrations of serum S100B protein in previously concussed and non-concussed athletes.

1.1 Athletic Head Injuries

Approximately 300,000 sport-related head injuries occur each year in the United States, and sport is the United States' second leading cause of traumatic brain injury among 15 to 24 year olds (Gessel et al., 2007). In Canada, over 11,000 people die every year from traumatic brain injury as a result of motor vehicle accidents, bicycle crashes, and sport-related injuries, and altogether, head injury is the most frequent direct cause of death in sport (Cantu, 1996). The majority of head injuries are concussions, and sports such as ice hockey and rugby have been found to have the highest incidence of concussion in high school, college, and amateur athletes, while soccer has reported the lowest incidence of concussion (Mendez et al., 2005). Other sports such as boxing and football have historically been shown to have high rates of head injuries (Cantu, 1996).

Athletes who sustain a concussion often become more susceptible to head injury and can be three times more likely to experience a second concussion in the same season (Mendez et al., 2005). Football studies found that players were four to six times more likely to sustain a concussion if they had a history of concussion (Cantu, 1996). Similar studies have indicated that 16.8%

of concussed high school athletes had previously suffered a sports-related concussion in the same season or the season prior (Gessel et al., 2007). High school athletes, in particular, were found to be more easily concussed than older athletes mainly because high school athletes tend to lack maturity of the adolescent central nervous system, fail to recognize that they have sustained a concussion, and are often subjective when reporting a concussion (Theye and Mueller, 2004). Cantu (1996) noted a decrease in the number of serious head injuries as a result of rule changes made to prevent injury, better equipment standards, improved physical conditioning, and enhanced on-the-field medical care. Nonetheless, an athlete's number of sports-related head injury poses a concern in terms of the subsequent short and long-term sequelae that arise following repeated injury (Mendez et al., 2005).

1.2 Concussion

According to Cantu (1996), compressive, tensile or negative pressure, and shearing forces on the brain are three distinct types of stress that can occur from an acceleration force to the head. The brain is typically protected from these rapid accelerations, decelerations, or rotations of the head by the cerebrospinal fluid that surrounds the brain acts as a protective shock absorber (Cantu, 1992). A concussion may result from direct head trauma following a collision, fall, or when sufficient force is applied to the brain without direct trauma, as in the case of whiplash (Cantu, 1996). The effects of applied forces to the brain are often manifested in recognizable signs and symptoms.

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1.2.1   Definition

In the past, the committee on Head Injury Nomenclature of the Congress of

Neurological Surgeons agreed upon the definition of concussion as "a clinical

syndrome characterized by immediate and transient post-traumatic impairment of

neural functions, such as alteration of consciousness, disturbance of vision or

equilibrium due to brain stem involvement" (Theye and Mueller, 2004). In 2001,

researchers at the First International Conference on Concussion in Sports

released a revised definition and stated that a sports concussion is "a complex

pathophysiological process affecting the brain, induced by traumatic

biomechanical forces" (McCrory et al., 2005). Common features of a concussive

head injury also outlined at the conference indicated that,

- "concussion may be caused by a direct blow to the head, face, neck, or

  elsewhere on the body with an impulsive force transmitted to the head,

- concussion typically results in the rapid onset of short-lived impairment of

  neurological function that resolves spontaneously,

- concussion may result in neuropathological change, but acute clinical

  symptoms largely reflect functional disturbance rather than structural

  injury,

- concussion results in a graded set of clinical syndromes that may or may

  not involve loss of consciousness, and resolutions of clinical and cognitive

  symptoms typically follow a sequential course, and

- concussion is typically associated with grossly normal structural

neuroimaging studies" (McCrory et al., 2005).

More recently, researchers at the Second International Conference on Concussion in Sports noted that post-concussive symptoms could be prolonged or persistent after some concussive injuries. For example, a direct or indirect rotational force to the head can lead to any of the following acute symptoms: brief loss of consciousness, light-headedness, vertigo, cognitive or memory dysfunction, tinnitus, blurred vision, difficulty concentrating, amnesia, headache, nausea, vomiting, photophobia, or balance disturbance (Wojtys et al., 1999). Delayed symptoms such as sleep irregularities, fatigue, personality changes, inability to perform daily activities, depression, or lethargy may also appear after the initial trauma, as well as post-concussion symptoms that include headaches at rest and during exertion, dizziness, fatigue, irritability, and impaired memory and concentration (Wojtys et al., 1999; Cantu, 1996). Of the aforementioned symptoms, high school athletes commonly reported headaches (40.1%), dizziness (15.3%), confusion (8.6%), loss of consciousness (3.9%), and amnesia (6.4%) after concussion (Gessel et al., 2007). Up to 80% of patients suffer from persistent symptoms after mild head injury, which can interfere with return to work or leisure activities (Stalnacke et al., 2005).

An injury to the head due to blunt trauma, acceleration, or deceleration forces may also induce a concussion that results in mild traumatic brain injury (mTBI). In these cases, observed or self-reported symptoms of transient confusion, disorientation, impaired consciousness, dysfunction of memory, loss

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of consciousness, signs of neurological or neuropsychological dysfunction, headache, dizziness, irritability, fatigue, or poor concentration are typically documented (Mendez et al., 2005). As a form of traumatic brain injury (TBI), concussions may then be explained in neurological terms as an altered state of consciousness or as a neuropsychological deficit in cognition (Wojtys et al., 1999). While concussion was historically thought to produce a temporary disturbance of brain function due to neuronal, chemical, or neuroelectrical changes without gross structural damage, researchers are now aware that structural damage and loss of brain cells can occur after a concussive injury (Cantu, 1996). Moreover, the damaging effects of concussion are cumulative, and precaution must be taken to avoid the effects of repeated injury. Athletes who return to competition and sustain a second head injury before symptoms associated with a first head injury have cleared are at greater risk of Second Impact Syndrome (SIS) (Cantu, 1996). SIS is characterized by a loss of the brain's ability to regulate its blood supply, which ultimately leads to vascular swelling in the cranium and an increase in intracranial pressure (Cantu, 1996).

1.2.2 Classification of Concussion in Sport

In the past, grading systems have been used to assess the severity of concussion. Grading systems used symptoms such as loss of consciousness to assign a grade to the severity of concussion, with a higher grade generally associated with a concussion of greater severity or death (Theye and Mueller, 2004). For example, Cantu Guidelines indicated that a mild concussion (Grade I)

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occurred without loss of consciousness and only a brief period of post-traumatic amnesia lasting less than 30 minutes, while a moderate concussion (Grade II) occurred if a period of unconsciousness of no more than 5 minutes and post-traumatic amnesia greater than 30 minutes but less than 24 hours was observed (Cantu, 1996). Lastly, a severe concussion (Grade III) occurred if an athlete experienced more than 5 minutes of unconsciousness and more than 24 hours of post-traumatic amnesia (Cantu, 1996). Other guidelines suggested that mild concussions are characterized by a brief period of disorientation or confusion without loss of consciousness or amnesia (Theye and Mueller, 2004).

Despite the many published guidelines, a behavior-sensitive approach is currently preferred that uses the number and duration of post-concussion symptoms as well as loss of consciousness as important criteria when diagnosing concussion (Theye and Mueller, 2004; Cantu, 2007). A multidisciplinary approach that focuses on combined measures of recovery to determine injury severity can help make return to play decisions on an individual-case basis (McCrory et al., 2005). Therefore, the practice of assigning different grades to the level of severity is no longer used and a new classification of concussion in sport has emerged. The new system classifies concussion as either simple or complex. Athletes who suffer a simple concussion have an injury that gradually resolves itself without complication over 7-10 days (McCrory et al., 2005). In these cases, the injury is managed with rest until all symptoms are resolved and a series of exertion tests have been completed before the athlete

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may return to sport (McCrory et al., 2005). In contrast, athletes who suffer persistent symptoms during rest and exertion, and who experience prolonged loss of consciousness and prolonged cognitive impairment after injury, are classified as having complex concussions (McCrory et al., 2005). Athletes who suffer multiple concussions over time or who sustain repeated concussions with less force are also categorized as complex concussions. Given the complex nature of injury, these cases are managed in a multidisciplinary manner (McCrory et al., 2005).

Presently, no objective anatomical or physiological measurements are available to determine if an individual has sustained a concussion, or to categorize the severity of concussion (Wojtys et al., 1999).

1.2.3   Physiology of concussion

Many theories exist to explain the circumstances surrounding concussion. The reticular theory presumes that a concussive blow temporarily paralyzes the brainstem reticular formation, while the centripetal hypothesis links concussion to mechanically induced strains that disrupt brain function (Mendez et al., 2005). A third theory, the pontine cholinergic system theory, concludes that activation of cholinergic neurons result in suppression of behavioral responses, and the convulsive hypothesis attributes concussion to generalized neuronal firing as seen by cerebral hyper-excitability followed by a period of depression after acute head injury (Mendez et al., 2005).

Concussion affects the brain at the cellular level. Physiological events

including neurometabolic cascades of neurotransmitters and ionic fluxes occur

following trauma to the head (Giza and Hovda, 2001). In these instances, injured

cells are exposed to massive ionic fluxes due to the release of excitatory amino

acids. Calcium (Ca^{2+}, in particular, accumulates within hours after injury and can

last for 2 to 4 days (Giza and Hovda, 2001). Influxes of Ca^{2+} occur when the

excitatory amino acid glutamate binds to N-methyl-D-aspartate (NMDA) receptors

that cause neuronal depolarization and further K$^+$ efflux and Ca^{2+} influx ionic

shifts (Giza and Hovda, 2001). An influx of Ca^{2+} can disrupt mitochondrial

oxidative metabolism, worsen the cell's energy crisis and ATP production, and

stimulate glycolysis (Giza and Hovda, 2001). As well, as reviewed in Giza and

Hovda (2001), an accumulation of Ca^{2+} can trigger pathways that lead to cell

death through the disruption of neurofilaments, microtubules, post-traumatic

neural connectivity, and activation of phospholipases, plasmalogenase, calpains,

protein kinases, nitric oxide synthase, and endonucleases. These changes may

also lead to the overproduction of free radicals, cytoskeletal reorganization, and

activation of apoptotic signals (reviewed in Giza and Hovda, 2001). The cells'

ionic imbalance demands additional ATP, and a subsequent increase in cerebral

glucose and glucose metabolism ensues. In turn, Na$^+$/K$^+$ pumps are activated to

restore ionic homeostasis (Glenn et al., 2003). This state of hyper-metabolism

occurs concomitantly with reduced cerebral blood flow, and prompts an energy

crisis that leaves the brain vulnerable to further injury. Following this period of

post-traumatic activation of glycolysis, glucose metabolism is depressed (Giza

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and Hovda, 2001). While the hyper-metabolism phase reflects increased energy

demands for reversal of ionic imbalances, the depression of glycolysis reflects

the decreases in the demand for energy (Glenn et al., 2003). Furthermore, the

energy demands remain high when mitochondrial function is impaired and that

injury events impair glycolysis (Glenn et al., 2003).

Following a concussive event, changes in cerebral glucose metabolism and

overall cerebral pathophysiology can be affected for weeks (Giza and Hovda,

2001). Cerebral oxidative metabolism can remain depressed for the first two

weeks after severe head injury, and the degree of its depression has been found

to correlate with poor long-term outcome (Glenn et al., 2003). Other factors such

as changes in NAD$^+$, NADH, and zinc influx may further worsen decreasing

levels of ATP, and ultimately, result in energy failure (Glenn et al., 2003). Ying

(2007) illustrates that the NAD$^+$ and NADH mediate glycolysis through their roles

as co-factors for the rate-limiting glycolytic enzyme glyceraldehyde-3-phosphate

dehydrogenase (GAPDH), as well as affect mitochondrial oxidative

phosphorylation. Thus, NAD$^+$ and NADH play important roles in the tricarboxylic

acid (TCA) cycle and the electron transport chain (ETC) in mitochondrial energy

metabolism, and as regulators of ATP metabolism and Ca^{2+} homeostasis (Ying,

2007). Lastly, post-concussive deficits are typically a result of temporary

neuronal dysfunction due to ionic shifts, changes in metabolism, damaged

connectivity, or changes in neurotransmission (Giza and Hovda, 2001).

Therefore, the first several days after a concussive injury are important as they

are characterized with dynamic changes in metabolism and blood flow.

1.2.4   *Diagnosis of Athletic Concussion*

Many resources are available to aid in making clinical diagnoses of

concussion, and yet a common approach to the identification of sports-related

concussion is the self-reporting of injury to team personnel. In circumstances

when a concussion results in loss of consciousness, post-traumatic amnesia, or

severe disorientation, self-reporting an injury can be fairly straightforward

(Williamson and Goodman, 2006). However, more than 90% of concussions are

mild and many of the aforementioned symptoms are not always evident, thus

rendering mild injuries difficult to diagnose (Williamson and Goodman, 2006).

Some commonly used laboratory tests to detect structural damage include the

magnetic resonance imaging (MRI), and the gold standard diagnostic tool to rule

out post-traumatic lesions, cranial computed tomography (CT) (Williamson and

Goodman, 2006). Given the risk of TBI, CT scans remain an effective method for

early detection of risks but may lack sensitivity to measure subtle deficits that

affect neurocognition and may not provide adequate information to support

return-to-play guidelines (Muller et al., 2007). In some cases CT scans are

expensive, not always available, and are impractical in an emergency setting.

Additional tests to measure the sensitivity of concussion include the

electrocardiogram (ECG), electroencephalogram (EEG) that measures brain

waves, postural stability sideline tests such as the Balance Error Scoring System

(BESS), and computerized tests that are sensitive to neurocognitive functioning

and measure the domains of memory, learning, reaction time, and speed at which information is processed (Theye and Mueller, 2004). During game or practice settings, sideline paper and pencil tests to determine mental status such as the Sport Concussion Assessment Tool (SCAT) are used as standardized evaluations for the immediate assessment of athletic concussion (Wojtys et al., 1999). SCAT evaluates measures of orientation, immediate memory, concentration, and delayed recall, and is used to detect the presence and severity of neurocognitive impairment associated with concussion. SCAT results provide immediate information to athletic trainers and other medical personnel responsible for making clinical decisions (Wojtys et al., 1999).

Given that the majority of athletic concussions are diagnosed as a result of identifiable signs and symptoms, imaging techniques, and tests based on cognition, the addition of a biological test to measure concussion may provide further insight into a player's decision to return-to-play. For these reasons, biochemical markers have been proposed as an alternative screening method for detection of intracranial injury. As each concussion is unique, a quick, sensitive, and objective biological screening test would be useful to demonstrate the physiological extent of athletic concussion.

1.2.5 Return-to-play After Concussion

After injury more than 50% of high school and collegiate athletes in the United States return to play in 9 days or less (Gessel et al., 2007). Many published guidelines recommend steps to follow when returning to play after an

athletic injury and return-to-play guidelines typically vary according to an athlete's

report of post-concussive symptoms (Collins et al., 1999). In cases of simple

concussions, athletes must rest until all symptoms have been resolved, and

follow-up with a graded program of exertion test before returning to sport

(McCrory et al., 2005). Athletes usually begin with light exercise and work

towards exercise on a stationary bicycle and treadmill. An athlete's response to

physical exercise and their reporting of symptoms during exercise aid in

determining their eligibility to return-to-play (Gall et al., 2004). In contrast,

decisions to return-to-competition in athletes with complex concussions or

athletes who suffer multiple concussions must take into account many factors

and are therefore managed in a multidisciplinary manner (McCrory et al., 2005).

Despite the many published recommendations, often return-to-play decisions are

based on experience rather than evidence, and it is important to note that return-

to-play guidelines may not be appropriate for all age groups because athletes of

varying ages may respond differently to head trauma or vary in recovery time

after injury (Theye and Mueller, 2004). For these reasons, serious decisions

regarding an athlete's return-to-play following a concussion should be advocated

to avoid the cumulative effects of repeated injury and long-term neurological

sequelae. Every effort must be met to protect an athlete's head as injury can

lead to dementia, epilepsy, paralysis, and death (Cantu, 1996). A biological tool

to assess concussion, and especially an objective tool to evaluate physiological

changes compared to baselines measures, will undoubtedly provide essential

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information to aid in the decision to return-to-play. In recent years, neurobiochemical markers of brain damage have emerged in experimental and clinical setting as useful tools to assess brain injury. One of these neurobiochemical markers, S100B protein, has been established in the literature as a marker of brain damage.

1.3. S100 Proteins

S100 proteins are named for their solubility in 100% saturated solution with aluminum sulfate (Moore, 1965). The S100 protein family consists of approximately 20 Ca^{2+} modulated proteins that have highly conserved biological roles among vertebrate species (Michetti et al. 2003). In human brain tissue, S100 proteins exhibit EF-hand, or helix-loop-helix, calcium-binding motifs and exist in astroglial and Schwann cells as alpha-beta (AB) heterodimers, or as alpha (AA) or beta-beta (BB) homodimers (Begaz et al., 2006). Of these isomers, the BB homodimer has a molecular weight of 21 kDa and is prevalent within glial and Schwann cells (Donato, 2001). In addition to the nervous system, S100 proteins are present extra-cranially in sources such as melanocytes, adipocytes, chondrocytes, and epidermal Langerhans cells. The beta isomer is also present in skeletal muscle, and skin cells, as well as in white and brown fat (Bloomfield et al., 2007). S100 proteins play a role in diverse cellular functions including intracellular and extracellular regulatory activities. Essentially, the S100 dimer binds to calcium and undergoes a conformational change that allows interaction with secondary effectors, thus having an affect on biological

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processes (Michetti et al., 2003). Some intracellular regulatory activities include regulation of protein phosphorylation, enzymatic activity, cytoskeleton components, and transcription factors, as well as Ca^{2+} homeostasis, cell proliferation, and cell differentiation. In contrast, extracellular functions include stimulation of neuronal survival and differentiation, astrocyte proliferation, and stimulation or inhibition of inflammatory cells (Zimmer et al., 1995). Although Ca^{2+} plays an important role in the function of S100 molecules, in some cases the biological activity of these Ca^{2+} binding proteins have been regulated by Zn^{+} and Cu^{2+} (Michetti et al., 2003).

1.3.1   *S100B*

As a member of the S100 protein family, S100B is a Ca^{2+}-binding protein with a molecular weight of 21 kDa found in glial cells of brain tissue in both central and peripheral nervous systems (Stranjalis et al., 2004; Zimmer et al., 1995). Initially, S100B was thought to be isolated to astrocytes and Schwann cells of brain tissue, but its presence is now known to exist in other tissues such as cartilage, skin, bone marrow, and fat (Berger et al., 2006). Nonetheless, the greatest expression of S100B is found in the nervous system (Nierwinska et al. 2008).

S100B can regulate many cellular functions including cell-to-cell communication, cell growth, cell structure, energy metabolism, contraction, and intracellular signal transduction (Zimmer et al., 1995). The majority of S100B proteins function intracellularly where they interact with target proteins to couple

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extracellular stimuli to cellular responses (Zimmer et al., 1995). Interestingly, changes in intracellular levels of Ca^{2+} alter the function of Ca^{2+}-binding proteins and their subsequent expression (Zimmer et al., 1995). Michetti et al. (2003) indicated that S100B acts similar to cytokines when secreted by astrocytes, and when released at physiological nanomolar concentrations have neurotrophic effects during development and nerve regeneration. At micromolar concentrations, however, S100B could be neurotoxic and participate in the pathophysiology of neurodegenerative disorders (Michetti et al. 2003). S100B has been established in the literature as a marker of neurologic injury in the perinatal period, an astrocyte-derived cytokine that promotes neuronal survival and development and the synthesis of beta-amyloid precursor protein in neurons and neurites, an indicator of advanced metastasis in melanoma patients, an indicator of the severity of depression, and has also been implicated in the pathogenesis of senile neuritic plaques used in the diagnosis of Alzheimer's disease (Friel et al., 2007; Marchi et al., 2004; Peskind et al., 2001).

According to studies investigating S100B, there are several mechanisms that influence the release of S100B into the extracellular space. For example, S100B may be associated with astrocytic activation and play a role in astrocytes' immediate response to injury through regulation of Ca^{2+} influx and stimulation of astrocytic proliferation through interaction with transcriptional factors (Bloomfield et al., 2007). As well, many biochemical molecules have influenced the release of S100B. For example, serotonin is known to influence the release of S100B via

stimulation of astroglial 5-HT1A receptors, corticotropin-like peptides, and adrenocorticotropic hormones (ACTH), and activation of glutamate receptors or adenosine has also been known to influence the release of S100B (Bloomfield et al., 2007). Upon release from the cytosol of damaged astrocytes, S100B crosses a disrupted blood-brain barrier into the extra-cellular space and then into the serum. S100B is also released into the cerebrospinal fluid via the arachnoid villi and then subsequently released into the blood (Bloomfield et al., 2007). Many studies have concluded that the exact half-life of S100B protein in the blood is still unclear but it is suggested to be close to 97 min with the target time for blood sampling as 24 h after TBI (Rainey et al., 2009). On the other hand, some studies indicate that the serum S100B elimination half-life is 2 hours (Ingebrigsten, 1999), 112 minutes (Ingebrigsten, 1999), or as short as 20-25 minutes and that blood sampling should occur as soon as possible after injury (Berger et al., 2006). It is important to note that a delayed rise in serum S100B occurring after 2 days is possible, and secondary brain injury can also increase serum S100B as much as 6 to 9 days after primary injury (Petzold et al., 2003). S100B is eliminated from the body in urine as a result of renal clearance (Jonsson et al., 2000).

1.4 Objective

The objective of this thesis is to examine the concentration of serum S100B protein in post-concussed and non-concussed Varsity athletes after exercising to exhaustion on a treadmill. The concentration of S100B will be quantified from pre

and post-exercise blood samples taken from post-concussed and non-concussed athletes. S100B will also be measured from a non-concussed no-exercise cohort that will serve as a control group. Lastly, this thesis will assess the aerobic capabilities of post-concussed and non-concussed athletes. To my knowledge there are few studies that have investigated the relationship between exercise and serum S100B, especially after a maximal aerobic fitness test (VO$_2$ max) that quantifies each individual's capacity for aerobic ATP synthesis while exercising at a standardized and controlled intensity. It is hopeful that findings from this thesis will contribute to the growing area of research on biochemical brain markers and it is anticipated that this thesis will demonstrate the usefulness of S100B as an additional objective tool for assessment of brain tissue damage and when making decisions to return-to-play after a sports-related head trauma (Stalnacke et al., 2003).

1.4.1   Hypotheses

Prior to obtaining the results for this study, it was hypothesized that the concentration of serum S100B after high-intensity exercise would be greater than the concentration of serum S100B before exercise in both post-concussed and non-concussed Varsity athletes. As well, it was hypothesized that no difference would be seen between baseline S100B in the non-concussed group, the control group, and the post-concussed group since post-concussed athletes had been cleared to return-to-play and were no longer experiencing any residual effects of concussion. Lastly, it was hypothesized that S100B in the post-concussed group

would be higher after exercise compared to S100B after exercise in the non-concussed group, which would indicate a possible additive effect of exercise and injury on S100B.

Chapter II: Review of Literature

This review of literature thoroughly investigates protein S100B and its role in a range of activities including exercise and the practice of sport. This review also provides evidence of the release of S100B after disruption of the blood brain barrier, and its use as a marker of disruption of the barrier after head injury and concussion. Many studies illustrate as much as a 10 to 15-fold increase above baseline levels of serum S100B after traumatic brain injury (Straume-Naesheim et al., 2008). The presence of S100B after minor head trauma is also associated with pathological findings on CT scans, prolonged in-hospital stays, prolonged absence from work, post-concussive complaints, and disability one year after initial head trauma (Straume-Naesheim et al., 2008). Furthermore, heightened levels can appear hours to days before changes in intracranial pressure, changes on neurological examinations, and changes on neuroimaging tests are seen. Therefore, S100B has been studied as a sensitive marker of CNS injury after mild head trauma and as a useful predictor of clinical outcome after brain injury (Bloomfield et al., 2007).

2.1 The Blood-Brain Barrier

The blood-brain barrier separates the central nervous system from the circulation. Essentially, the barrier is responsible for selective transport of chemicals in and out of the central nervous system and for protection following chemical fluctuations after meals, exercise, and from other circulating agents (Nierwinska et al., 2008). The barrier is highly permeable to H_2O, CO_2, O_2, and

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most lipid-soluble substances, and slightly permeable to electrolytes such as

Na$^+$, Cl$^-$, and K$^+$. The barrier, however, is almost impermeable to plasma proteins

and large organic molecules (Nierwinska et al., 2008). Breakdown of the blood-

brain barrier is widely known to be associated with brain damage. If the blood-

brain barrier is damaged during head injury, proteins may gain access to the

peripheral circulation where they can be sampled in serum. Since S100 proteins

are known markers of blood-brain barrier integrity, their presence in the

peripheral circulation may reflect functional or morphological disruption of the

barrier and possible damage to the central nervous system (Nierwinska et al.,

2008). Moreover, mathematical modeling of S100B kinetics across the blood-

brain barrier indicates that serum S100B is a marker of increased blood-brain

barrier permeability up to a level of 0.34 ng/ml, where higher values are related to

neuronal damage and poor patient outcome (Marchi et al., 2004). Although

levels of S100 protein appear to be directly correlated with the integrity of the

blood-brain barrier, some research has indicated that these levels do not

correlate with neuronal damage (Marchi et al., 2004). It is important to note that

during an increase in blood-brain barrier permeability, neuronal cell death does

not occur during the insult but after a delay. An increase in the permeability of

the blood-brain barrier has also been seen in diseases such as neoplasia,

ischemia, hypertension, dementia, epilepsy, infection, multiple sclerosis, and

trauma. Disease can secondarily affect the cerebral blood flow and vascular tone

in the brain, which further influences selective transport across the blood-brain

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barrier (Marchi et al., 2004).

2.2 Head Injury & S100B

If the blood-brain barrier is damaged during head injury, proteins may gain access to the peripheral circulation where they can be sampled in serum. Following minor head injury, protein S100B can be measured in serum and its concentration may be used as a biochemical marker for prediction of outcome (Woertgen et al., 1999). Many studies have shown that an inverse relationship exists between serum S100B levels and outcome following severe head injury such that patients with higher concentrations of S100B after head injury required a longer period of inpatient observation and were associated with worse neuropsychological outcome six months after head injury (Townend and Ingebrigsten, 2006). In addition, S100B is an established serum marker of primary and secondary brain damage, failure to return to work or activities has been found to correlate with elevated S100B, and neuropsychological deficits have occurred at S100B levels above 500 ng/L after minimal head trauma (Stranjalis et al. (2004); Waterloo et al., 1997). In these cases, the relationship between S100B and unfavorable short-term outcome suggested the need for closer observation, medical tests, or hospitalization (Stranjalis et al., 2004). Lastly, Poli-de-Figueiredo et al. (2006) demonstrated that protein S100B has a very high sensitivity and negative predictive value. Therefore, the role of S100B may be important in ruling out the need for CT scan after minor head injury, especially when trauma incidence is high and medical resources are limited (Poli-

de-Figueiredo et al., 2006).

S100B has been used in many sport-specific studies as a marker of head injury. According to the Stalnacke et al. (2006), most hockey and basketball players demonstrated significantly higher post-game S100B values compared to pre-game values. When S100B was quantified in a player with a Grade 2 concussion, a higher than usual post-game value was measured as well as a higher overall change in the concentration of S100B compared to changes seen in non-concussed players (Stalnacke et al., 2006). Furthermore, two players who suffered Grade 1 concussions displayed high post-game values of S100B but changes from pre to post did not differ from the changes experienced by non-concussed players (Stalnacke et al., 2006).

2.3 Exercise & S100B

A relationship exists among exercise and protein S100B. Many studies have focused on the changes in blood proteins in athletes who participate in a variety of different sports. S100B has been measured after activities involving major physical work such as soccer, ice hockey, basketball, running, and swimming.

2.3.1 *Physical Activity and S100B*

During exercise O$_2$ and CO$_2$ molecules are transported freely across the blood-brain barrier, while carriers embedded within the membrane assist other molecules. Although S100B is quickly released from the brain and into the blood when the blood-brain barrier is disrupted, the effect of physical activity on the

serum level of S100B and the source of its release into the serum are uncertain (Marchi et al., 2004). It is known, however, that S100 proteins do not typically cross the blood-brain barrier under normal physiological conditions and that the barrier can be disrupted specifically as a result of prolonged moderate exercise in warm conditions (Straume-Naesheim et al., 2008). During low to moderate exercise intensities, changes in membrane permeability are not always as obvious despite the use of muscle tissues and these intensities are not likely to damage cells and affect the permeability of the blood-brain barrier (Nierwinska et al., 2008). Even though S100B is released from additional sources outside of the nervous system, the concentration of S100B from these sources are small in comparison to concentrations released from astroglial and Schwann cells (Straume-Naesheim et al., 2008). For example, Straume-Naesheim et al. (2008) established that S100B values after exercise were lower than values after minor head trauma and Mussack et al. (2003) found a short-lived, yet higher, increase in serum S100B after an exercise session with repetitive controlled headers in comparison to simply an exercise session. Intense physical activity also promotes the release of inflammatory factors such as cytokines that are also released in high amounts during trauma. These factors may disrupt nerve cells and show similar cell-activating effects. An increase of this nature may explain the effects seen after exercise as well as the source of its release from the nervous system (Straume-Naesheim et al., 2008). Finally, if the blood-brain barrier is altered during exercise, normal brain function may be disrupted which

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can lead to feelings of central fatigue. Therefore, serum S100B can be used as a marker for disruption of the blood-brain barrier and as an index of brain trauma in individuals who suffer head injuries during sports.

2.3.2   Sport and S100B

S100B has been studied in athletes involved in a variety of different sports. Studies have illustrated significant correlations between elements of game-associated activities and S100B. For example, studies investigating boxers and other high cardiovascular output activities demonstrated a significant increase in serum S100B in activities that involve repetitive, jarring movement, or contact to the head such as boxing, sparring, running, and jogging. Similar results by Otto et al. (2000) found that boxers and runners have enhanced levels of S100B in serum after sport activities. While increased levels of S100B after boxing may be due to brain injury, it is unlikely that running also induces brain injury. Therefore, increased S100B evident after running activities may result from astroglial activation, astroglial destruction, or blood-brain barrier disruption (Marchi et al., 2004). In contrast, Hasselblatt et al. (2004) suggested that increased S100B after running simply originates from extra-cranial sources but Marchi et al. (2004) advise that these levels are not influenced by release from extra-cranial sources. High levels of S100B measured in runners after a race decreased within 20 hours, were weakly related to body weight, and were not associated with sex, age, or training status (Hasselblatt et al., 2004). Various physical activities such as long-distance running, swimming, and basketball are events where head

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traumas and other sudden head-accelerating events like heading are rare, yet increases in S100B after these activities are still evident. In these instances, the effect of physical activity on serum levels of S100B and the source of its release are unresolved. Extra-cranial sources of S100B are well known, but concentrations in these cells are very small compared with those in astroglial and Schwann cells (Straume-Naesheim et al., 2008).

Athletes participating in other sports such as basketball, soccer, and ice hockey have also demonstrated increased S100B after a competitive game as well as positive correlations between game-associated activities and S100B. For instance, serum S100B was increased in male and female soccer players after a competitive game, and increases in S100B correlated significantly with game-related activities such as the number of headers and the number of other trauma events (falls or collisions) for male and female soccer players (Stalnacke et al., 2003; Stalnacke et al., 2006). Stalnacke et al. (2006) also found short-term increases in S100B after 55 minutes of controlled heading in soccer players, after direct head trauma such as heading, and after acceleration-deceleration of the body without head trauma (falls, collisions, jumps) (Stalnacke et al., 2006). Finally, Stalnacke et al. (2008) found higher serum S100B in soccer players after a regular match irrespective of whether players experienced head impacts, and 35% of cases demonstrated S100B values above the suggested cutoff level used for severity screening of patients with minor head trauma in hospitals (Straume-Naesheim et al., 2008). A somewhat smaller increase was found after high-

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intensity exercise without heading (Straume-Naesheim et al., 2008).

S100B studied in basketball players revealed statistically significant correlations between changes in S100B and the number of jumps of players, also known as the most frequent acceleration-deceleration events. In addition, changes in S100B were correlated with the number of jumps for male basketball players and with the number of other trauma events (Stalnacke et al., 2003). Statistically significant correlations were evident between S100B levels post-game and the number of jumps, while no significant correlation was found between the pre-game levels and the number of jumps as well as between the overall change in the concentration of S100B and the total number of acceleration-deceleration events (Stalnacke et al., 2003). Although increases of S100B were seen after acceleration-deceleration events, these changes in S100B were smaller than the changes measured after concussion or mTBI.

Overall, Stalnacke et al. (2006) illustrated that changes in S100B in elite female soccer players were similar to changes seen in male elite soccer players, ice hockey players, basketball players, and swimmers, as well as in males after long distance running. Therefore, changes in serum S100B were similar in both female and male athletes and across sporting activities (Stalnacke et al., 2006; Stalnacke et al., 2003). No differences in S100B levels were seen between high-intensity exercise groups and in athletes after a regular match (Straume-Naesheim et al., 2008). Factors such as exertion, stress, and increased circulating levels of epinephrine have also been shown to increase blood-brain

barrier permeability and subsequently an increase in serum S100B. These physiological responses occur during a competitive match, and thus performing a controlled high-intensity exercise may reflect the responses seen in a regular competitive match. Therefore, if S100B is to be used to detect brain injury in athletes during competition, the confounding effects of exercise must be considered (Straume-Naesheim et al., 2008).

2.4 S100B in Normal Controls

Given that the majority of samples will be pre-exercise and controls, it is expected that samples will yield low concentrations with baseline concentrations similar to each other. This assumption is based on findings from previous literature that quantified S100B in a variety of individuals including controls and baselines. For example, Korfias et al. (2006) quantified S100B in participants with extra-cranial injuries where values above 0.5 ug/L were considered pathological, below 0.15 ug/L normal, and between 0.50-0.15 ug/L borderline. Moreover, Portela et al. (2002) reported a median S100B concentration of 0.105 ug/L and interquartile range 0.015-0.202 ug/L in healthy adults aged 16 to 20 years and a median S100B concentration of 0.100 ug/L and interquartile range 0.045-0.150 ug/L in healthy adults aged 21 to 25 years. Finally, Poli-de-Figueiredo et al. (2006) reported a median S100B concentration of 0.04 ug/l in a healthy control group, Stalnacke et al. (2006) reported baseline S100B concentrations of 0.11 ug/l in male soccer players, Stalnacke et al. (2003) reported baseline S100B concentrations of 0.22 ug/L in hockey and basketball

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players, and Dietrich et al. (2003) reported a mean serum S100 baseline of 70.66

pg/ml in swimmers before a race. With the use of a sensitive assay, S100B can

be found in very low concentrations in normal controls (Bloomfield et al., 2007).

2.5 Study Rationale

The development of effective injury prevention depends upon a greater

understanding and knowledge of concussion rates, patterns, and risk factors

(Gessel et al., 2007). Moreover, injury prevention depends upon the ability to

make sound decisions when returning-to-play after injury. An athlete who is

symptomatic from a head injury must not participate in contact or collision sports

for at least one week and until all cerebral symptoms have cleared. Whether it

takes days, weeks, or months to reach an asymptomatic state, an athlete must

never be allowed to practice or compete while experiencing post-concussion

symptoms (Cantu, 1996). An objective biological indicator of head injury would

provide considerable evidence to confirm injury, especially given that the majority

of concussions are based on self-reporting of signs and symptoms. Furthermore,

an objective biological test may reflect an athlete's unique physiology of

concussion and provide additional insight into the decision to return-to-play

following injury. A better understanding of the roles and effects of exercise on

levels of S100B will certainly be met in studying S100B in athletes without head

injuries and after normal sport practice. As well, studying post-concussed

athletes before and after exercise will help to tease out the effects of exercise

and the effects of concussion on serum concentrations of S100B. In the future,

S100B might be used to reliably predict secondary brain injury and enable physicians to implement therapeutic interventions in a timely manner (Bloomfield et al., 2007).

2.6 Limitations

There are several limitations that are addressed. Although several studies have illustrated the role of S100B as a biochemical marker of brain tissue damage, S100B is also released as a function of an increase in the permeability of the blood-brain barrier during and after exercise or stress (Marchi et al., 2004). Increases in S100B have also been documented in patients with multi-traumas, fractures, and during surgery, all of which indicate that S100B may have low specificity for use as a marker of brain damage (Stalnacke et al., 2006). Finally, in addition to expression seen in glial cells of the central and peripheral nervous systems, S100B may be expressed in melanocytes, adipocytes, and chondrocytes outside of the nervous system, as well as in response to trauma without head injury or neuronal damage (Laterza et al., 2006; Strauma-Naesheim et al., 2008). However, despite these extra-cranial increases, S100B levels reported after many activities such as swimming, running, and boxing are much lower than levels reported after minor head injury, and no biomarker has consistently demonstrated the specific ability to predict post-concussion syndrome after mild traumatic brain injury (Begaz et al., 2006). Thus, the specificity of S100B as an indicator of CNS injury may be compromised by extra-cranial sources that release S100B in the absence of brain injury (Bloomfield et

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al., 2007).

Each independent concussion is influenced by a variety of factors and every individual responds and recovers differently to/from a concussive injury. In other words, variability among participants is already present. For example, each participant will vary in terms of their exercise abilities, their response to exercise, the time from injury to data collection, and the time from data collection to sample analysis. Higher transient increases in serum S100B have been found in young players after an exercise session with repetitive controlled headers in comparison to an exercise session, reasons for which may be that young players are more inexperienced and lack mature technique for the skill of heading (Mussack et al., 2003). Overall, exercise and sport have been shown to raise S100B serum levels, and ranges of different activities or different sports have influenced distinct profiles of these changes. Therefore, the specificity of S100B as a marker of brain injury is still uncertain and further analysis of the role of S100B after exercise and head injury would contribute to the growing body of research on this topic (Stalnacke et al., 2004).

2.7 Delimitations

There are several delimitations that are identified in this study. First, participants were collegiate athletes during the 2008-2009 academic years with the exception of the control group who were not collegiate athletes but students who attended Lakehead University. The post-concussed group consisted of participants who were diagnosed with a concussion within the past 12 months by

a physician and had since returned to competition in their respective sport. The

non-concussed and control groups consisted of athletes and students who were

not diagnosed with a concussion within the past 12 months. The no-exercise

non-concussed group did not partake in aerobic fitness testing.

Chapter III: Methodology

This chapter outlines the methodology used for participant recruitment, participant selection, VO$_2$ max testing, blood collection, and protein analysis. The chapter concludes with a description of the statistical tests used for quantitative analyses.

3.1 Participants

A total of 23 participants were evaluated in this study. Participants included a convenience cohort of 13 collegiate athletes who played on a variety of Varsity teams during the 2008-2009 academic season including ice hockey, volleyball, wrestling, soccer, cross-country running, Nordic skiing, with the exception of one athlete who played ultimate Frisbee on the university's club team. The post-concussed group consisted of 4 athletes who were diagnosed with a concussion within the past 12 months by a physician and who were asymptomatic at rest and have since returned-to-play in their respective sports. The non-concussed group consisted of 9 athletes who were not concussed within the past 12 months. A third group, also known as the non-concussed no-exercise group, served as controls and consisted of 10 students from Lakehead University who did not participate in VO$_2$ max testing and who were not diagnosed with a concussion within the past 12 months.

3.2 Research Design

This study followed a between-group, repeated measures design where blood samples were drawn from post-concussed and non-concussed participants

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before and after running to exhaustion on a treadmill. The design included two independent variables and one dependent variable. The first independent variable, group, consisted of the three groups to which participants belonged - post-concussed, non-concussed, or control. The second independent variable, time, consisted of the repeated measure time, or pre and post exercise. Finally, the dependent variable measured in this study was the concentration of serum S100B protein. Various methods have been developed to measure S100B in serum including a radio-immune assay (RIA), immuno-radio-metric assay (RMA), fluoro-immune assay (FIA), enzyme-linked immunoabsorbent assay (ELISA), and optic immunization (Bloomfield et al., 2007). Of these methods, an ELISA was chosen as it is known as a technique that is simple, inexpensive, convenient, and highly sensitive whereas others such as the 100-immuno-radio-metric (IRMA) assay are less sensitive and have lower detection limits that lack the sensitivity to assess normal control subjects (Bloomfield et al., 2007).

3.2.1   Informed Consent

Participants completed a Physical Activity Readiness Questionnaire, or PAR-Q, before participation in the maximal aerobic fitness test. Participants received a cover letter that outlined the rationale as well as requirements and expectations for participation in this study (**Appendix A**). A consent form was distributed and was signed by the participant before involvement in VO$_2$ max or blood collection (**Appendix B**). A second consent form for participation in VO$_2$ max with direct spirometry was also signed and returned to the researcher

(**Appendix C**). Participants were asked a series of questions concerning their activities within the past 24 hours such as any vigorous activity, food consumption, caffeine consumption, and narcotics or alcohol consumption. Participants also noted any injuries that may have affected their performance and any medication they were taking for a medical condition. Participation was voluntary and they were able to withdraw from the test at their volition. The Research Ethics Board at Lakehead University approved the ethics of this study.

3.3 Descriptive and Anthropometric Measures

Descriptive characteristics such as gender, age (years), sport, and history of concussion were recorded. Participants were asked to identify the number of times they were concussed within the past 12 months, the number of concussions they had sustained prior to the last 12 months, and if they missed playing time as a result of their injury. Anthropometric measures such as height (cm) and weight (kg) were also recorded (**Appendix D**). Finally, participants were asked to identify their smoking status on a General Health Questionnaire and to identify if they had any of the following medical conditions: angina pectoris, asthma, cardiac conditions, diabetes, high blood pressure, Raynaud's phenomenon, rheumatoid arthritis, or any other ailment that might affect their output or participation in this study (**Appendix E**).

3.4 Aerobic Fitness

During high-intensity exercise a greater amount of oxygen is supplied to muscles in the body. The highest amount of oxygen that can be taken in,

transported, and used to produce aerobic ATP during heavy exercise relative to body weight can be quantified using a VO$_2$ max treadmill test and is represented by VO$_2$ max, or maximal oxygen consumption, measured in ml/kg/min (Plowman and Smith, 2008). Therefore, VO$_2$ max provides a quantitative measure of a person's capacity for aerobic ATP synthesis and is an important determinant of how well a person can sustain high-intensity exercise. Attainment of a high VO$_2$ max has important physiological meaning, especially given its role in sustaining energy metabolism and the integration of responses from the various body systems (McArdle et al., 2007). In certain cases, a plateau of oxygen consumption may not be reached and attainment of maximum effort may be limited by injury, illness, or fatigue. Maximal oxygen consumption in these cases is referred to as peak VO$_2$ (McArdle et al., 2007).

A treadmill test was chosen to ensure that exercise intensity was standardized for each participant. Moreover, a treadmill test was chosen rather than a bicycle ergometer test since Marchi et al. (2004) found no increase in serum S100B after exertion on a stationary bicycle. As well, low to moderate exercise intensities are not likely to damage cells or to affect the permeability of the blood-brain barrier (Nierwinska et al., 2008). A treadmill running test to exhaustion was chosen to simulate high-intensity exercise.

3.4.1. Modified Bruce Protocol

The VO$_2$ max took place in the Exercise Physiology Laboratory in the C.J. Sanders Fieldhouse at Lakehead University. Given that the test was a maximal

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cardio-respiratory fitness test, a supervisor was present for the duration of testing and all required safety precautions were met.

A modified Bruce protocol was used to assess aerobic fitness (**Appendix F**). The original Bruce Protocol is a standardized treadmill test for assessment of cardiovascular fitness. In comparison to the Bruce Protocol, the modified Bruce Protocol is adjusted so that the treadmill begins in a horizontal position rather than on an incline, and only the slope of the treadmill is increased during the first few intervals (McArdle et al., 2007). As a test of maximum capacity, exercise was performed at the highest intensity and the participant was subject to progressive increases in treadmill elevation until exhausted and unable to continue. Oxygen consumption was assumed to increase rapidly during the first few progressive increases and then fail to increase as rapidly or to the same extent during the final few increases in elevation (McArdle et al., 2007). That being said, gradual increases in treadmill elevation required greater energy output and demand on aerobic ATP synthesis (McArdle et al., 2007). The region where oxygen consumption reached a plateau or increased only slightly during additional increases in exercise intensity represented the participant's maximal oxygen consumption or VO$_2$ max (McArdle et al., 2007). The test ended when VO$_2$ max was reached or if the participant grabbed onto the handrails and/or was too exhausted to continue. The test also ended if the researcher concluded that the participant was in cardiac or respiratory distress. Participants were able to voluntarily stop the test at any time. Testing protocols and expectations were

introduced and explained to all participants prior to the test.

Physiological baselines measures such as resting blood pressure (mmHg) and heart rate (beats per minute (bpm)) were taken for each participant (**Appendix G**). The test began with a five-minute warm-up on the treadmill. A heart rate monitor was fitted around the participant's chest, and a facemask was attached to the COSMED Pulmonary Function Equipment used to analyze and monitor oxygen consumption and heart rate. In addition, heart rate and VO_2 values were manually recorded every 30 seconds throughout the test and during the cool down period. Spotters were used as a safety precaution in case the participant slipped or fell on/off the treadmill. Immediately after the test, participants began a 5-minute cool down period where oxygen consumption and heart rate were monitored. Final blood pressures and heart rates were taken within 5 to 10 minutes post-exercise. Participants were supervised in the laboratory until all physiological measures returned to baseline and it was deemed safe for the participant to leave.

3.5 Blood Sampling

Blood collection took place in the Exercise Physiology Laboratory located in the C.J. Sanders Fieldhouse at Lakehead University.

3.5.1   Blood Collection

A phlebotomist from LifeLabs Medical Laboratory mobile services collected blood by venipuncture. A total of 10 ml or 10 cc of blood was drawn from each participant. The phlebotomist collected a 5 ml "pre-exercise" blood sample 5

minutes before warm-up. A 5 ml "post-exercise" blood sample was taken within 15 minutes after the completion of the VO$_2$ max as the biological half-life of serum S100B has been reported to be as short as 25.3 minutes (95% CI, 15.3-35.3 minutes)(Straume-Naesheim et al., 2008). Blood samples were collected in serum separator tubes, or SSTs. After collection, tubes were inverted five times and placed in a vertical position for 30 minutes to allow for coagulation.

3.5.2   *Serum transport and storage*

Serum samples were transported from the Exercise Physiology Laboratory in the C.J. Sanders Fieldhouse to the laboratory in the Northern Ontario School of Medicine at Lakehead University. Samples were centrifuged for 10 minutes at 1300 RCF (g) to avoid hemolysis. Serum samples were stored in aliquots at -80°C until the time of assay.

3.6 Serum Protein Analysis

Protein analysis took place in the laboratory at the Northern Ontario School of Medicine. Blood samples were analyzed using an immunoassay kit that followed an ELISA, or Enzyme Linked ImmunoSorbent Assay, procedure. Essentially, an ELISA is used to detect the presence of an antibody or antigen in a sample through a series of antibody-antigen binding and washing steps followed by the addition of a substance to convert the antibody-linked-enzyme to a detectable signal (BioVendor, 2009). In this case, a sandwich enzyme immunoassay technique was used to detect and quantify S100B in human serum.

3.6.1   Human S100B ELISA

A total of 5 kits were used to analyze the concentration of S100B protein in serum samples. Each microplate contained 96 wells and consisted of a set of standards, a high quality control, a low quality control, and samples that included post-concussed, non-concussed, and controls. Standards were based on the beta-beta homodimer in S100B purified from human brain tissue (animal based), while the quality controls were human serum based. Human S100B master standard at 4000 pg/ml was reconstituted with dilution buffer and deionized water to prepare serial dilutions of standard stock at 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 200 pg/ml, 100 pg/ml, and 50 pg/ml. Quality controls were also reconstituted from a lyophilized form using deionized water. All of the standards, quality controls, and samples were diluted 4X with dilution buffer and 100 ul of each were pipetted in duplicates into a 96-well microplate pre-coated with polyclonal anti-cow S100B antibodies. After 120 minutes of incubation on an orbital shaker at 300 rpm, the plate was washed and a conjugate solution of monoclonal anti-human S100B antibodies labeled with horseradish peroxidase (HRP) was added to each well. After 90 minutes of incubation and shaking on an orbital shaker at 300 rpm with captured S100B, the plate was once again washed and the substrate solution TMB, or tetramethylbenzidine, was added to each well. After 15 minutes of incubation, the reaction was stopped with the addition of an acidic stop solution that caused noticeable change in colour from blue to yellow. Absorbance was measured spectrophotometrically at 450 nm and output was

generated using the software provided. Given that absorbance is proportional to the concentration of S100B, a standard curve was constructed by plotting absorbance values against concentrations of standards. Concentrations of the unknown samples were determined using this standard curve (BioVendor, 2009).

Each microplate contained duplicate wells of standards, quality controls, and samples to verify the intra-plate variability. A number of independent kits were used as replicates to examine the variability between plates. The software provided with the microplate reader (KC4™) created a standard curve using the known concentrations of the set of standards, and then performed automatic calculations of the unknown sample concentrations. Results are reported as concentration of S100B in pg/ml and standard curves were created by plotting the mean absorbance at 450 nm of standards (y-axis) against log of the known concentration of standards (x-axis) using the four-parameter algorithm (BioVendor, 2009). According to BioVendor (2009), the analytical limit of detection calculated from the real S100B values in wells was 5 pg/ml, and the assay sensitivity of 20 pg/ml takes the 4X dilution of samples into consideration.

3.7 Statistical Analyses

As a result of the high variability of the O_2 analyzer when testing VO₂ max in trained individuals, the researcher selected a data transformation protocol based on Hoehler (1995) (**APPENDIX H**). In this procedure the last ten estimates of oxygen consumption are fitted using a logistic regression data transformation to ensure an optimized asymptotic prediction. As a result of the unequal sample

sizes between the two groups, and therefore assumed unequal variances, non-parametric statistics were used for analysis. A SAS program performed a Wilcoxon two-sample test to calculate the sum of the scores and the sum of the ranks in both groups in order to test for a difference between the predicted VO$_2$ max of post-concussed and non-concussed groups. Statistical significance was accepted if $p < 0.05$.

Chapter IV: Results & Discussion

4.1 Descriptive and Anthropometric Measures

Descriptive characteristics such as gender and age as well as anthropometric measures such as height and weight are recorded in **Table 1**. The post-concussed sample consisted of two males and two females, the non-concussed sample consisted of six males and three females, and the control group consisted of five males and five females from a university age cohort.

Table 1. Descriptive measures (gender, age) and anthropometric measures (height, weight) in post-concussed, non-concussed, and control groups.

Post-Concussed			
Gender	Height (cm)	Weight (kg)	Age (yrs)
F	166.5	55.5	22
M	180	95	25
F	170	65	22
M	169.5	77	21

Non-Concussed			
Gender	Height (cm)	Weight (kg)	Age (yrs)
F	178	73	21
M	172	65	23
F	177	70	20
M	178.5	83	20
M	181.5	86	19
M	174	68	19
M	177	80	22
F	176	66	22
M	192	100	23

Controls			
Gender	Height (cm)	Weight (kg)	Age (yrs)
F	160	62	23
F	178	72	21
F	165	60	25
M	181	87	22
M	173	70	24

M	170.5	80	24
M	181	93	26
M	172	70	24
F	167	64	24
F	180	95	25

Half of the participants who were concussed within the past twelve months

had a history of concussion (three or more), while the other half had no prior

history of concussion. Of all post-concussed athletes, the most recent

concussion occurred eight weeks prior to testing. All twenty-three participants

were identified as non-smokers, one participant had Raynaud's phenomenon,

and four participants identified asthma from the following medical conditions on

the General Health Questionnaire: angina pectoris, asthma, cardiac problems,

diabetes, high blood pressure, Raynaud's phenomenon, rheumatoid arthritis. No

other ailments or disorders were reported that might have affected the output or

participation in this study.

4.2 Aerobic Fitness

Physiological measures including blood pressure and heart rate taken

before and after exercise are seen in **Appendix I**. Similar physiological

measures are evident across both groups, and in accordance with Gall et al.

(2004), exercise capacity appears to be unaffected in concussed athletes who

are asymptomatic at rest. Moreover, heart rates at rest were consistent across

both groups, a finding similar to Gall et al. (2004) who found no difference in

heart rate at rest between concussed athletes and their matched controls. Unlike

Gall et al. (2004), however, the maximum heart rate was not higher in post-

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concussed athletes which may be explained by the time elapsed since injury as
athletes in Gall et al. (2004) performed the test at one week after being
asymptomatic at rest. In addition to physiological measures, VO$_2$ max estimates
obtained from the COSMED, scores recorded by the researcher, and the
transformed VO$_2$ max scores of post-concussed and non-concussed groups are
seen in **Appendix J.** COSMED estimations of VO$_2$ max were verified using a
logistic regression equation that fit the last ten estimates of O$_2$ consumption to an
S-shaped curve (Hoehler, 1995). Raw VO$_2$ scores and data transformation
results for one participant is seen in **Figure 1**.
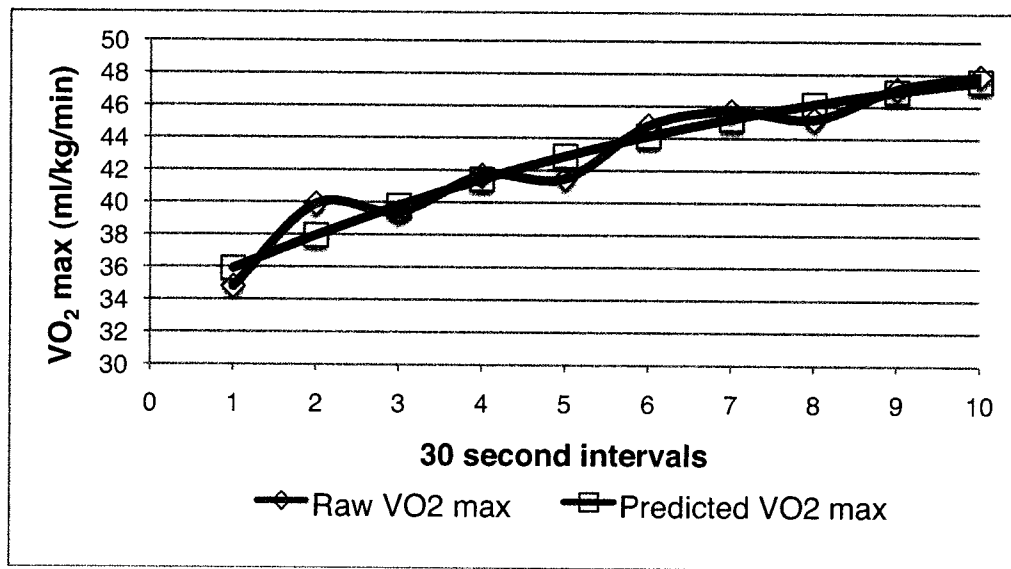
Figure 1. Oxygen consumption recorded in 30-second intervals and
transformed data using logistic equation protocol based on Hoehler
(1995) to ensure an optimized asymptotic prediction.

No significant difference in predicted VO$_2$ max was seen between post-
concussed and non-concussed groups (U=27, p > 0.05) **(Figure 2)**.
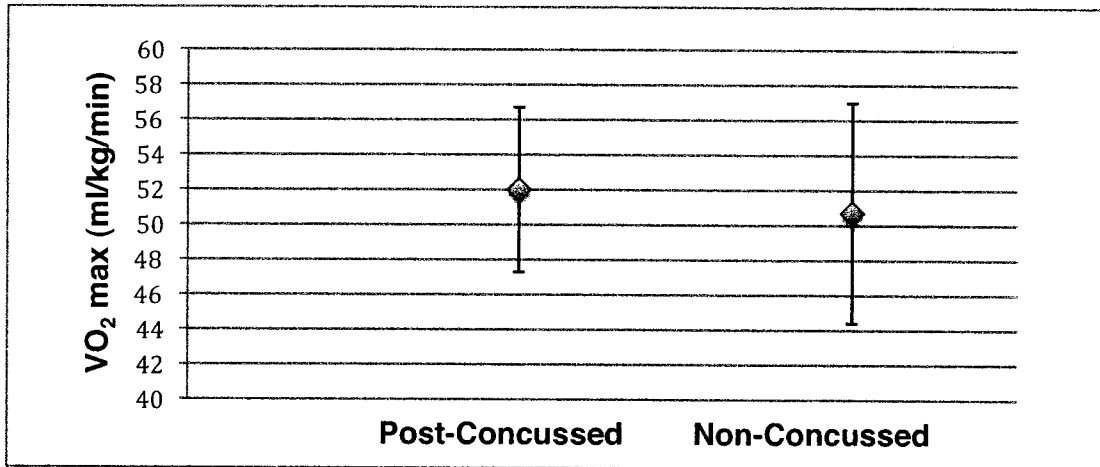
Figure 2. Mean VO$_2$ max in post-concussed and non-concussed athletes.

The post-concussed group (n=4) had a transformed VO$_2$ max mean of 52.0 ± 4.7

ml/kg/min while the non-concussed group (n=9) had a transformed VO$_2$ max

mean of 50.7 ± 6.3 ml/kg/min. Overall, mean VO$_2$ max for Varsity athletes (n=13)

was 51.1 ± 5.7 ml/kg/min.

A number of tables of normative values for VO$_2$ max have been published.

Table 2 outlines the aerobic capacities obtained in this study compared to

normative values categorized according to sex and age published by the Cooper

Institute for Aerobics Research (Heyward, 1998).

Table 2. Treadmill VO$_2$ max results and normative VO$_2$ max data categorized
according to sex and age.

Aerobic Fitness		
Participants	**Results: VO$_2$ max**	**VO$_2$ max (Heyward, 1998)**
Males Age: 3-19 years	53.4 ml/kg/min 50.6 ml/kg/min	**Superior**: > 55.9 ml/kg/min **Excellent**: 51.0 - 55.9 ml/kg/min **Good**: 45.2 – 50.9 ml/kg/min **Fair**: 38.4 – 45.1 ml/kg/min **Poor**: 35.0 – 38.3 ml/kg/min **Very Poor**: < 35.0 ml/kg/min

Males **Age: 20-29 years**	58.3 ml/kg/min 56.7 ml/kg/min 56.2 ml/kg/min 55.1 ml/kg/min 49.5 ml/kg/min 47.6 ml/kg/min	**Superior**: > 52.4 ml/kg/min **Excellent**: 46.5 – 52.4 ml/kg/min **Good**: 42.5 – 46.4 ml/kg/min **Fair**: 36.5 – 42.4 ml/kg/min **Poor**: 33.0 – 36.4 ml/kg/min **Very Poor**: < 33.0 ml/kg/min
Females **Age: 20-29 years**	54.0 ml/kg/min 53.0 ml/kg/min 49.3 ml/kg/min 41.1 ml/kg/min 39.9 ml/kg/min	**Superior**: > 41.0 ml/kg/min **Excellent**: 37.0 – 41.0 ml/kg/min **Good**: 33.0 – 36.9 ml/kg/min **Fair**: 29.0 – 32.9 ml/kg/min **Poor**: 23.6 – 28.9 ml/kg/min **Very Poor**: < 23.6 ml/kg/min

While there are many published tables of normative values for VO$_2$ max where values are categorized according to age and gender, it is less common to find normative values for athletes who participate in specific sports (Mermier et al., 2008). Many tables illustrating VO$_2$ max for athletes are not specific to athletes who are currently competing, use athletes of varying ages, or use data that mixes professional, elite, collegiate, and Olympic athletes (Mermier et al., 2008). Many factors must be considered when analyzing published tables such as the athlete's familiarity with the testing procedures, the time of testing and whether it was pre-season, mid-season, or post-season, as well as the athlete's overall general health and injury status.

In this study, the mean VO$_2$ max of collegiate athletes was similar to the mean VO$_2$ max of 55.1 ± 7.1 ml/kg/min in collegiate athletes on Varsity football, basketball, wrestling, hockey, weight lifting, and track and field teams reported by

Lukaski et al. (1983). Lukaski et al. (1983) also found that Varsity athletes had a

much higher mean VO$_2$ max compared to the mean VO$_2$ max of 47 ± 6.0

ml/kg/min in non-athletes. Overall, VO$_2$ max scores observed in this study were

similar to sport-specific VO$_2$ max results found in collegiate athletes from other

published literature (**Table 3**).

Table 3. Treadmill VO$_2$ max results and sport-specific VO$_2$ max data from the literature. Note: Sport-specific VO$_2$ max categorized according to sex and age.

Aerobic Fitness		
Sport	**Results: VO$_2$ max**	**VO$_2$ max (Published literature)**
Basketball	54.0 ml/kg/min	43.0-60.0 ml/kg/min (Wilmore and Costill, 2005)
Cross-country running	56.2 ml/kg/min	79.4 ml/kg/min, 78.5 ml/kg/min in middle distance & 74.4 ml/kg/min in marathon runners (Mermier et al., 2008; Pollock et al., 1975)
Ice hockey	47.6 ml/kg/min	50.0-63.0 ml/kg/min (Wilmore and Costill, 2005)
Nordic skiing	49.3 ml/kg/min 53.4 ml/kg/min & 56.7 ml/kg/min	82.0 ml/kg/min (Saltin and Astrand, 1980)
Soccer	50.6 ml/kg/min	54.5-61.9 ml/kg/min, 62 ± 4.1 ml/kg/min; range 49.9-63.9 ml/kg/min, 58.4 ± 0.83 ml/kg/min; range 53-66.7 ml/kg/min (Mermier et al., 2008; Raven et al., 1976)

Volleyball	53.0 ml/kg/min, 41.1 ml/kg/min & 39.9 ml/kg/min	43.2 ml/kg/min (Spence et al., 1980)
Wrestling	58.3 ml/kg/min & 55.1 ml/kg/min	57.0 ml/kg/min (Saltin and Astrand, 1967)

Post-concussed and non-concussed collegiate athletes demonstrated similar capacities for oxygen consumption since VO$_2$ max scores of post-concussed athletes did not differ significantly from VO$_2$ max scores of non-concussed athletes. Given the prerequisite that participants in the post-concussed group must have returned-to-play in their respective sports, the participants were accustomed to high-intensity exercise. Moreover, post-concussed athletes were concussed between September 2008 and February 2009 and testing did not take place until April, May, and June 2009. As a result, the timeline from injury to testing was different for each participant with the most recent injury having occurred eight weeks prior to testing. Therefore, as expected, the ability to perform high-intensity exercise did not differ between groups.

4.3 Human S100B ELISA

Post-exercise S100B was not greater than pre-exercise S100B in post-concussed or non-concussed athletes. As well, S100B in post-concussed athletes was not higher than S100B in non-concussed athletes or controls.

In theory, if samples are assayed identically and processed in exactly the same manner then sample assay responses are directly comparable to each other. Furthermore, if samples are processed in the same manner the variance in protein quantity is the only possible cause for difference in final absorbance. In this study samples were plated in duplicates, which should have induced similar, if not identical, absorbance. Absorbance results showed consistency in the method where absorbance of standards, quality controls, and samples in duplicates are matched. **Table 4** illustrates the mean absorbance, standard deviation, and coefficient of variation of standards and quality controls obtained from the ELISA as well as the expected average absorbance according to the Certificate of Analysis. The mean absorbance for standards and quality controls was similar to the expected absorbance for standards and quality controls noted in the certificate of analysis.

Table 4. Mean absorbance, standard deviation, and coefficient of variation of standards and quality controls from the ELISA and expected absorbance at 450 nm according to the Certificate of Analysis.

ELISA – 450 nm				
ID & Expected Concentration/ Dilution (pg/ml)	Expected absorbance A$_{450}$ average – Certificate of Analysis	Mean absorbance	Standard deviation	CV %
Standard 2000	3.118	2.667	0.033	1.246
Standard 1000	1.718	1.627	0.086	5.257
Standard 500	0.879	0.73	0.054	7.453
Standard 200	0.475	0.346	0.003	0.817
Standard 100	0.247	0.171	0.011	6.616
Standard 50	0.155	0.08	0.018	22.236

Quality Control High	1.502	0.924	0.209	22.651
Quality Control Low	0.425	0.202	0.034	16.701
Blank	0.063	0.043	0.003	6.719

Furthermore, concentrations results showed consistency in the method where concentration of standards and quality controls are matched. Expected concentrations of standards, obtained concentrations of standards from the certificate of analysis, as well as observed concentrations, standard deviations, and coefficient of variation from the ELISA are seen in **Table 5**. The mean concentration for standards and quality controls was similar to the expected concentration for standards and quality controls noted in the certificate of analysis. Given the consistency of references, it is evident that the researcher's use of pipettes, mixing of reagents, and/or other lab techniques utilized in the ELISA procedure is reasonable.

Table 5. Expected S100B concentrations of standards, obtained S100B concentration of standards from the certificate of analysis, observed mean S100B concentrations, standard deviations, and coefficients of variation from the ELISA.

ELISA – 450 nm					
ID & Expected Concentration/ Dilution (pg/ml)	[Obtained] (pg/ml) – Certificate of Analysis	[Observed duplicate] (pg/ml)		[Observed mean] (pg/ml)	Standard deviation	CV %
Standard 2000	1998	2025.7	1962.1	1993.85	44.976	2.256
Standard 1000	1012	974.74	1058.2	1016.41	58.927	5.798
Standard 500	473	493.73	449.78	471.756	31.081	6.588
Standard 200	228	242.51	239.86	241.185	1.875	0.777
Standard 100	95	115.83	100.92	108.376	10.544	9.729
Standard 50	43	<41.58	<41.58			
Quality Control	870	576.35	746.59	582.989	121.01	20.76

High 836 (669-1004)		553.53	455.50			
Quality Control Low 205 (164-246)	198	166.97 97.026	141.62 134.13	134.938	28.919	21.43

A standard curve was constructed where mean absorbance was plotted against log of the known concentrations of standards (**Figure 3**). The following equation was used to calculate data points for the standard curve.

$$y= (a-d) / ((1+ (x/c) \wedge b) + d)$$

Coefficients used in the equation as well as the coefficient of determination (R^2) that represents how well the outcomes are likely to be predicted by the model are presented in **Table 6.** Note that R^2 is close to 1, where an R^2 of 1 indicated that the regression line was perfectly fit to the data points.

Table 6. Coefficients used to calculate the standard curve.

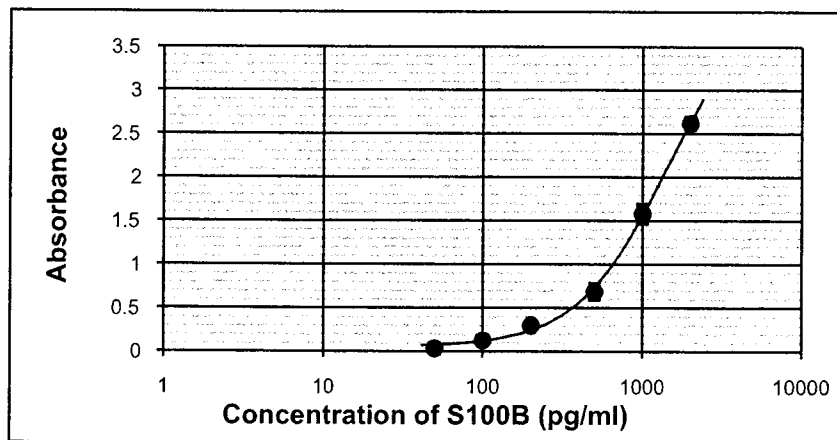| ID	A	B	C	D	R	R²	Err.
STD	0.051	1.537	1420.26	4.152	0.999	0.998	0.037

Figure 3. S100B standard curve is plotted using the four-parameter function as a proportion of the log of the known concentration of S100B and absorbance at 450 nm. Results are reported as concentration of S100B in pg/ml.

In contrast to absorbance of standards and quality controls, absorbance of samples did not produce detectable S100B concentrations. The concentration of S100B in samples was less than 41.6 pg/ml or lower than the minimum concentration plotted on the standard curve. Thus, no difference in the concentration of S100B before and after exercise could be determined in post-concussed and non-concussed samples. These results suggest that the concentration of S100B present in samples was too low to be detected using the Human S100B ELISA and that the exercise performed in this study did not increase S100B to detectable levels. According to the literature, S100B is found in low or undetectable levels in plasma in normal subjects. Moreover, 95% of patients with no previous history of neurological injury demonstrate levels of S100B below 0.12 ug/l, and sex and age in normal controls are not likely to significantly influence serum S100B levels (Marchi et al., 2003; Nygaard et al., 1997). Given that the majority of the samples were pre-exercise and controls, it was expected that the samples would yield low concentrations and that these baseline concentrations would be similar to each other. Furthermore, baseline S100B concentrations in the post-concussed group were expected to be low as participants had since returned-to-play and were no longer experiencing any residual effects of concussion. In this case, however, the immunoassay lacked the sensitivity to detect very low concentrations of S100B or subtle changes of S100B in the samples, and the exercise protocol was inadequate to induce a

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detectable measure of S100B in serum.

Given that the blood-brain barrier can be disrupted during physical activity, a treadmill test was chosen as it represented the high-intensity exercise experienced during competition. Although running was thought to be adequate to provoke a change in the blood-brain barrier, it is possible that the exercise did not change the permeability of the blood-brain barrier, or that the exercise did change the permeability of the blood-brain barrier but not enough in terms of intensity or duration to release S100B into serum. The VO$_2$ max test utilized in this study lasted no more than 15 minutes, and even though studies such as Marchi et al. (2004) found increased serum S100B after activities such as boxing, sparring, running, and jogging, and Otto et al. (2000) measured S100B in healthy boxers after five-minute rounds and in healthy sprinters after two-minute sprint intervals, it is likely that increased S100B was seen in the aforementioned examples due to exercise protocols that underwent more than one physical activity and recovery period. A rise of S100B during these exercises was also thought to be provoked by axial vibration of the brain, a concept that was later examined by Dietrich et al. (2003) who found an increase in S100B in athletes after exercise of a longer duration such as a non-impact 7,600-meter swimming race. Therefore, Dietrich et al. (2003) concluded that there is a potential acute influence of prolonged exercise on serum S100B not related to central nervous system injury, and instead, that physical activity produced an increase in S100B independent of trauma caused by axial vibrations in the brain. A treadmill VO$_2$

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max test was thought to be sufficient exercise to elicit a release of S100B from extra-cranial sources as the amount of aerobic work in a maximal test is often indicative of aerobic work generated in a competitive sporting environment (Straume-Naesheim et al., 2008). Other factors such as exertion, stress, and increased levels of epinephrine are known to be associated with competition and have also been known to increase the permeability of the blood-brain barrier. These factors were thought to be present during a treadmill VO₂ max and would have therefore contributed to an increase in the barrier's permeability. Another possible reason for a lack of S100B in blood samples is the amount of water that was ingested before and after the treadmill VO₂ max. Nierwinska et al. (2008) suggested that water ingestion could limit exercise-induced increases in serum S100B due to the limiting osmotic movement of fluid across the blood-brain barrier, and that changes in the permeability of the blood-brain barrier to S100B may give misleading results in exercising individuals under conditions that lead to heat stress (Nierwinska et al., 2008). Lastly, S100B may not be present if its release from tissue into the blood was delayed. Studies have illustrated that S100B in participants with severe head injury can be released following a delay of up to a few hours (Petzold et al., 2003). Although a delayed release of S100B is possible, the majority of literature indicated that the half-life of S100B can be as short as 25 minutes and blood collection should take place within 15 minutes after injury or exercise.

4.4 Limitations & Recommendations

The following limitations and recommendations were identified in this study.

4.4.1   Participation Recruitment

A larger sample size is needed for future studies. Although a larger number of concussed athletes were recruited, many were not available during May 2009 and June 2009. As well, testing dates conflicted with some prospective participants due to the complexity of scheduling participants, phlebotomists, supervisors, and researchers. In this study, the most recent concussion occurred eight weeks prior to testing. Although post-concussed participants must have been cleared to return-to-play for ethical purposes, greater effort should be made to identify prospective post-concussed participants immediately following their injury where data collection would take place as soon as they returned-to-play. These issues would undoubtedly be addressed for future studies such that data collection would be ongoing throughout the academic year and all samples would be immediately aliquoted and frozen at -80°C until analysis.

4.4.2   VO$_2$ max and Blood Collection

Following data collection the researcher became aware that the speed of the treadmill used in this study was 2.5 miles faster than the required speed in the Modified Bruce Protocol. However, all participants used the same treadmill and experienced the same change in speed. Even though it is possible that participants attained VO$_2$ max more quickly, the logistic regression transformation verified the COSMED estimation of VO$_2$ max. Finally, in some cases

phlebotomists were unable to draw the full 5 cc of blood. For example, only a third of the blood collection tube was filled for the post sample for one non-concussed participant.

4.4.3   Serum S100B and Protein Analysis

The immunoassay in this study is known to detect S100B in individuals suffering from neurological diseases or brain damage as well as to detect low concentrations in normal individuals. According to BioVendor (2009), the Human S100B ELISA immunoassay can detect approximately 43% of the normal population and variable results may be explained by improper or inadequate washing, improper mixing of standards, quality controls, or samples, improper preparation or storage of a reagent, omission of a reagent or step, assay performed before reagents were allowed to come to room temperature, and improper wavelength when reading absorbance (BioVendor, 2009). Recommendations for future studies include spike and recovery experiments to validate the kit using samples specific to the study. For example, Friel et al. (2007) used this technique to validate the Human S100B ELISA using human amniotic fluid. Another possible limitation is the number of freeze/thaw cycles, however great effort was made to keep all samples frozen at -80°C until time of assay. Lastly, better temperature control of samples throughout data collection and processing as well as independent assays to act as replicates in order to establish inter-assay variability are suggested to improve future studies.

Chapter V: Conclusions

No difference in aerobic capacity was seen between post-concussed and non-concussed groups, and overall both groups demonstrated VO$_2$ max similar to previous published literature in collegiate athletes. Moreover, S100B did not increase after exercise, S100B was not greater in post-concussed athletes compared to non-concussed athletes, and baseline S100B did not differ between post-concussed, non-concussed, and controls groups. Although no change in S100B was detected, it is in fact possible that such changes exist but that the immunoassay lacked the sensitivity to detect minute changes of S100B at such low concentrations. Furthermore, the exercise protocol used in this study, a VO$_2$ max treadmill test, was not a strong enough stimulus in terms of exercise intensity or exercise duration to elicit detectable levels of serum S100B. Future studies should incorporate different exercise stimuli to determine the level of physical activity and exertion needed to provoke detectable levels of S100B in the laboratory setting. A better understanding of the effects of S100B after exercise in non-concussed individuals is necessary to further explore the influence that exercise alone has on of serum levels of S100B, and especially to tease out the independent effects of exercise and concussion in order to make objective return-to-play decisions. Future studies should focus on athletes in collegiate cohorts in order enhance a body of literature that otherwise focuses on adult populations, as well as to develop prevention and injury management strategies aimed at a target cohort. Finally, consideration of the limitations and

implementation of the recommendations identified in Chapter IV is needed prior

to further research.

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APPENDICES

Appendix A

Cover Letter

Lakehead
U N I V E R S I T Y

<div align="right">School of Kinesiology

(807)343-8544
(807)343-8944</div>

Dear Prospective Participant,

    I am conducting a study to examine the relationship between head trauma, such as a concussion, and levels of protein S100B found in the bloodstream. The goal of this study is to create blood profiles of protein S100B from samples taken before and after exercise at a controlled intensity, as well as to compare blood profiles and the changes in S100B in concussed participants and their matched controls. It is hopeful that these blood profiles will help to understand the effects of exercise after concussion and to illustrate the need for a biological assessment tool in the evaluation of an athlete's decision to return-to-play following a head injury.

    As a participant, a trained phlebotomist will draw a 5 ml blood sample before and a 5 ml blood sample after participation in a treadmill VO_2 max test. During the test, participants will run up progressively steeper hills until exhausted and unable to continue. This test will provide a quantitative measure of the participant's capacity for aerobic, high-intensity exercise.

    Each 5 ml blood sample will take approximately 10 minutes to complete and the VO_2 max test itself approximately 10 minutes. Please note that additional warm-up and cool-down time will be required before and after the test.

    Collected data will be kept under strict confidentiality. Individual blood samples and VO_2 max data will be coded by an assigned subject number ensuring anonymity and confidentiality. Data will be stored at Lakehead University for five years post-completion. A summary of the findings after the completion of the results may be obtained. Participation is voluntary and you are under no obligation to complete the study. Risks in this study are minimal. There are no physical or psychological risks associated with participation in this study beyond which would normally be expected during blood-taking methods and during treadmill VO_2 max testing. This study has been approved by Lakehead University's Research Ethics Board. If you require additional information, please contact the Research Ethics Board at (807) 343-8283 or one of the following researchers.

Lindsay Jarvis
lnjarvis@lakeheadu.ca
(807) 343-8414

Dr. William Montelpare
wmontelp@lakeheadu.ca
(807) 343-8481

Consent Form 1

Lakehead
U N I V E R S I T Y

School of Kinesiology

(807)343-8544
(807)343-8944

Consent Form

My signature on this form indicates that I agree to participate as a participant in the blood biomarkers and concussion research project of Miss Lindsay Jarvis at Lakehead University. I understand that my participation in this study is conditional upon the following:

1) I have read and understood the cover letter and have had the study explained to me.
2) I fully understand what I will be required to do as a participant in the study.
3) I am a volunteer participant and I may withdraw from the study at any time without any consequence.
4) There are no physical or psychological risks associated with participation in this study beyond which would normally be expected during blood-taking methods or treadmill VO_2 max testing.
5) My data will be confidential and stored at Lakehead University for five years.
6) I will receive a summary of the project, upon request, following the completion of the project.

I agree to participate in this study.

_____   _____
Signature of Participant   Date

I wish to obtain a summary of the findings: Yes   No

If yes,

Email Address: _____

Mailing Address: _____

_____

955 Oliver Road Thunder Bay Ontario Canada P7B 5E1 www.lakeheadu.ca

Consent Form 2

Lakehead
UNIVERSITY   School of Kinesiology

(807)343-8544
(807)343-8944

Letter of Informed Consent

Measuring Maximal Oxygen Consumption using Treadmill and Direct Spirometry

I, _____ consent to participate in this exercise which will require the measurement of Aerobic Fitness based on a direct spirometer VO_2 max test.

I understand the following:

a) That there is very little risk of injury associated with testing for healthy individuals. There will be trained "spotters" assisting the VO_2 max test.
b) That there may be some physical discomfort due to exercising to the point of temporary exhaustion.
c) That I am obligated to immediately inform the researchers present of any unusual pain, discomfort, fatigue or any other symptom(s) that I incur during or after the testing.
d) That I can withdraw from the testing at any time.
e) Individual data will be kept confidential. Publication of results will not reveal my subject identity since subjects will be referenced by number.

_____   _____
Signature of Participant   Date

_____   _____
Signature of Parent/Guardian   Date
(If Participant is under the age of 18 years)

_____   _____
Signature of Witness   Date

2. I have explained the nature of the testing to the athlete and believe he/she has understood it.

_____   _____
Signature of Researcher   Date

Appendix D

Data Collection Sheet 1

Data Collection Sheet 1

Participant Info		
Name		
Gender	Male	Female
Age (years)		
Weight (kg)		
Height (cm)		
Sport		
History of Concussion	Yes	No
	How Many?	
PAR-Q	Yes	No
Change in health since PAR-Q?	Yes	No
Date		
Time of Day		

APPENDIX E

General Health Questionnaire

GENERAL HEALTH QUESTIONNAIRE

Date:

Personal Data

Name
Telephone Number
Email Address
Date of Birth (dd/mm/yyyy)
Sex   Female   Male
Country of Birth

Living Conditions

Smoking Status   Smoker   Ex-Smoker   Non-Smoker
If smoker/ex-smoker, indicate the number of years as a smoker:

Medical Conditions

Has a doctor diagnosed you to have any of the following medical conditions:
Angina Pectoris
Asthma
Cardiac Problems
Diabetes
High Blood Pressure
Raynaud's Phenomenon
Rheumatoid Arthritis
Other ailments (please
specify):

Modified Bruce Protocol

Modified Bruce
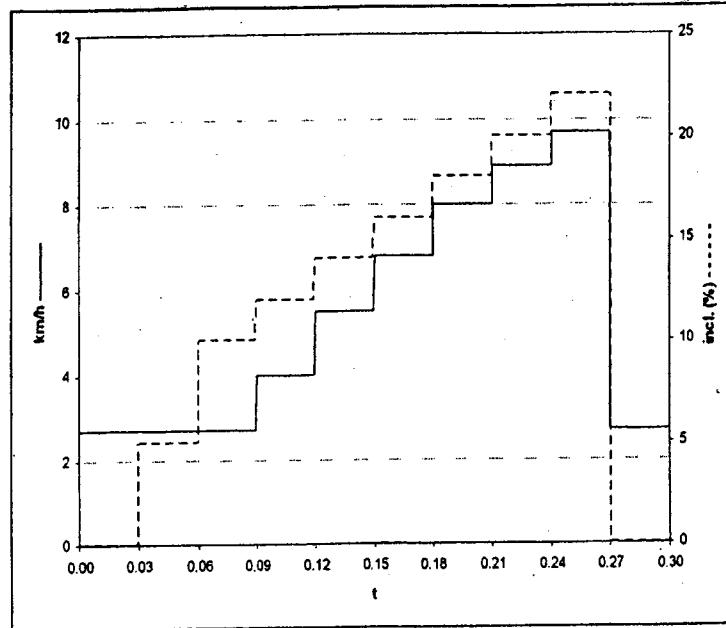
Time (mm:ss)	Speed (km/h)	Speed (mph)	Incline (%)
00:00	2.7	1.7	0
03:00	2.7	1.7	5
06:00	2.7	1.7	10
09:00	4.0	2.5	12
12:00	5.5	3.4	14
15:00	6.8	4.2	16
18:00	8.0	5.0	18
21:00	8.9	5.5	20
24:00	9.7	6.0	22
27:00	2.7	1.7	0 (recovery)

The protocol can terminate before the end if the end of test condition is reached, or you stop it manually.

APPENDIX G

Data Collection Sheet 2

Data Collection Sheet 2

Physiological Measures				
Heart Rate (bpm)		VO$_2$ (ml/kg/min)	Blood Pressure (mmHg)	
baseline HR			baseline BP	
1 min warm-up				
1 min exercise				
2 min exercise				
3 min exercise				
4 min exercise				
5 min exercise				
6 min exercise				
7 min exercise				
8 min exercise				
9 min exercise				
10 min exercise				
11 min exercise				
12 min exercise				
13 min exercise				
14 min exercise				
15 min exercise				
16 min exercise				
1 min post-exercise			1 min post-exercise	
5 min post-exercise			5 min post-exercise	
10 min post-exercise			10 min post-exercise	

Logistic Regression VO_2 max Transformations (Hoehler, 1995)

```
data curve;
input x y;
x2 = x * x;
xy = x * y;
cards;
1 34.8
2 39.9
3 39.4
4 41.7
5 41.5
6 44.8
7 45.7
8 45.2
9 47.1
10 47.9
;
proc means data=curve noprint;
 var y x x2 xy;
 output out=sums sum=y x x2 xy max=ymax; /* ymax = asymptote */

data _null_;
 set sums;
 slope = (xy - x * y / _freq_) / (x2 - x * x / _freq_);
 y_int = y / _freq_ - slope * x / _freq_;
 mid = (ymax / 2 - y_int) / slope;
 call symput('mid',mid);
 call symput('ymax',ymax);

proc nlin data=curve;
 parms a=0 b=&mid c=&ymax;
 var0 = exp(a * (b - x));
 var1 = 1.0 + var0;
 var2 = -c * var0 / (var1 * var1);
 model y = c / var1;
 der.a = (b - x) * var2;
 der.b = a * var2;
 der.c = 1.0 /var1;
output out=p  p=predict r=residual stdi=stderr;
proc print data=p;
var x y predict residual stderr;
```

Physiological Measures

Physiological measures include blood pressure (mmHg) and heart rate (beats per minute) taken before and after exercise in post-concussed and non-concussed participants. Baseline physiological measures are also included for controls.

Post-Concussed

ID	Blood Pressure (mmHg)		Heart Rate (bpm)	
	Pre	Post	Pre	Post
1	110/62	105/62	72	120
2	112/56	112/40	64	132
3	112/70	120/60	80	112
4	118/80	120/62	56	116

Non-Concussed

ID	Blood Pressure (mmHg)		Heart Rate (bpm)	
	Pre	Post	Pre	Post
1	122/68	120/64	80	100
2	116/78	106/70	88	116
3	102/70	112/66	60	84
4	134/74	130/64	70	114
5	122/68	126/66	56	120
6	124/78	106/74	76	128
7	114/72	118/60	64	160
8	110/84	102/74	102	118
9	138/82	128/58	76	116

Controls

ID	Blood Pressure (mmHg)	Heart Rate (bpm)
1	122/84	88
2	110/60	84
3	102/80	68
4	113/70	80
5	118/74	62
6	134/88	64
7	135/78	80
8	118/74	68

9	104/60	82
10	114/84	66

APPENDIX J

VO$_2$ max Transformations (Logistic Regression)

VO$_2$ max COSMED output, final VO$_2$ max recorded by the researcher, and predicted VO$_2$ max using a logistic regression equation (**Appendix I**).

Post-Concussed			
ID	COSMED (ml/kg/min)	Observed by Researcher (ml/kg/min)	Logistic Regression (ml/kg/min)
1	56.0	53.6	49.2505
2	47.9	47.9	47.5827
3	87.1	87.1	52.9685
4	58.9	58.9	58.2600

Non-Concussed			
ID	COSMED (ml/kg/min)	Observed by Researcher (ml/kg/min)	Logistic Regression (ml/kg/min)
1	41.3	40.1	41.1184
2	55.6	55.6	55.1051
3	67.0	57.3	54.0420
4	57.5	54.6	56.2120
5	53.8	53.5	53.3551
6	58.4	51.3	50.6409
7	57.0	56.6	56.7084
8	43.7	43.7	39.9095
9	50.8	45.9	49.4727