8-1-2014

The Interrelationships Among a Concussion-Related Biomarker, Head Hits, And ImPACT Test In Collegiate Football Players

Lucas Bianco
University of Nevada, Las Vegas, biancol2@unlv.nevada.edu

THE INTERRELATIONSHIPS AMONG A CONCUSSION-RELATED BIOMARKER, HEAD HITS, AND IMPACT TEST IN COLLEGIATE FOOTBALL PLAYERS

By

Lucas Bianco

Bachelor of Science in Athletic Training
Lynchburg College
2012

A thesis submitted in partial fulfillment
of the requirements for the

Master of Science -- Kinesiology

Department of Kinesiology and Nutritional Sciences
School of Allied Health Sciences
Division of Health Sciences
The Graduate College

University of Nevada, Las Vegas
August 2014

THE GRADUATE COLLEGE

We recommend the thesis prepared under our supervision by

Lucas Bianco

entitled

The Interrelationships Among a Concussion-Related Biomarker, Head Hits, and Impact Test in Collegiate Football Player

is approved in partial fulfillment of the requirements for the degree of

Master of Science - Kinesiology

Department of Kinesiology and Nutrition Sciences

Janet Dufek, Ph.D., Committee Co-Chair

Barbara St. Pierre Schneider, Ph.D., Committee Co-Chair

James Navalta, Ph.D., Committee Member

Michelle Samuel, M.S., Additional Committee Member

Darren Liu, DrPH, Graduate College Representative

Kathryn Hausbeck Korgan, Ph.D., Interim Dean of the Graduate College

August 2014

ABSTRACT

THE INTERRELATIONSHIPS AMONG A CONCUSSION-RELATED BIOMARKER, HEAD HITS, AND IMPACT TEST IN COLLEGIATE FOOTBALL PLAYERS

by

Lucas Bianco

Head injuries are prevalent in collegiate athletics with concussions being common among contact sports, such as football. Concussion assessment and diagnosis is complicated by the lack of objective and aassessment techniques. The purpose of the study was to determine if there is a relationship between the level of a concussion-related biomarker, self-report (subjective) head hits, video-analyzed head hits, and ImPACT test scores in college football athletes before and after a football practice session.

After being recruited and consented to participate, 29 Division I college football athletes provided a blood sample via finger stick two days before a 2014 spring football season practice and within within 4 hours post-practice.

The blood samples were processed to generate serum samples, and these serum samples were tested using a S100B ELISA kit to measure the amount of S100B. A computerized concussion diagnosis test (ImPACT) was completed by each subject before and after the practice. Video recordings of the practice were used to objectively count the number of hits that each participant sustained during the practice.

The major results are as follows. None of the participants suffered a concussion based on current diagnostic concussion assessments, including a symptom checklist, Standardized Assessment of Concussion (SAC), and Balance Error Scoring System (BESS). The participant who suffered head hits (as determined by video review) during

practice had a serum S100B level that increased from 39.5 pg/ml pre-practice to 85.3 pg/ml post-practice. In comparison, three other participants who did not sustain head hits (as determined by video review) had decreased or a minimal increase in the serum S100B level.

Due to the limited number of samples (15 out of 76) that had a CV $\leq$ 15%, the relationships between biomarker difference scores and head hits and ImPACT scores were not conducted. However, the relationship between video-analyzed head hits and the five ImPACT test composite scores was a negative correlation: $r = -0.10$, $p = 0.29$; $r = -0.11$, $p < 0.01$; $r = -0.17$, $p = 0.02$; $r = -0.01$, $p = <0.01$; $r = -0.15$, $p = 0.10$). The subjective Head Hit Index (HHI) was negatively correlated with 3 of the 5 composite scores ($r = -0.38$, $p = 0.04$; $r = -0.12$, $p < 0.01$; $r = 0.08$, $p < 0.01$; $r = 0.05$, $p = <0.01$; $r = -0.22$, $p < 0.01$). The verbal memory score of the ImPACT test had the highest correlation ($r = -0.38$, $p = 0.04$) with the subjective HHI.

In conclusion, an increased serum S100B level may be linked to sub-concussive head hits during collegiate football practice. Because an increase in serum S100B level likely represents trauma to the brain, sub-concussive hits may cause mild brain injury.

The major clinical implication of this study's findings relates to the objective clinical data that an athletic trainer needs for diagnosing brain trauma. For an athletic trainer to provide comprehensive care to football players, the athletic trainer needs objective clinical data, such as biomarker data. However, the collection of blood samples on the sidelines is a challenge. Based on this study's findings, the collection of blood via finger stick is feasible. Therefore, in the future, the athletic trainer might be able to obtain biomarker data to aid in the diagnosis of brain trauma.

ACKNOWLEDGEMENTS

I would like to thank all of my committee members, Dr. Janet Dufek, Dr. St. Pierre Schneider, Dr. James Navalta, Ms. Michelle Samuel and Dr. Darren Liu for their support and trust during this process. Each member took time from his/her schedules to allow me to question them, present to them and hear their advice. Even though I only had a class with Dr. Dufek most of my education during the past two years has come from outside the classroom with this group of professors.

This entire committee and thesis would not have been possible without the belief of Dr. Dufek. She took on primary responsibilities as the chair of my thesis committee at the beginning of the process and guided me through the creation of my committee. In chairing my thesis committee she was putting herself in a slightly uncomfortable position in which she was dealing with concepts that were out of her area of expertise. Having a professor challenge herself in this way took some pressure off of me and instilled a confidence in me to complete the process to the best of my ability.

Dr. St. Pierre Schneider was asked to become a co-chair on the committee early in the process and accepted the increased involvement. From that point, she made multiple resources available to me. The most helpful resources that she offered me were her time and knowledge. Having her on my committee put me in a position to complete the project while learning multiple laboratory skills that I would not have been exposed to otherwise. This experience was very helpful toward my overall growth as a graduate student and researcher.

TABLE OF CONTENTS

List of Tables

List of Figures

CHAPTER 1

INTRODUCTION

Sports are organized physical activities in which people of all ages compete. There are many positives for participation in sport including promoting health and wellness, improving academic achievement, raising self-esteem, and increasing psychosocial skills.[1,2] Paired with these positives is the inherent risk of injury during sports which could leave an athlete unable to continue living a healthy lifestyle. When the limits of a sport are pushed to improve performance, higher risks are taken for higher rewards.

Our society has gravitated to the sports world as a form of entertainment making professional sports organizations multi-billion dollar industries. Sports are part of the college experience with athletes working toward an undergraduate degree while also competing on the regional or national stage. The public attention on sports at the professional and collegiate level creates a level of stardom for the athletes.

Despite the stardom that these individuals reach due to their personal accolades, physiologically they are still at risk for sports-related injuries. Football is the most common collegiate sport with head injuries resulting in diagnosed concussions.[3, 4, 5] As defined, a concussion "is the historical term representing low-velocity injuries that cause brain 'shaking' resulting in clinical symptoms and that are not necessarily related to a pathological injury."[5] Already dealing with the challenges and pressures of school, stresses of life and the sport of football these athletes need prompt and proper care of their injuries. These athletes deserve accurate and appropriate diagnoses and treatments of their injuries, allowing them to continue to perform at a high level while not jeopardizing their long-term health.

An estimated 3.8 million concussions occur each year as a result of sport and physical activity.[5] The occurrence of concussions in football cannot be overtly observed, and during a competitive game, many athletes are not willing to report their symptoms, which would lead to them being removed from the game.[6] Therefore, the identification of objective measurements of a concussion is an expanding research area. Football has used injury surveillance data to make rule changes to tackling techniques to help protect players from sustaining cervical neck injuries, concussions, and other head injuries.[6] These changes are preventive in nature similar to advancements in football helmets. Research should continue to explore preventive techniques for concussions. But like all injuries, concussions cannot be completely prevented. That is why accurate and rapid concussion assessment has been another common area for research. When a concussion occurs, a healthcare provider needs to diagnose a concussion precisely and quickly to start the athlete on the correct management program.

To accurately and quickly assess an athlete, several diagnostic tests including ImPACT, BESS, Sensory Organization Test (SOT), SAC and Sport Concussion Assessment Tool (SCAT 3) have been used. The SAC and SCAT 3 assess cognitive function and can be administered on the field. The ImPACT test also assesses cognitive function, but must be completed in the locker room or clinic. The BESS and SOT are used to measure balance and vestibular awareness of a patient with a head injury. The BESS can be completed on the field while the SOT is completed in a laboratory.

However, as the use of these diagnostic tests becomes the more widely accepted standard, the tests' validity has been called to question.

The physiological effects of a concussion are starting to be more clearly understood. Physiological changes occur during a concussion but structural changes are not always present to provide a reason for the physiological changes. A metabolic response in the brain is thought to be responsible, and research is emerging describing what happens structurally in the head during a concussion. Current literature is examining Blood-Brain Barrier Disruptions (BBBD) as a way of measuring traumatic brain injuries (TBI).[7-12] Blood biomarkers are present after an event in the body and are based on what chemicals or enzymes appear in blood.[7] The blood biomarker predominantly used in research of BBBD has been the enzyme, S100B. BBBD or an increased permeability of the brain vasculature has been linked to a variety of neurological disorders including seizures, Alzheimer's disease, stroke, and traumatic brain injury.[13]

Considering the occurrence of concussions in football, current concussion assessments and research involving blood biomarkers in traumatic brain injuries, a logical next step is to transition these methods into the sports world. To date, the relationship between the blood biomarker, S100B, with a concussion diagnosis has not been examined during football practice. There was recently a study that examined S100B levels in individuals with a diagnosed concussion.[14] Therefore, the overall purpose of the current study was to examine the relationship between the change in the concussion-related biomarker level, S100B, and the change in selected diagnostic measurement thought to be associated with a concussion in football players. Specifically, this study aimed to examine the relationship between the change in the blood S100B level and 1) change in

ImPACT Test scores, 2) head hit number and 3) head hit intensity. The study's main hypothesis was that the change in blood S100B levels will be negatively correlated to the change in scores from three sections of ImPACT Test and positively correlated to the HHI, Video Analysis Score and scores from two sections of the ImPACT Test.

DEFINITION OF TERMS

Blood-Brain Barrier- Protective collection of blood vessels and glia of the brain that act as a cushion for the brain and prevent unwanted substances from entering in the brain.

Concussion- brain injury and is defined as a complex pathophysiological process affecting the brain, induced by biomechanical forces.

Clinical Diagnosis- The act or process of identifying or determining the nature and cause of a disease or injury through evaluation of patient history, examination, and review of laboratory data. The opinion derived from such an evaluation.

ELISA- The enzyme-linked immunosorbent assay (ELISA) is a test that uses antibodies to the concentration of a substance within a sample.

Football- American football

Head Hits- Contacts during a football event that involve the head in impacts between other players and/or the ground.

Hits- Contacts during a football event caused from impacts between the player's body and another player and/or the ground.

S100B- a calcium binding protein, of the S-100 protein family. S100B is secreted by astrocytes or can spill from injured cells and enter the extracellular space or bloodstream. Serum levels of S100B increase in patients during the acute phase of brain damage.

Sub-Concussive Hits- Hits below the threshold for a diagnosis of a concussion.

Symptom- A symptom is subjective, observed by the patient, and cannot be measured directly.

CHAPTER 2

REVIEW OF LITERATURE

Injuries are one of the risks of sport, a risk that athletes accept every time they step on the playing field. When the head is involved in a sports injury, the risk of returning to play is considered higher for the athlete because of the possibility of further damage to the brain. The sport of football has the most documented concussions per season.[3,4] Concussions occur at every level of football: professional, college, interscholastic, and sandlot. At the college level, an estimated 4.8% to 6.3% of athletes suffer a concussion per year.[3,4] Since the 2004 college football season, the concussion rate has been consistently increased to the 2011 season. During the 2013 college football season, there were 192 publicly announced concussions from the 120 teams in the Division I Bowl subdivision collected from individual team injury reports, NCAAFootball.com, USA Today, ESPN Stats & Information and media reports. Since the number of concussions is not being reduced, it is the responsibility of the sports medicine healthcare providers to ensure that the concussions that do occur are assessed and diagnosed appropriately to allow the athlete the proper time to recover and return-to-play.

Concussion in Sport

Advances in research, injury surveillance data and the media reports have heightened the awareness about sports-related concussions. The diagnosis of a concussion is: "continually being refined in the medical world".[5, 15, 16, 17] Over time, as more data and research are collected on concussions, the diagnosis of a concussion is becoming more evidence based. However, since concussions are currently diagnosed using several factors and are a “clinical diagnosis,” many concussions may still go undiagnosed or head injuries that are not concussion may be over diagnosed.

Definition

In 2012, the 4th International Conference on Concussion in Sport released a definition of concussion as part of its consensus statement: “concussion is a brain injury and is defined as a complex pathophysiological process affecting the brain, induced by biomechanical forces.”[5] The 4th International Conference on Concussion in Sport also released common features that incorporate clinical, pathologic and biomechanical constructs used to define a concussion:

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

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

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

4. “Concussion results in a graded set of clinical symptoms that may or may not involve loss of consciousness. Resolution of the clinical and cognitive symptoms typically follows a sequential course. However, it is important to note that in some cases symptoms may be prolonged.”[5]

There needs to be a force to cause a coup or countercoup injury, a forceful blow to a moveable head for coup, or the head hits a non-moving object for countercoup. Both of these types of injuries generate a stressor on the brain tissue to lead to injury. Concussions can be from a coup or countercoup injury. A compressive stressor involves crushing forces that are not absorbed by protective tissues. A shearing stressor moves across the tissue parallel fibers, and tensile forces are a direct stretching of the tissues.

Due to the nature of the sport of football, the risk for head injuries is greater than other sports.[18, 19, 20] The risk of concussion is greatest in football and rugby, followed by hockey and soccer.[4] Impacts occur at a higher frequency and greater intensity during games in comparison with practices. The position an athlete plays on the field may affect the type and magnitude of the contacts received during a game.[18] During every play of a football game, there is the possibility of head injury. Contacts or impacts can come from a defensive player tackling an offensive player or from an offensive player blocking a defensive player. Impacts can be head to head, head to body, head to ground or even football to head. Any force that acts on the head causing the brain to jar or shake in the skull can result in a concussion.

Risk Factors

Risk factors including age, sex, genetic predisposition and sport may affect concussion risk, severity, or recovery.[5, 15, 16, 17] For example, the recovery from a concussion takes longer in an adolescent (10-19 yrs) in comparison with an adult on the basis of neuropsychological testing.[21, 22,23] The adolescent's brain during growth is more susceptible to injury.[21] Additionally, female athletes may be at greater risk for concussion and increased severity and duration of symptoms than male counterparts that have suffered a concussion.[4, 5, 24,25] However, this difference may be influenced by a reporting bias in which males are less likely to report symptoms in comparison with females.[3,6,26,27] An individual's genetic makeup has been associated with a greater risk for sustaining concussion. Genes, including Apolipoprotein E epsilon4 allele, catechol O-methyltransferase, Dopamine D2 receptor, and Angiotensin-converting enzyme, have been implicated but a direct cause-relationship is unclear.[28-31]

Injury Surveillance in Football

Concussions occur at every level of football: NFL, college, interscholastic, and sandlot. Because the population of athletes participating in college and interscholastic football (1,268,000) is much higher than the number of NFL athletes (1,700), the number of concussions is also higher. In a given year, 3.6% to 5.6% of the 1.2 million interscholastic football athletes sustain a concussion, corresponding to an estimated 43,200 to 67,200 injuries. At the college level, an estimated 4.8% to 6.3% of athletes suffer a concussion per year.[20]

The concussion rate is increasing in the NFL. Casson et al[19] collected concussion injury data recorded by NFL team physicians and athletic trainers over two consecutive 6 year periods (1996-2001 and 2002-2007). There were 0.38 documented concussions per NFL game in 2002-2007—7.6% lower than the 0.42 in the earlier period (1996-2001).[19] The injury rate was lower in quarterbacks and wide receivers but significantly higher in tight ends during the second 6 years.[19] Concern about the long-term effect of concussions in professional football has increased since news reports of football players, such as

Junior Seau, Mike Webster, Terry Long, Justin Strzelczyk, Andre Waters, and Chris Henry, who have suffered from neurological diseases after their careers ended. It is unclear whether or not there is a cause and effect relationship between concussions and neurological diseases.[16]

In the years 2004 to 2009, the rate of concussion during collegiate games per 1,000 athlete exposures for football was 3.1. Comparatively, men's lacrosse = 2.6, men's ice hockey = 2.4, women's ice hockey = 2.2, women's soccer = 2.2, wrestling = 1.4, men's soccer = 1.4, women's lacrosse = 1.2, field hockey = 1.2, women's basketball = 1.2, and men's basketball = 0.6, accounting for between 4.0 and 16.2 percent of the injuries for these sports as reported by the NCAA Injury Surveillance Program by the Datalys Center.[4]

Pathophysiology

Anatomically concussions are not the same, in that there is not obvious specific structural damage to brain tissue that can be detected by imaging to diagnosis the injury as a concussion. A concussion sustained by an athlete presents uniquely to the healthcare team leaving the diagnosis of the injury up to the use of multiple assessment tools to provide a clinical decision.[5,8,32-34]

Functional disturbances rather than structural abnormalities cause the beginning of a concussion.[5,35] Immediately following head impact, axons act in spastic patterns and cannot communicate properly with other axons. Consequently, certain neurons are overloaded with an electrical stimulus that does not allow the mitochondria to maintain homeostasis within the cell. This impaired mitochondrial function leads to the consumption of more glucose than normal by the damaged neurons. This increased glucose consumption induces an accumulation of lactate, which can damage the neuronal membrane. Due to the mitochondria dysfunction, the cerebral blood flow decreases causing damaged neurons to die.[5,36,37] This metabolism disturbance caused by the initial mitochondrial dysfunction is the main biochemical explanation for most post-concussive signs and symptoms.[36]

Biomarkers can provide feedback that physiological changes have happened when anatomical damage cannot be measured. S100B is the principal low-affinity calcium-binding protein in astrocytes.[37] The biomarker S100B has been used to measure blood brain barrier disruptions (BBBD).[38-41] S100B is released into the blood stream after BBBD.[38-41] Increase levels in S100B would provide evidence that a BBBD had occurred.

Physiologically, a concussion is a complex disturbance in the cellular metabolism. This disturbance is caused by biomechanical forces.[5,35] By measuring the physiological changes of brain tissue after a concussive hit an objective threshold for a concussion could be researched.

Signs and Symptoms

Concussion symptoms are self-limiting and experienced differently in each patient. The subjective symptoms reported by one patient may not be the same in another patient and may be different in severity and duration. Concussion symptoms are observed within the first 24 hours following head trauma.[5,21] These symptoms include headache, nausea, dizziness, balance problems (lasting 3-5 days), blurred vision or other visual disturbance, confusion, memory loss and fatigue. The majority of concussions occur without loss of consciousness or obvious neurological deficits.[21]

Some of these signs and symptoms, summarized in Table 1, are more commonly reported than other symptoms. Even though the mechanism of injury may be the same for three individuals one may have few low severity symptoms, one may have many severe symptoms and one may not have any symptoms. There are individuals who will have certain symptoms for longer periods of time or who will not have all of the possible symptoms. The acute concussion effects of symptoms are not predictable, and most symptoms typically resolve within two weeks.[20]

TABLE 1. Signs and Symptoms of Post-Concussion Syndrome

Loss of consciousness Confusion Post-traumatic amnesia Retrograde amnesia Disorientation Delayed verbal and motor responses Inability to focus Headache Nausea/Vomiting Excessive drowsiness	Visual Disturbances (Photophobia, blurry Phono/photophobia vision, double vision) Disequilibrium Feeling “in a fog,”“zoned out” Vacant stare Emotional lability Balance Problems Dizziness Slurred/incoherent speech

The signs and symptoms of a concussion can last for long periods of time, with cases of symptoms lasting up to months or years. One main sign that seems to be the last restored in recovery from a concussion is cognitive dysfunction. Teasdale and Engberg et al[22] compared cognitive exam scores of 520 young men (16 to 24 yrs) who had been admitted to the hospital for concussion. Of the concussed men, 26.5% had a lower cognitive exam score than their non-concussed counterparts when tested over a seven day period following the concussion.[22] These data suggest that cognitive dysfunction can have longer effects than other signs and symptoms following a concussion.

In NFL players, the signs and symptoms of a concussion commonly consist of nausea, headache, dizziness, fatigue, drowsiness, feeling like in a fog, difficulty concentrating, sensitivity to light, sensitivity to noise, blurred vision and feeling slowed down. [19] The most common symptoms are headache and dizziness, while the most common signs are problems with information processing and immediate recall.[19]

Management at the Collegiate Level

The management of concussions/head injuries at the collegiate level is completed by the healthcare provider at each institution. The NCAA 2012-2013 guidelines include the following:

> "Institutions shall have a concussion management plan on file such that a student-athlete who exhibits signs, symptoms or behaviors consistent with a concussion shall be removed from practice or competition and evaluated by an athletics healthcare provider with experience in the evaluation and management of concussions. Student-athletes diagnosed with a concussion shall not return to activity for the remainder of

that day. Medical clearance shall be determined by the team physician or his or her designee according to the concussion management plan”.[4]

“In addition, student-athletes must sign a statement in which they accept the responsibility for reporting their injuries and illnesses to the institutional medical staff, including signs and symptoms of concussions. During the review and signing process, student-athletes should be presented with educational material on concussions.”[4]

In 2008 a new rule was added to NCAA college football:

“No player shall initiate contact and target an opponent with the crown of his helmet. No player shall initiate contact and target a defenseless opponent above the shoulders (i.e., whether or not with the helmet.) *Even though rare, an offensive player can be penalized should he use his helmet to punish a player.”[4]

Current Assessment Techniques for Diagnosis and Monitoring Recovery

The current assessment techniques for a concussion are symptom checklists, neurophysiological tests, balance function examinations and sideline assessment tools. In the field of sports medicine, evidence-based practice is one of the best ways to progress the credibility and effectiveness of professionals and their skills in the field. Concussion assessment and diagnosis is another area that demands more research to allow clinicians to make highly educated decisions based on the risks and benefits of assessment techniques.

Even though the symptom checklist is a useful tool in concussion assessment, there is no gold standard by which symptoms are assessed to help diagnosis a concussion with greater accuracy. Randolph et al[27] completed a study to develop a graded symptom checklist that included the symptoms most related to a concussion. This study included 641 high school and college athletes that completed the Concussion Symptom Inventory.[27] This tool included a 27 symptom checklist, administered at baseline then post-concussion, post-game and 1,3 and 5 days after concussion. As a result of the study, a 12-symptom variable checklist with a grading scale of 0-6, 0 being absent symptom and 6 being serve, was created.

The ImPACT test is a computer-based test battery consisting of a concussion symptom inventory and six modules measuring neurocognitive function. Schatz et al[42] tested the sensitivity and specificity of the ImPACT test in concussed athletes. The positive predictive value was 89.4%, and the negative predictive value was 81.9%.[42] The use of the ImPACT test was validated when compared to written tests and the SAC. A meta-analysis found no statistically significant difference in sensitivity 14 days after injury among written tests, computer-based tests, and the SAC.[43] The ImPACT test is considered the cornerstone of a concussion assessment and a return-to-play protocol providing comparable scores to other cognitive diagnostic tests. Therefore, concussion guidelines suggest that all concussion policies include a neurocongnitive exam.[5,16-18]

Since a concussion can lead to impaired balance, postural stability testing is completed using either the BESS or the Sensory Organization Test (SOT) as a diagnostic tool. The BESS requires the individual to stand in three different positions (double leg, single leg, and tandem stance) on a stable platform then on an unstable platform which can be done on the sideline of an athletic event.[5,16-18] The evaluator keeps track of errors in balance totaling the errors at the end. The errors are defined as (1. hands lifted off iliac crest, 2. opening eyes, 3. step, stumble, or fall, 4. moving hip into greater than 30 degrees abduction, 5. lifting forefoot or heel, 6. remaining out of testing position greater than 5 seconds) from the BESS directions. SOT employs computerized force plate technology together with a servo-controlled visual surround and support surface to evaluate a person's ability to utilize and integrate visual, vestibular, and somatosensory information while maintaining upright stance in altered sensory environments.[44] The SOT challenges more aspects of an individual's balance functions than the BESS. Therefore, the SOT is the preferred test but the equipment is not portable making the BESS the tool for sideline use.[44]

The SAC can be used to assess orientation, memory, concentration, and delayed recall in individuals with a suspected concussion. The SAC has been validated as a sideline diagnostic tool for athletes who are junior high school age and older competing in any sport.[15] The Sport Concussion Assessment Tool (SCAT) combines a symptom checklist, concentration and memory tasks (Maddock's questions), the SAC, the BESS, and the Glasgow Coma Scale. The SCAT2/ SCAT 3 have not been validated; however, it is widely used and the most sophisticated sideline assessment tool available.[5] Current assessment tests are summarized in Table 2.

TABLE 2. Current Concussion Assessment Tests

Diagnostic Test	Purpose
Symptom Checklist	Records signs & symptoms.
SAC	Evaluate memory, concentration, and organization.
SCAT	Evaluate memory, concentration, and organization.
BESS, SOT	Evaluate vestibular system.
ImPACT	Evaluate cognitive function.

Investigational Objective Assessment Techniques

Biomechanical Analysis of Football Contact

The biomechanical analysis of football contact is under investigation as a possible objective measure of a concussion. Biomechanical analysis of football contact involves the measurement of linear acceleration, rotational acceleration, jerk, force, impulse, and impact duration associated with head contact. A certain magnitude of these measures may be related to the likelihood of a concussion. Based on position athletes experience different amount and type of biomechanical force more regularly. Linear acceleration is greatest in defensive linemen and offensive skill players, and in any player when contact is at the top of the helmet.[46] Defensive linemen also had the largest recorded rotational acceleration, while offensive skill positions have the highest-magnitude jerk, force and impulse and the shortest impact duration.[46]

If the magnitude of a contact can be measured on the field it can provide the clinician with warning that a player may have suffered a concussion. Biomechanics of concussion has been evaluated to find a threshold of concussive injury in all football players. Guskiewicz et al[46] studied the biomechanics of football hits with the Head Impact Telemetry (HIT) System to measure impact magnitudes and impact location in hits to athletes resulting in a concussion. Impact magnitudes measured from the 13 concussive hits provided a range of 60.51 to 168.71 *g*.[46] There was no significant relationship between impact magnitude (linear or rotational acceleration) or impact location and change scores for symptom severity, postural stability, or neurocognitive function ($p > 0.05$).[46] Researchers concluded, there are too many factors, history of concussion and frequency of sub-concussive hits, that make it difficult to establish a biomechanical threshold of a concussion.[46]

For the past 10 years, the traumatic brain injury research community has been involved in the discovery of different blood biomarkers for traumatic brain injuries. The research has attempted to correlate brain injury with different biomarkers in the body. The research is still new and needs more evidence, but correlations between biomarkers and traumatic brain injury have been shown. Biomarkers that have been studied are: glial fibrillary acidic protein, Ubiquitin C-terminal hydrolase L1, Neuron Specific Enolase, spectrin breakdown products, and Tau.[38-41,48-50]

Concussion Blood Biomarkers

In recent years there have been a number of technologies under development to objectively aid in the diagnosis of traumatic brain injury.[51] Some of these include advances in MRI, quantitative electroencephalogram, visual tracking[36,52], and serum based biomarkers of brain injury.[38-41] Using the logistical constraints mentioned previously and the need for the test not to be confounded by conditions of physical activity (sleep deprivation, stress, fatigue, etc.), one of the more promising options is the development of a simple blood test to detect brain specific proteins after a traumatic brain injury.[50] Blood-based tests have been successful in the diagnosis of other disease conditions, such as cardiac disease and cancer so it is possible biomarkers could be used to identify traumatic brain injury as well.

The research into serum based biomarkers of brain injury has examined several blood biomarkers. Advances in this area will come from a better understanding of specific levels of serum based biomarkers of the brain. Knowing specific levels, such as normal baseline levels, reference levels correlated with physical activity without concussion, levels correlated with traumatic brain injury and finally correlation between levels and diagnostic concussions will advance understanding of the concussion mechanisms.

The blood biomarker S100B, a brain specific biomarker, has been researched for its ability to detect traumatic brain injury compared to the capabilities of a computed tomography scan.[7] The benefit of using the less invasive, less time consuming and less expensive blood test compared to a computed tomography (CT) scan would be to make the diagnostic process more efficient.

S100B levels have been compared to brain injury diagnostic imaging techniques. Mueller et al[7] completed a study of 226 patients who arrived at a hospital with a head injury and a Glasgow Coma scale score of 13 out of 15 the patient had their blood drawn and computed tomography scan completed. The level of S100B was sensitive in 95% of patients with intracranial injury but was specific in 31% of patients who did not have an injury but were above the 0.10 ng/ml cutoff.[7] The participants in this study had suffered traumatic brain injuries, but none of them were assessed for concussions and there was no cutoff for what the level of S100B should be in a concussion.

Zongo at el[49] reported similar findings with 1,560 patients that had minor head injury. With a cutoff of 0.12 ng/ml, for a BBBD, traumatic brain injuries on CT were identified with a sensitivity of 99.1% (95% confidence interval [CI] 95.0% to 100%), a specificity of 19.7% (95% CI 17.7% to 21.9%), a negative predictive value of 99.7% (95% CI 98.1% to 100%), a positive likelihood ratio of 1.24 (95% CI 1.20 to 1.28), and a negative likelihood ratio of 0.04 (95% CI 0.006 to 0.32).[49]

To provide more information about S100B, studies have been completed to determine average levels in the blood. In healthy individuals S100B levels have been found to be within the range 0.02 to 0.15 ng/ml.[11]

Marchi et al[53] measured the S100B levels of college football athletes pre- and post-game for five games of a season. The change in S100B levels between pre- and post-game measures were correlated with the severity of hits sustained during the game. The severity of hits was quantified with the HHI which gave a score of 0 to 6 based on the number of hits and how distinctively each athlete remembered their hits. “Players with a HHI of 6 had a significantly higher S100B post-game – baseline than players with a HHI of 0 ($p = 0.03$). There was also a significant positive trend ($p < 0.01$) correlating S100B post-game – baseline and HHI.”[31] These results suggest increased S100B levels are related to head contacts.[53]

Sub-concussive Hits

Concussion can cause short and long term effects to the brain and general healthcare of the patients that sustain them. The long term effects of concussion are not completely understood because of the many variables of a concussion. It is not known if one concussion causes adverse effects in a lifetime or if it takes more than three concussions to negatively impact the brain. It might not have to do with the number of concussions at all and may have to do more with the number of hits an athlete receives over a playing career. These hits that do not lead to a diagnosis concussion could still be slowly causing irreversible brain damage.

Sub-concussive hits are considered part of the game and have no obvious short-term effects. With the increase in concussion research, sub-concussive hits have gained attention as a new area of focus, specifically examining the role of sub-concussive hits in long term effects on neurological defects. Blood bio-markers may have a significant role in quantifying an acceptable amount of sub-concussive contacts accumulated during a practice or game. The S100B enzyme can indicate a disruption in the blood-brain barrier once a level of 0.10 or 0.12 ng/ml is reached.[53] Disruptions in the blood-brain barrier have been linked to a variety of neurological disorders including seizures, Alzheimer's disease, stroke, and traumatic brain injury.

Recovery

Recovery from a concussion varies. During the first week after injury the brain undergoes a dynamic restorative process. In all injuries there is an injury response. Similar for other parts of the body the brain must heal. During this healing time there is a great risk for reinjury which could result is further damage.[23] Recovery, return to pre-functioning, assessed using symptom ratings or brief neuropsychological measures typically occurs within 2-14 days, but can take up to 3 months as evidenced by neuropsychological reaction time tests.[43,45,54]

Athletes with a concussion should rest physically and cognitively until symptoms have resolved at rest and then with exertion. Rehabilitation following concussion progresses through a stepwise graded fashion.[5,16-18]

Future Diagnostic Techniques

Concussions do not present as structural defects in current imaging techniques, such as CT scans and Magnetic Resonance Imaging (MRI), and therefore, cannot be diagnosed using such techniques.[5,16-18] The majority of concussions occur without loss of consciousness or obvious neurological deficts.[5,16-18] The assessment of a concussion includes somatic, cognitive and neurobehavioral symptoms. The use of a graded check-list or scoring scale for signs and symptoms from the concussed individual is important in a diagnosis of a concussion because it supplies an objective number for assessment.

Brain imaging techniques have been used in research to provide an objective measure of what happens to the neuroantomy after a concussion. One of the brain imaging techniques that has been used is functional MRI. Functional MRI takes an image of the brain while the subject is completing a task. This provides specific information about neural function and, is well suited to detect functional abnormalities associated with concussion. Jantzen et al[36] examined eight college football athletes who received preseason functional MRI, while four of eight received another functional MRI one week after they suffered a concussion. The four controls received a second functional MRI at the end of their season.[36] The results from the study showed "specific neural signatures" in the four athletes that had concussions.[36] When compared with control subjects, concussed players had marked within-subject increases in the amplitude and extent during the functional task.[36] Functional MRI has been successful as a research diagnostic tool but the practical application may be limited to the testing procedure and cost.

Summary

Concussion injuries on the most simplistic level are recognized by the presence of a mechanism of injury and subsequent signs and symptoms. Current assessment techniques of concussion injuries are based on symptom checklists, neurophysiological tests, balance function examinations and sideline assessment tools. Future assessment techniques have targeted the possibility of creating a threshold of objective measurements based on physiological changes after a concussion. The use of a range of acceptable and unacceptable levels of biomarkers could advance the assessment and management of concussion injuries on the sports field.

CHAPTER 3

METHODS

The overall purpose of this study was to examine the relationship between the change in the concussion-related biomarker, S100B, and the change in selected diagnostic measures thought to be associated with a concussion in football players. Specifically, this study examined the relationship between the change in the blood S100B level and 1) change in ImPACT Test scores, 2) head hit number and 3) head hit intensity. The study's main hypothesis was that the change in blood S100B levels would be negatively correlated to the change in ImPACT Test scores and positively correlated to hit number and hit intensity.

Participant Characteristics

The participants (*N* = 30) were male, 18-30 years old (21±1 years, 189±8 cm, 109±21 kg). Ethnicities included 57% Caucasian, 23% African American, 17% Pacific Islander 17%, and 3% Hispanic. Each participant was a Division I football athlete who was on the 2014 spring practice roster. The recruiting process took place 1-2 weeks before the selected test practice day. This research study was approved by the university Institutional Research Board (approval #1309-4571) and the University Institutional Biosafety Committee (approval #13-09-05). Each participant read and signed an institutionally approved informed consent form during the recruiting process. No athletes suffered significant bleeding during the practice and/or serious orthopedic injuries.

Participants played a variety of positions on the football team as is summarized in Table 3.

Figure 1 shows a flow diagram of participant involvement. All but one subject completed the study. Athletes who were recovering from recent acute injuries or long term rehabilitation from surgery or did not participate in practice were used as controls (*n* = 4).

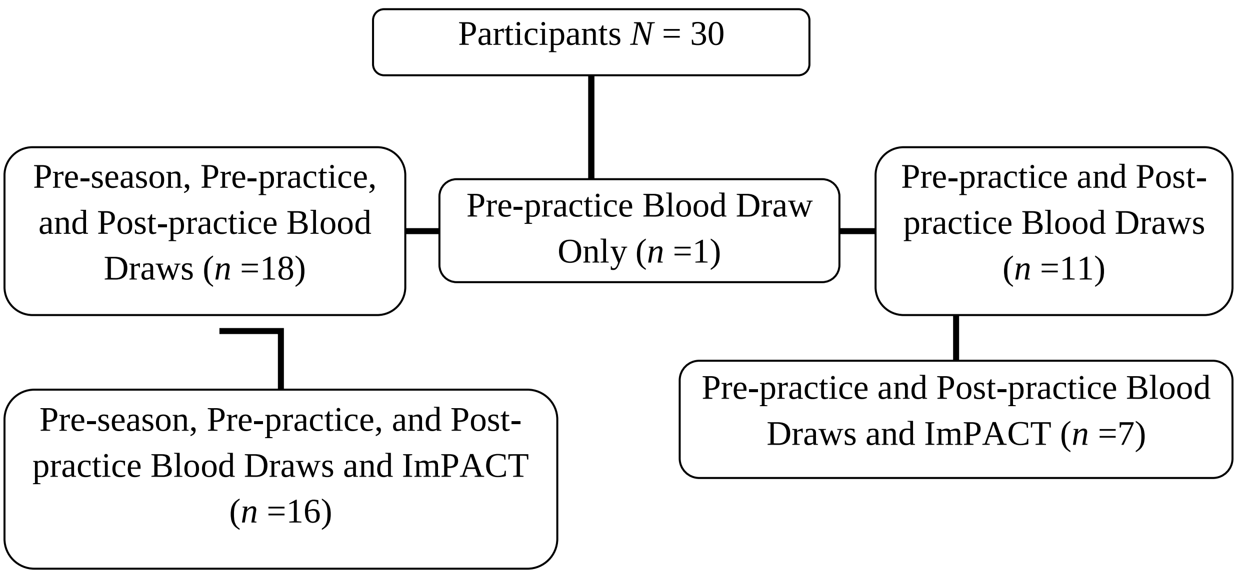

Figure 1. Flow Chart of Involvement of Participants in Blood Draws and ImPACT Test.

Table 3. Number of Participants by Football Position.

Position	Number of Participants
Offensive Lineman	10
Linebacker	6
Wide Receiver	3
Defensive Lineman	3
Tight End	2
Running Back	2
Defensive Back	2
Quarterback	1

Procedure

Prior to the 2014 spring football season, each athlete completed a pre-participation physical exam under the medical direction of the team physician. As part of the university concussion policy: baseline SAC, BESS, and ImPACT tests were completed during the same time as the pre-participation physical exam. Information from this examination was used as comparative data for the current study.

A single practice in the 2014 Football Spring Season served as the test practice. Pre-season blood draw occurred 2 weeks before the practice chosen for the study, and the pre-practice blood draw occurred 2 days before the practice chosen for the study. Within 2 hours post-practice, each eligible participant had blood drawn again, using the same protocol as the pre-season and pre-practice draw. Blood was aseptically drawn through a finger stick using a single-use Capiject Safety Lancet (Terumo Medical Corporation, Somerset, NJ, USA, 1.5mm W x 1.5mm D) into a capillary blood collection tube (Multivette 600, Sarstedt Aktiengeselischaft & Co, Numbrecht, Germany). The blood sample was transported on ice to an on-campus laboratory and centrifuged for 10 minutes at 239 g-force to separate the serum. The serum was frozen and stored in the laboratory freezer at –70 degrees Celsius.

The team videographer and crew filmed the practice. Two athletic trainers reviewed the video and counted the number of contacts delivered and received by each participant.

Immediately before or after the post-practice blood draw, participants completed a questionnaire about the number and intensity of the hits received or delivered during the selected practice. Symptoms and HHI scores were collected from this post-practice survery. Within the next 2 days after the study practice, participants completed the ImPACT test.

Practice Selection

Several factors contributed to the decision of selecting which practice to use as the test practice. Due to the nature of the study and the emphasis on concussions, the practice needed to include full contact tackling drills for a higher probability of head injuries. The practice that was selected was in the middle of the 2014 spring season to ensure that tackling drills would be incorporated. Through discussions with a football coach, it was concluded that the practice chosen would be the first moderate contact practice which had a higher probability for head injuries. Later in the 2014 spring season other practices included more contact drills but the timeline of this study limited selection of those practices.

Change in Serum S100B Level

Pre-season, pre-practice and post-practice serum S100B levels were measured using a BioVendor human S100B sandwich enzyme immunoassay 96-well plate kit (CAT# RD192090100R; Asheville, NC). With the limited amount of research in testing serum S100B in football athletes, this immunoassay kit was selected based on its availability. The manufacturer's procedure was followed. Briefly, 60 µl from each serum sample was prepared as a 1:4 or 1:2 solution using the dilution buffer. Then 100 µl of the diluted sample was pipetted into two separate wells to perform the assay in duplicate. After the samples were pipetted into the wells, reagents were added to the wells to promote binding of the S100B in the serum sample to the anti-S100B antibody coating on each well. After the addition of reagents, the wavelength setting of a microplate reader (Molecular Devices SpectraMax 340PC384 Absorbance Microplate Reader 340pc 384) was set at 450 nm and 630 nm to detect the presence of S100B in the wells. Additionally, the manufacturer's standard reagents were used to generate a standard curve. This standard curve was used to calculate the specific pre-season, pre-practice and post-practice S100B levels. The kit's detection range was 15 pg/ml.[55]

All participants were recruited on a volunteer basis. After the practice chosen for this study was completed, participants were categorized based on the number and intensity of head hits received during the practice both subjectively from the HHI and objectively from the Video Analysis Score. The scores for each group recognized participants with no head hits, minimal head hits, and major head hits. The participants without head hits as determined by video served as the asymptomatic group and were expected to have lower or no increase in the S100B level from pre-practice to post-practice.

ImPACT Test Score

The ImPACT test (ImPACT Applications, Inc., Pittsburgh, PA, USA), is a computer-based test battery consisting of a concussion symptom inventory and six modules measuring neurocognitive function. The five composite score categories are Verbal Memory, Visual Memory, Motor Speed, Reaction Time and Impulse Control. This test was administered at two time points: 1) within 8 months before the 2014 football season (designated as the baseline measurement) and 2) within 1-2 days after the selected practice (designated as the post-practice measurement). Participants took the test, based on their schedule, on one of three computers. The testing environment was similar at each computer.

Head Hit Index

A questionnaire of possible head injury predictors, the HHI, was administered to the participants within 2 hours after the end of the practice. The HHI has been used by Marchi et al[53] in a study to measure the number and intensity of hits during a football game. Participants were asked to track the frequency and intensity of significant hits they experienced during the practice. The questionnaire consisted of: 1) number of hits experienced; 2) number of episodes of hits involving the head; 3) number of significant episodes of hits to the head; and 4) presence of acute symptoms (e.g., headache, neck pain, nausea). A HHI score, ranging from 0 to 6, was derived from (a×b) where a) is the number of head collisions and b) is the overall severity of these head hits. A score of 0 would have denoted a participant experiencing no head hits while a score of 6 would be from a participant who felt he had a higher number of intense head hits.

Video Analysis

The video was watched by two reviewers blinded to the study. They were asked to record the number of body contacts and number of head contacts as well as severity for those hits they witnessed for each study participant. Each participant received a score of 0-2 points with 0 meaning no head hits, 1 meaning head hits with minor intensity and 2 meaning head hits with major intensity. To assess interreliability of the two reviewers' scores, a percentage difference was calculated for each participant. The percentage difference was ≤ 10% for 25 out of 29 participants.

Data Reduction

Change in Serum S100B Levels

The levels of S100B were computed and then entered into an Excel worksheet using Microsoft Office 2010 (Microsoft; Redmond, WA). All graphs and statistical calculations were completed using Excel worksheets.

The correlation between change in S100B levels and change ImPACT or hit intensity and number was not completed because values of S100B were below the detection level of the assay and/or the coefficient of variance (CV) of duplicate samples was above 15%. A CV of 15% or less is needed to ensure reliability.

ImPACT

The five composite scores (Verbal Memory, Visual Memory, Motor Speed, Reaction Time, and Impulse Control) for the pre-season and post-practice measurement were obtained from the ImPACT test website. The five composite scores were compared to symptoms and hit number and intensity.

Head Hit Index

Each participant was assigned a score, 0-6, which was a subjective measurement of number and intensity of hits during the practice. Participants that scored higher, (>3) on the HHI subjective test felt that they had more head hits (>10 head hits) and subjectively felt that their hits were a greater intensity compared to the intensity of a "normal" hit.

Video Analysis

Each participant was assigned a score of 0-2, which was an objective measurement of the number and intensity of hits during the practice. After receiving the video recording of the entire practice on a DVD, two separate research members watched the practice on a computer. The research members recorded the number of head hits or body hits and the intensity of those hits from minor or major. Participants with more than half of their hits being major head hits were scored a 2, minor head hits were scored a 1, while participants with no head hits were scored a 0.

Data Analysis

The S100B data are presented per participant because of the low sample number (useable data, n = 4). Using Excel (Microsoft; Redmond, WA), correlations and t-tests were run on the ImPACT results, HII score and Video Analysis Score data collected.

The data from the ImPACT test scores were kept on the ImPACT testing website accessible only by password. Correlations were conducted with HHI score/ Video Analysis Score and each composite ImPACT test score. Symptoms from the ImPACT test were also used to create graphs to organize the number of participants that were either asymptomatic or symptomatic and their head hits number and intensity. Another graph was created to represent the number of asymptomatic or symptomatic participants that passed or failed the ImPACT test.

CHAPTER 4

RESULTS

The overall purpose of this study was to examine the relationship between the change in the concussion-related biomarker, S100B, and the change in selected diagnostic measures thought to be associated with a concussion in football players. There were four participants' samples that were complete and reliable S100B data to relate to the hit intensity and number and ImPACT test results.

S100B Level

From the 30 football players who participated in the study, a total of 77 samples of blood were collected 18 pre-season, 30 pre-practice and 29 post-practice. These 77 blood samples were processed to serum and tested to quantify the S100B level. From the 77 blood samples, 76 duplicate serum samples were tested using the ELISA kit. One participant's pre-practice blood draw sample was not measured in the ELISA because no post-practice blood draw sample was collected, and there would have been no comparative data for the participant.

The S100B level for each sample was tested using two separate ELISA kits due to the number of samples (76) tested. The standard curves for both kits were reliable. The R^2 values for the two standard curves were 1.0 and 0.998. The standard curve for the first kit was created using a dilution factor of 1:4 with each sample having the same dilution factor. The second kit used a 1:2 dilution factor for the standard curve and samples.

After testing was completed, the results showed that there were 31 samples with a CV > 15 %. There were 19 samples that had a CV of 0.0 %. Another 10 samples were out of the assay's detection range. Therefore, 60 out of 76 samples could not be used in data analysis.

Table 4 shows the S100B levels for the four participants whose samples yielded reliable data. Only one of the four participants (participant #11), assigned to the asymptomatic group because he did not play in the practice, had acceptable CV levels (≤ 15%) for all three blood draws. The S100B levels for participant 11 were 87.2 pg/ml, 109.7 pg/ml and 38.5 pg/ml for pre-season, pre-practice, and post-practice, respectively.

Five participants' samples for the pre-practice blood draw had acceptable CV levels, and the S100B levels were 99.9 pg/ml; 109.7 pg/ml; 75.8 pg/ml; 64.4 pg/ml, and 39.5 pg/ml. Ten participants' samples for the post-practice blood draw had acceptable CV levels, and the S100B levels were 31.2 pg/ml; 43.2 pg/ml; 109.8 pg/ml; 38.5 pg/ml; 38.2 pg/ml; 49.9 pg/ml; 74.9 pg/ml; 41.0 pg/ml; 55.1 pg/ml and 124.8 pg/ml.

Table 4. Change in S100B Levels.

S100B Levels (pg/ml)				
Participant	Pre-Season	Pre-Practice	Post-Practice	Post-Pre
11	87.2	109.7	38.5	-71.2
16	Not Used	75.8	79.4	3.6
18	Not Used	64.4	41.0	-23.4
29	Not Collected	39.5	124.8	85.3

Only participant number 29 had objectively recorded head hits during the practice. All four participants passed the ImPACT test.

Relationship of Head Hits Number/ Intensity and ImPACT Test

All 29 subjects, that completed the post-practice blood draw, had their head hits quantified subjectively through the post-practice survey and objectively through video analysis. Table 5 includes the correlations between participants that completed the ImPACT test within two days after practice (n=23). The ImPACT test score was compared to the participant's baseline ImPACT test score. None of the ImPACT composite scores were significantly correlated with number and intensity of head hits. The Video Analysis Score was negatively correlated with all of the composite ImPACT test scores. The subjective HII was negatively correlated with 3 of the 5 composite scores and the verbal memory score had the greatest correlation (r =-0.38, p = 0.04) with the subjective HII (Table 5).

Table 5. Relationships Between Head Hits and Difference in Pre-Season and Post-Practice ImPACT Test Composite Scores.

Relationships Between Head Hits and Difference in Pre-season and Post-Practice ImPACT Test Composite Scores					
	Verbal Memory	Visual Memory	Visual-Motor Speed	Reaction Time	Impulse Control
Subjective HIT Index	r = –0.38 p = 0.04	r = –0.12 p < 0.01	r = 0.08 p < 0.01	r = 0.05 p < 0.01	r = –0.22 p < 0.01
Video Analysis	r = –0.10 p = 0.29	r = –0.11 p < 0.01	r = –0.17 p = 0.02	r = –0.01 p < 0.01	r = –0.15 p = 0.10

Relationship of Head Hits Number/ Intensity and Symptoms

The number and intensity of head hits measured subjectively by the participants or objectively by Video Analysis Score had weak correlations with the symptoms reported by each participant The HHI formulas are given in Table 6 This score was calculated from the answers supplied by each participant in the post-practice survey, Figure 7. HII and Post-Practice Survey number of symptoms had an $r = 0.09$, $p < 0.01$ Figure 2 graphically represents of the number of asymptomatic and symptomatic participants based on the Post-Practice Survey and their HII Score. HII and ImPACT number of symptoms had an $r = –0.10$, $p = 0.06$ Figure 3 illustrates, a graph of the number of asymptomatic and symptomatic participants based on the ImPACT Symptom Score and their HII score. Video Analysis Score and Post-Practice Survey number of symptoms had an $r = 0.17$, $p = 0.74$ Figure 4 illustrates, a graph of the number of asymptomatic and symptomatic participants based on the Post-Practice Survey and their Video Analysis Score. Video Analysis Score and ImPACT Symptom Score has an $r = 0.10$, $p = 0.31$ Figure 5 illustrates, a graph of the number of asymptomatic and symptomatic participants based on the ImPACT Symptom Score and their Video Analysis Score.

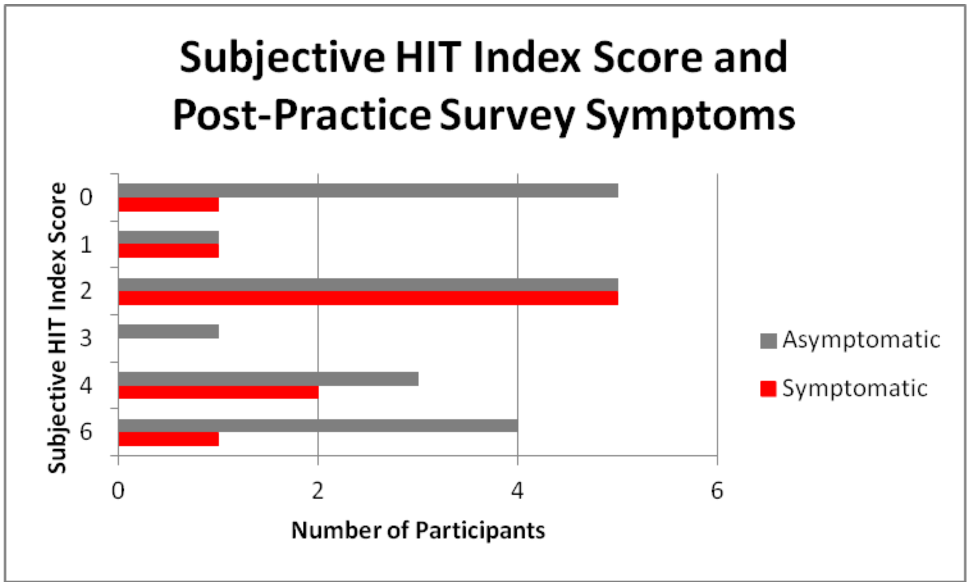

Figure 2. Symptom Score From Post-Practice Survey Compared to the Sujective HIT Index Score.

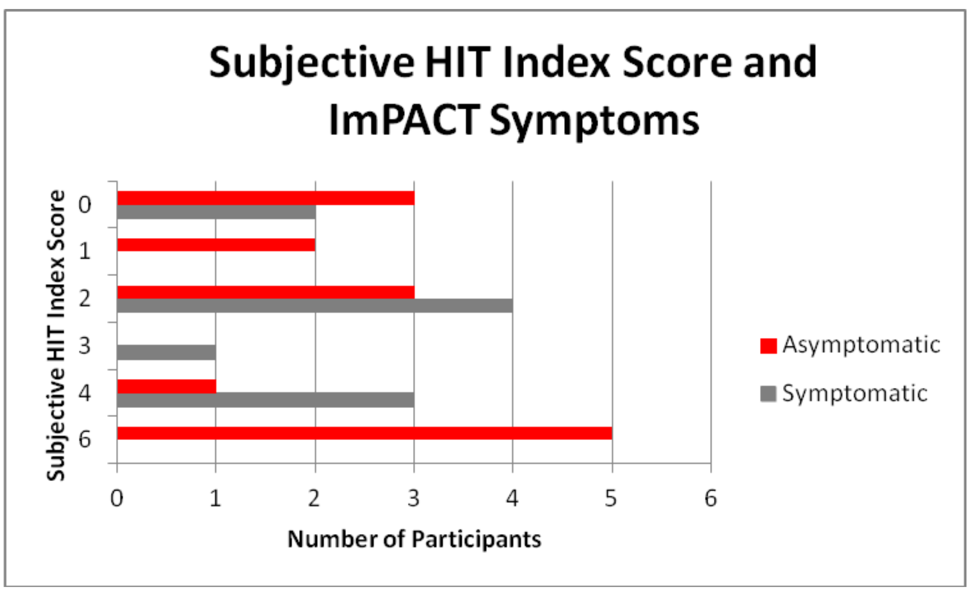

Figure 3. Symptom Scores From the ImPACT Test Compared to Subjective HII Index Score.

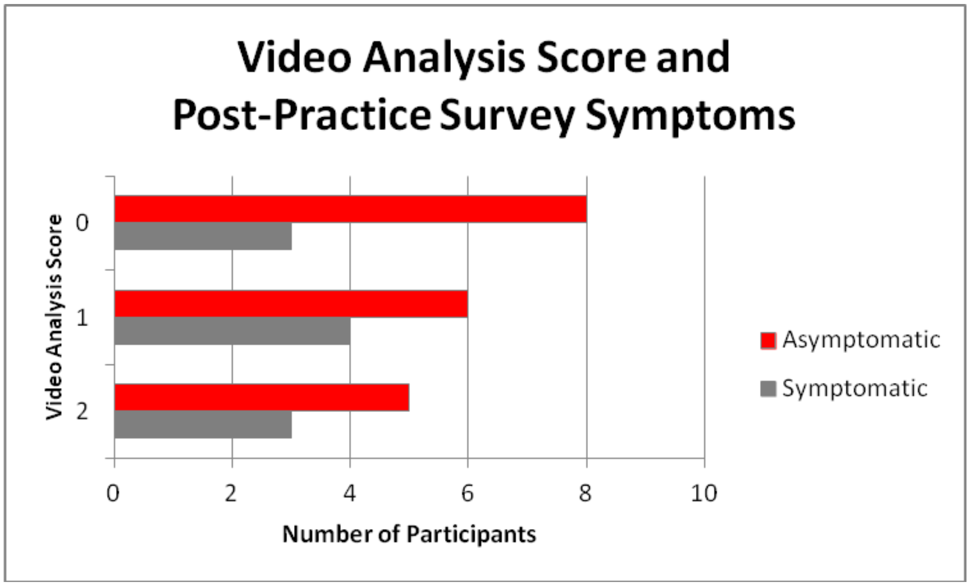

Figure 4. Symptom Score From Post-Practice Survey Compared to the Video Analysis Score.

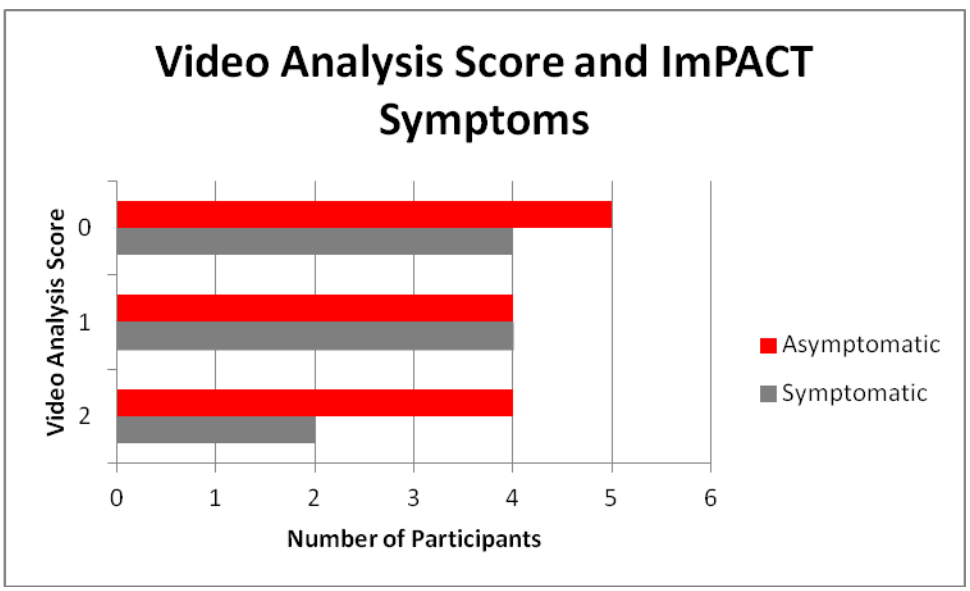

Figure 5. Symptom Scores From the ImPACT Test Compared to the Video Analysis Score.

Relationship of Symptoms and ImPACT Test Composite Scores

Participants that had symptoms were more likely to fail at least one section of the ImPACT Test. Seven of the eleven participants that failed the ImPACT Test had symptoms while eleven participants that passed the ImPACT Test were asymptomatic (Figure 6).

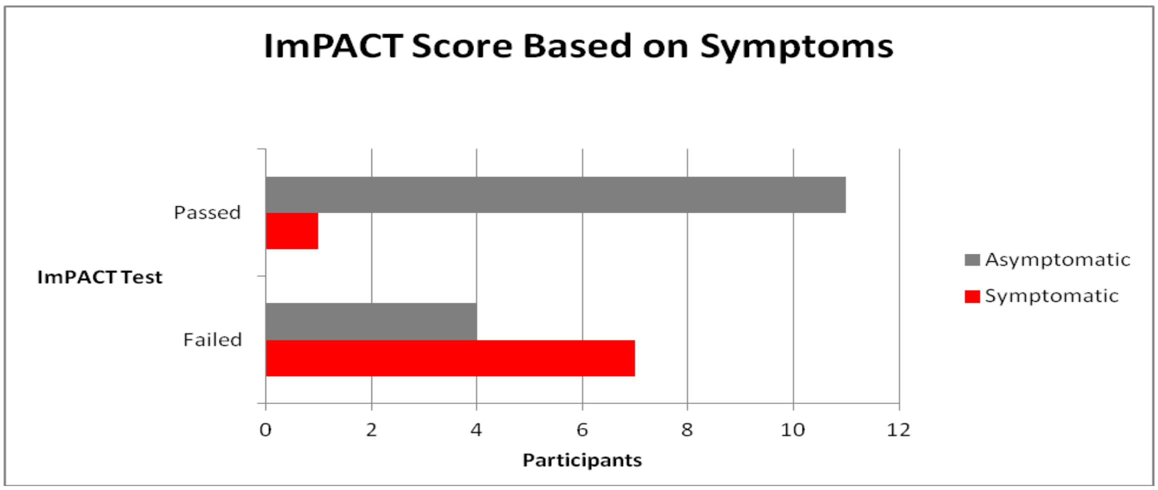

Figure 6. Participant Symptoms and the ImPACT Results.

CHAPTER 5

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

The overall purpose of this study was to examine the relationship between the change in the concussion-related biomarker, S100B, and the change in selected diagnostic measures thought to be associated with a concussion in football players. Specifically, this study aimed to examine the relationship between the change in the blood S100B level and 1) change in ImPACT Test scores, 2) head hit number, and 3) head hit intensity. The study's main hypothesis was that the change in blood S100B levels would be negatively correlated to the change in ImPACT Test scores and positively correlated to hit number and hit intensity.

Summary

S100B Level

The S100B levels were low in all of the samples, which was expected since no concussions or traumatic brain injuries were observed in participants. Six pre-practice S100B levels were higher than post-practice S100B levels. One possible explanation for higher pre-practice levels is that these levels were not a true baseline measurement. S100B levels can be elevated after normal physical activity without head hits.[14,53] Ten of the participants had finished a weight training session within 30 minutes of completing the pre-practice blood draw. Then these same participants did not participate in the test practice at the same physical activity intensity as the weight training session completed prior to pre-practice blood draw because they were limited at practice due to injuries. For example, participant 11 who completed a weight training session before the pre-season and pre-practice blood draws had S100B levels of 87.2 and 109.7 pg/ml, respectively. However, he did not participate in the test practice and did not engage in physical activity before the post-practice blood draw, which led to a post-practice S100B level of 38.5 pg/ml.

Previous studies involving measurements of S100B level in football athletes have used venipuncture blood draw[14, 53]. In contrast, finger stick blood draw was used for data collection in this study. The amount of whole blood drawn for data collection was 600 µl which supplied roughly 180 µl of serum from each participant. In comparison, venipuncture blood draw collects 10 ml of whole blood, which is processed into approximately 2.8 ml of serum. The limited amount of blood drawn by finger stick might have contributed to the difficulty in reliably detecting S100B in the samples.

Although only four participants' samples yielded usable and reliable S100B data, the trend in the data suggests a link between higher post-practice S100B levels and head hits. Participant 29 was the only one of the four participants to sustain head hits. This participant's post-practice S100B level was 124.8 pg/ml, which was higher than that of the three other participants. In addition, his S100B difference was greatest too.

ELISA Kit

The ELISA kit yielded undetectable S100B levels. For the first kit, a dilution factor of 1:4 was used as recommended by the vendor. However, the majority of samples yielded undetectable levels. Alternatively, or the second kit, a more concentrated sample (1:2) was used, but did not yield values within the detection range. Although the kit is sensitive at 15 pg/ml, [55] this sensitivity might not have been applicable to this study's samples.

ImPACT Test Scores

The ImPACT test is a computerized program that can take 20-30 minutes to complete. It measures how the test taker's brain is working. Each section challenges the brain with different types of tasks that evaluate memory, speed, coordination and reaction time. Based on the time commitment and challenging tasks of the ImPACT, this was the part of the study that participants choose not to complete.

Normally there are only two reasons a student-athlete would complete an ImPACT test. The test is completed either as a baseline measurement as part of a mandatory pre-participation physical or as a diagnostic test during a concussion evaluation. In both of these situations the level of focus would most likely be higher than if the test was being completed for a research study with no direct implications on the test taker.

The ImPACT test does have calculations included that can be used to determine a ratio of speed versus accuracy during the exam. This calculation can be compared to the baseline measurement to see if an individual had a similar ratio in both tests. If the ratio is lower the test taker was not as accurate at the speed he completed the test as the first time he took the test. A higher ratio would indicate that he was more accurate at the speed at which he completed the test.

The different tasks completed during the ImPACT test were created to evaluate visual and verbal memory, speed and reaction time. Five different composite scores are assigned based on the test results. In the data collected from the participants in this study the most common composite score with the greatest difference compared to their baseline was visual memory which had a mean difference in score of 11.91± 14.24 points.

Head Hit Number and Intensity

An objective score was given to the video analysis of number and intensity of head hits from the practice used in this study; this was termed the Video Analysis Score. The number of hits was recorded on video during the practice. Two athletic trainers watched the video to record the number of hits either being a head hit or a body hit then ranked the severity of each hit. The subjective score of head hit number and intensity came from the post-practice survey.

The athletes in this study reported a higher number of hits in total as well as head hits compared to the number reported from the Video Analysis Score of the practice. This along with the subjective information about the symptoms shows the variability involved with subjective data.

The difference between using a practice versus a game adds to the relevance of these data. The intensity of a game would be considered "high" by the coaching staff in this study. The intensity for the practice chosen for this study was considered "moderate" because of the limited amount of "live" or game-like drills. The tackling form in this practice was more controlled overall than during a game. The athletes in "non-live" drills were instructed to "thud" the opposing player, which meant not taking them to the ground. The coach explained that a thud tackle works on body control and wrapping up the opponent. In a game where the goal is to get the opponent on the ground or jarring the ball out of their possession the technical form tackle is sometimes replaced with a more explosive tackle. Since some athletes and coaches feel that these more explosive tackles may provide a competitive advantage in certain situations these hits are going to be executed. In practice, however injuries, including head injuries can be decreased if technical form tackles are used.

Signs and Symptoms

One of the main reasons this research study was conducted was to explore objective measurements that could be used to diagnosis a concussion. The goal was to add to the research on objective measurements used in the clinical diagnosis of concussion. The data on the symptoms patients felt after the practice was able to add to the research on the use of subjective measurements with regard to concussions.

In this study, symptoms were measured twice once right after the test practice and then again after the ImPACT test. The time between the practice and ImPACT test was less than two days. Participants had changes in the number and severity of symptoms during that time period. Some of the symptoms reported could have been attributed to exams and projects that the student-athletes were completing that week due to midterms. Specifically, “trouble falling asleep” or “sleeping less than usual” could be attributed to long nights of studying or writing papers and not be related to concussion symptoms. The change in symptoms from the post-practice survey to the ImPACT test symptoms checklist could be due to the participants feeling different at one time compared to another or the participant may not have been telling the truth on one or both of the symptom questionnaires. One of the questionnaires was completed in front of research team members while the other was completed on the computer where the participant may have felt that their information could not be connected to them individually. Either way following the NCAA guidelines these participants should have been removed from play because they were having symptoms consistent with a concussion.[4]

Symptoms are an important part of any injury. If a physically active individual states they are not feeling normal it is the duty of the medical professional to evaluate them. The concern would be that sometimes symptoms are not disclosed due to the fear of being held out of sport. Also many athletes don't know when a symptom is normal or gets to the point that it is worse than normal, especially with head injuries. That is why basing concussion evaluation solely on symptoms is safe but may be not the best treatment plan.

Limitations

There are three major limitations of this study. The use of a single finger stick blood draw may be a limitation. This technique was selected so that the amount of blood drawn was low and an athletic trainer could draw the blood. However, finger stick blood draw may not be appropriate when measuring circulating S100B levels. Future studies are needed in which serum S100B levels obtained from venipuncture and finger stick blood draws are compared.

The ELISA kit used in this study may have been a limitation. Samples were below the kit's detection range (15 pg/ml),[55] this detection range limited the number of useable results. Future studies need to be completed with ELISA kits that have a lower detection range. This would provide information for samples with a lower S100B level.

The length and nature of the ImPACT test was another limitation. The ImPACT test takes between 20-30 minutes to complete and must be done while sitting at a computer. Normally, each football player completes only one ImPACT test annually as a baseline. Six participants declined to complete the post-practice ImPACT test. These participants explained that they declined to complete the test because of the time commitment and nature of the test.

Conclusions and Recommendations

Conclusions

An increased serum S100B level may be linked to head hits during collegiate football practice. In this study, the participant who suffered head hits (as determined by video review) during practice had a serum S100B level that increased 85.3 pg/ml after practice. In comparison, three other participants who did not sustain head hits (as determined by video review) had decreased or a minimal increase in the serum S100B level. Further research is needed to determine whether the participants in this study sustained mild brain injury.

For an athletic trainer to provide comprehensive care to football players, the athletic trainer needs objective clinical data. As further research is done on the blood biomarkers as a diagnostic assessment tool for head injuries, it will be important to explore how these data are collected. This study did show that finger stick blood can be performed in the clinical setting with limited complications.

Recommendations

Future research should examine blood S100B levels in college football players with diagnosed concussions. Specifically, a pre-season blood S100B level as a baseline is obtained, and then blood S100B levels are obtained after any possible concussion or hit prompting evaluation from the medical staff. After obtaining these levels, the relationship between blood S100B levels and a clinically diagnosed concussion can be determined.

For S100B to be a relevant concussion blood bio-marker more research must be completed to demonstrate that blood can be practically and reliably tested. The promise of an objective test that can take the guessing out of concussion diagnosis outweighs the challenges associated with the use of S100B. As more studies are completed that support the effectiveness of S100B these practical issues will need to be addressed. The ultimate goal for this line of research is to create and test a portable device that has the capabilities to measure S100B in minutes providing the healthcare provider with a number that can be used to diagnosis a concussion in the clinical setting.

APPENDIX

Ten Participants' S100B Level Samples from Post-Practice

Of the post-practice serum samples, there were 10 samples with a CV ≤15%. Using these samples, the investigator performed a Pearson's Correlation between post-practice S100B levels and HII, Video Analysis Score, ImPACT Verbal Memory Composite Score, ImPACT Visual Memory Composite Score, ImPACT Motor Speed, ImPACT Reaction Time, and ImPACT Impulse Control. Table 7 shows the data per participant. Alpha was set at 0.05.

The analyses that yielded significant positive moderately to high correlations are ImPACT Video Analysis Score, ImPACT Visual Memory, and ImPACT Impluse Control. In relation to the visual memory score, the positive relationship means that participants that scored higher on their post-practice ImPACT test than their pre-season ImPACT had a higher levels of S100B post-practice. This is opposite of what was hypothesized. This relationship could be due to the fact that no participants in this study suffered a concussion which would have caused their ImPACT scores to decrease. The one participant that had a high S100B level compared to normal values did well on the ImPACT test. Either this participant had a higher S100B baseline level or he was able to focus to do well on the ImPACT test. This participant has had two concussions over the past year which, based on previous studies, should have no effect on S100B levels.

Table 7. S100B Post-Practice Levels, Hit Data and ImPACT Composite Scores.

Participant	Post S100B	HIT Index	Video Analysis	Verbal Memory Composite	Visual Memory Composite	Motor Speed	Reaction Time	Impulse Control
2	31.2	6	1	-21	39	-1.82	0.1	-2
4	43.2	2	0	-23	17	-13.78	0.09	0
8	109.8	1	2	N/A	N/A	N/A	N/A	N/A
11	38.5	0	0	7	18	3.45	-0.13	1
12	38.2	1	1	-3	20	0.05	-0.05	5
15	49.9	2	1	12	4	-0.28	0.06	0
16	79.4	0	0	-1	-4	-1.35	0.08	2
18	41.0	0	0	-7	26	-8.28	0.04	0
28	55.1	6	2	-6	25	-1.97	-0.04	-1
29	124.9	6	2	-4	-22	1.8	-0.08	4

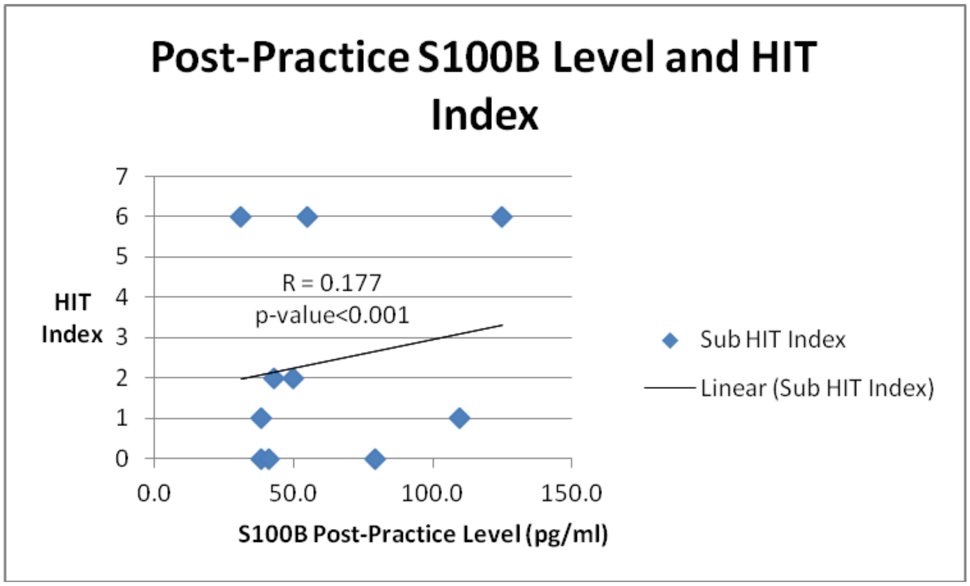

Figure 7. Relationship of Post-Practice S100B Level and HHI Score.

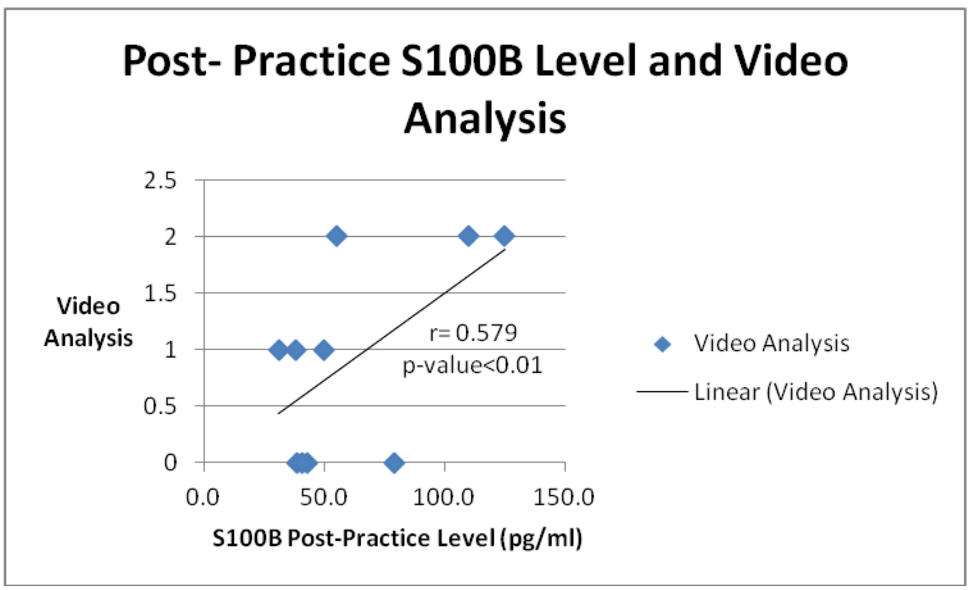

Figure 8. Relationship of Post-Practice S100B Level and Video Analysis Score.

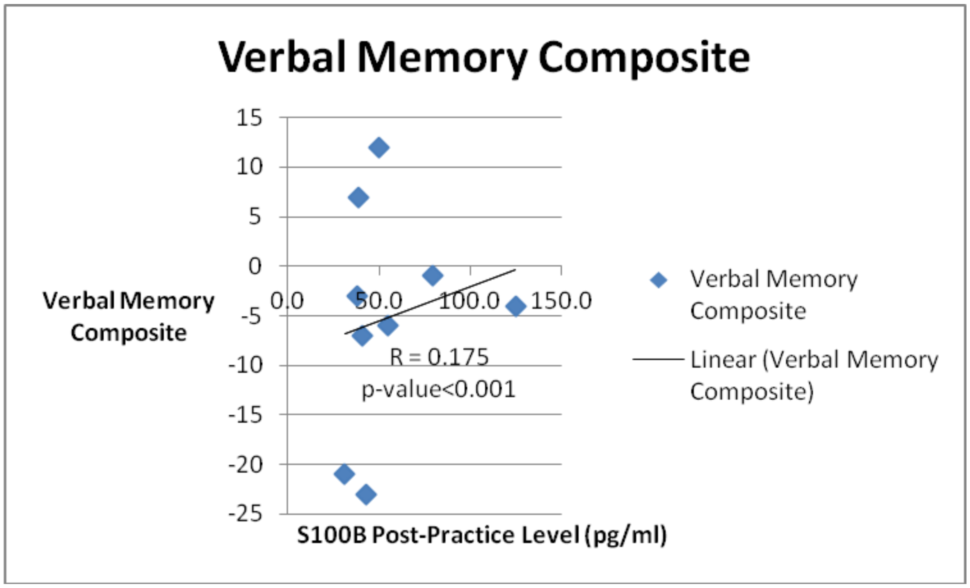

Figure 9. Relationship of Post-Practice S100B Level and Verbal Memory Composite Score.

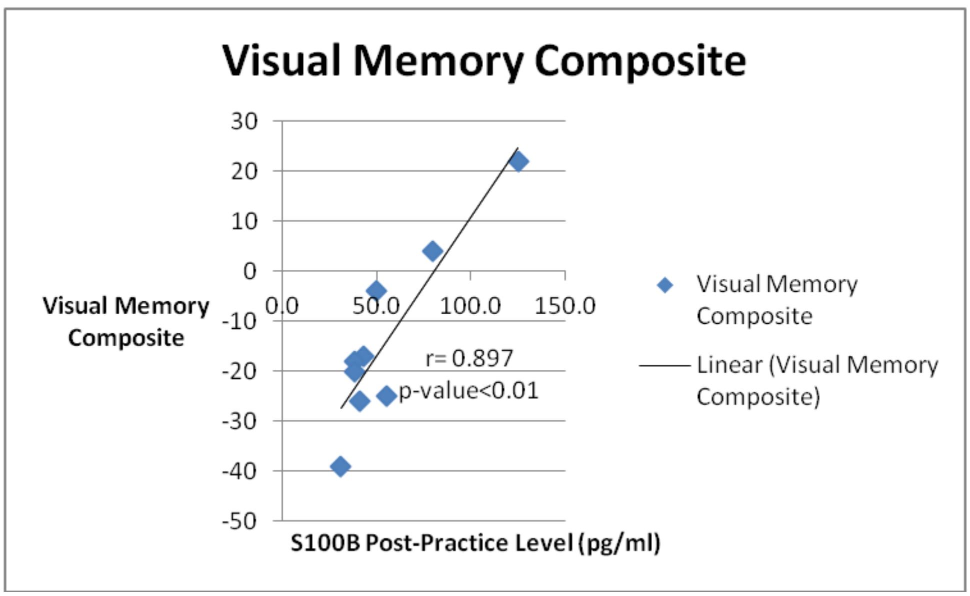

Figure 10. Relationship of Post-Practice S100B Level and Visual Memory Composite Score.

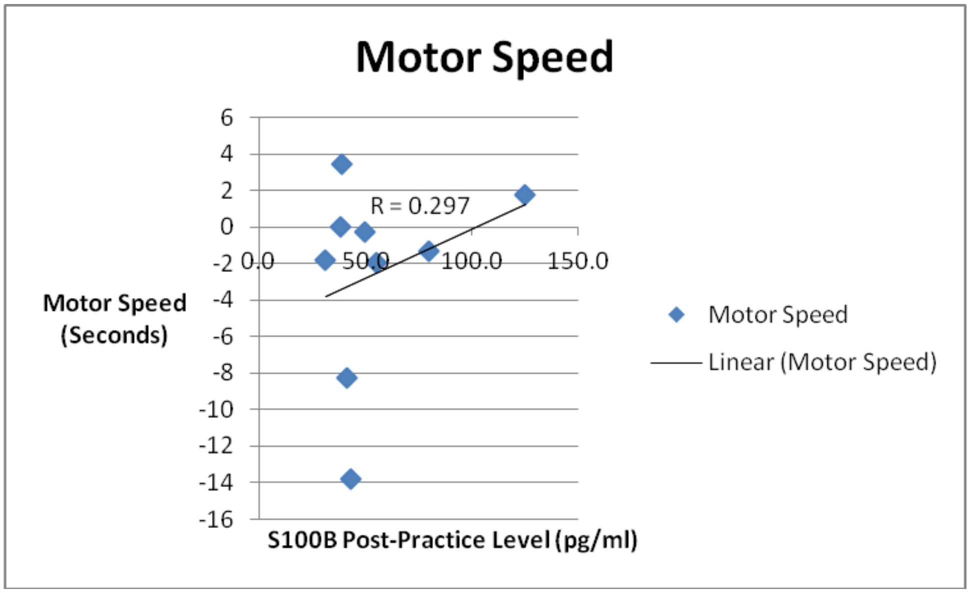

Figure 11. Relationship of Post-Practice S100B Level and Motor Speed.

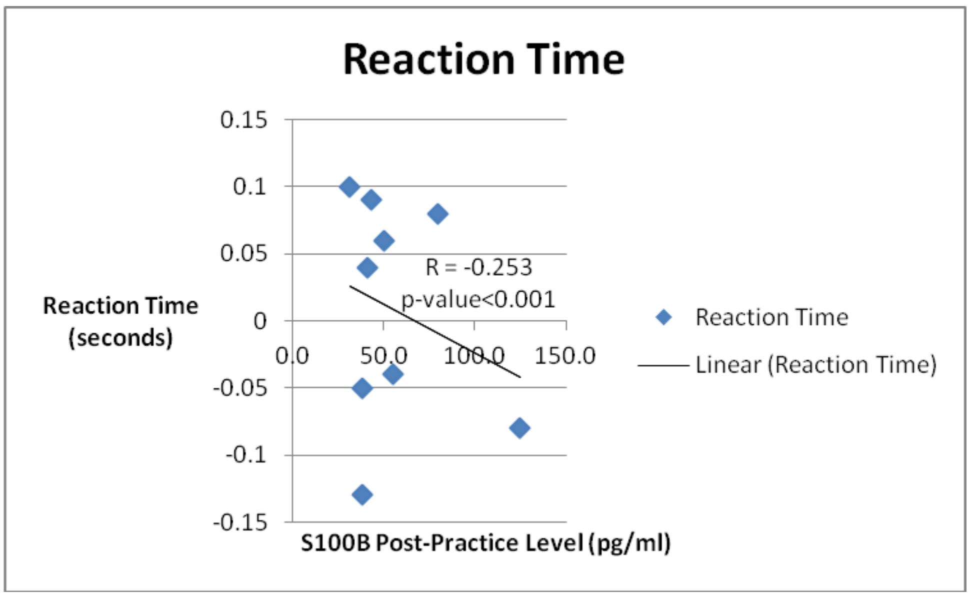

Figure 12. Relationship of Post-Practice S100B Level and Reaction Time.

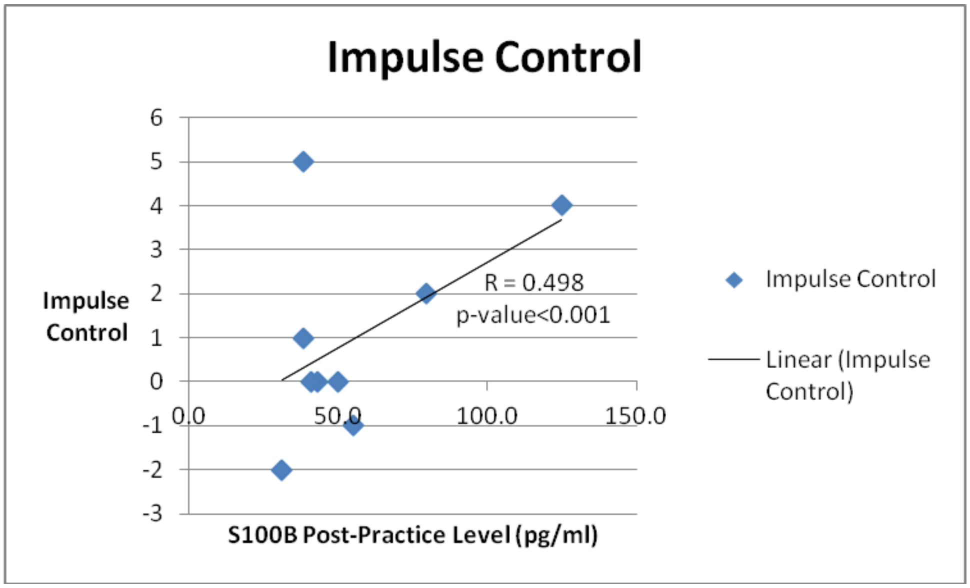

Figure 13. Relationship of Post-Practice S100B Level and Impulse Control.

Table 6. Head Hit Index

HEAD HIT INDEX

HHI (A x B)	A=0 (no hits)	A=1 (1-4 hits)	A=2 (5-20 hits)	A=3 (>20 hits)
B=0 (negligible, body-helmet or ground-helmet contacts)	0 No Head Hits	0 Negligible Hits	0 Negligible Hits	NA
B=1 (player acknowledges the head hit)	0 No Head Hits	1 Few Head Hits	2 Several but normal hits	3 > 20 normal hits
B=2 (player distinctively remember the head hit)	0 No Head Hits	2 Few Harsh Head Hits	4 Player who had several hits including harsh ones	6 Player who had >20 hits of which several were harsh

Post Practice Questionnaire

1. How many contacts/collisions did you experience during the practice?
   A. None B. 1-4 C. 5-20 D. More than 20
2. How many of those contacts/collisions involved your head?
   A. None B. 1-4 C. 5-20 D. More than 20
3. How severe were the contacts/collisions involving your head on a scale from 0-4? (0- no contacts/collisions involving head, 2- worst head contact/collision ever)
   A. 0 B. 1 C. 2
4. Do you have any symptoms of a concussion currently? Please rate your symptoms.

Nausea	0	1	2	3	4	5	6
Headache	0	1	2	3	4	5	6
Dizziness	0	1	2	3	4	5	6
Fatigue	0	1	2	3	4	5	6
Drowsiness	0	1	2	3	4	5	6
Feeling like in a fog	0	1	2	3	4	5	6
Difficulty concentrating	0	1	2	3	4	5	6
Sensitivity to light	0	1	2	3	4	5	6
Sensitivity to noise	0	1	2	3	4	5	6
Blurred vision	0	1	2	3	4	5	6
Feeling slowed down	0	1	2	3	4	5	6

Figure 14. Post Practice Survey

UNLV
UNIVERSITY OF NEVADA LAS VEGAS

INFORMED CONSENT

Department of Kinesiology and Nutritional Sciences

TITLE OF STUDY: The Interrelationships Among Concussion Bio Marker, Head Hits, ImPact Test in Collegiate Football Players

INVESTIGATOR(S):

For questions or concerns about the study, you may contact Dr. Dufek at (702) 895-0702 or Dr. St. Pierre Schneider at (702)895-1216 or Lucas Bianco at **(845)728-4176.**

For questions regarding the rights of research subjects, any complaints or comments regarding the manner in which the study is being conducted, contact **the UNLV Office of Research Integrity – Human Subjects at 702-895-2794, toll free at 877-895-2794 or via email at IRB@unlv.edu.**

Purpose of the Study

You are invited to participate in a research study. The purpose of this study is to examine the relationship between the change in the concussion-related biomarker, and the change in selected diagnostic measures thought to be associated with a concussion in football players.

Participants

You are being asked to participate in the study because you fit criteria: 18-30 years of age, division I collegiate football athlete.

Procedures

If you volunteer to participate in this study, you will be asked to do the following: allow trained professionals to draw your blood (600 µl), about the same amount as less than 1/10 of a teaspoon, from your finger at most twice before the season then before and after one practice during the 2013-2014 football season. Answer truthfully on a post-practice questionnaire about the hits you received or gave out during the practice. Note that the video from the game will be reviewed to document the number of hits you gave or received. Your baseline ImPact test score from your medical record will be compared to a second ImPact concussion test taken within two weeks of the post game blood draw.

Benefits of Participation

There will not be direct benefits to you as a participant in this study. However, we hope you learn about the incidence of concussions at the collegiate football level as well as the new diagnostic tools currently being studied to assist in the assessment and diagnosis of concussions.

Risks of Participation

There are risks involved in all research studies. This study may include only minimal risks. Anticipated risks include possible minimal discomfort from the blood draw. With any breach of skin there is a possibility of infection.

Cost /Compensation

There will not be financial cost to you to participate in this study. The study will take 45 minutes of your time over 3-4 days. You will not be compensated for your time.

Confidentiality

All information gathered in this study will be kept as confidential as possible. None of the research results including the ImPact test score or symptoms checklist will be collected or used by the athletic training staff, nor will it become a part of your athletic file. No reference will be made in written or oral materials that could link you to this study. Paper files will be shredded, electronic files will be deleted and any backup storage media destroyed. Left over blood samples will be stored indefinitely in the BHS 118, 120 and or 122 labs.

The data recorded in this research study will not be shared with any coaching or medical (e.g., Team Physician, Athletic Trainer) staff. We want you to talk with those staff members about any injury or game issues that you would normally talk to them about even if you have told us about those issues because we will not tell them about any of your responses.

Voluntary Participation

Your participation in this study is voluntary. You may refuse to participate in this study or in any part of this study. You may withdraw at any time without prejudice to your relations with UNLV. You are encouraged to ask questions about this study at the beginning or any time during the research study.

Participant Consent:

I have read the above information and agree to participate in this study. I have been able to ask questions about the research study. I am at least 18 years of age. A copy of this form has been given to me.

______________________ __________

Signature of Participant Date

____

Participant Name (Please Print)

REFERENCES

1. Larson R, Seepersad S. Adolescents' Leisure Time in the United States: Partying, Sports, and the American Experiment. 2003; 99: 53-63. In: Verma S, Larson R. *New Directions for Child and Adolescent Development: Volume 99. Examining Adolescent Leisure Time Across Cultures: Developmental Opportunities and Risks*. San Francisco, CA: Jossey Bass.
2. Mahoney JL, Larson RW, Eccles JS. *Organized Activities as Contexts of Development: Extracurricular Activities, After-School and Community Programs*. Mahwah, NJ: Erlbaum; 2005: 3-22.
3. McCrea M, Hammeke T, Olsen G, Leo, Guskiewicz K. Unreported Concussion in High School Football Players. *Clin J Sport Med.* 2004; 14(1): 13-17.
4. Klossner D. Sports Medicine Handbook. *The National Collegiate Athletic Association. 23rd Edition*. 2012; 53-57.
5. McCrory P, Meeuwisse WH, Aubry M, Cantu B, Dvořák J, Echemendia RJ, Turner M. Consensus Statement on Concussion in Sport: The 4th International Conference on Concussion in Sport held in Zurich. *Br J Sports Med*. 2012; 47(5): 250-258.
6. Greenwald RM, Chu JJ, Beckwith JG, Crisco JJ. A Proposed Method to Reduce Underreporting of Brain Injury in Sports. *Clin J Sport Med*. 2012; 22(2): 83-85.
7. Müller K, Townend W, Biasca N, Undén J, Waterloo K, Romner B, Ingebrigtsen T. S100B Serum Level Predicts Computed Tomography Findings after Minor Head Injury. *J Trauma Acute Care Surg*. 2007; 62(6): 1452-1456.
8. Schmid KE, Tortella FC. The Diagnosis of Traumatic Brain Injury on the Battlefield. *Front Neurol*. 2012; 3(39): 90.
9. Savola O, Pyhtinen J, Leino TK, Siitonen S, Niemelä O, Hillbom M. Effects of Head and Extracranial Injuries on Serum Protein S100B Levels in Trauma Patients. *J Trauma Acute Care Surg*. 2004; 56(6): 1229-1234.
10. Rothermundt M, Missler U, Arolt V, Peters M, Leadbeater J, Wiesmann M, Rudolf S, Wandinger KP, Kirchner H. Increased S100B Blood Levels in Unmedicated and Treated Schizophrenic Patients are Correlated with Negative Symptomatology. *BMC Physiol*. 2001; 6(4): 445-449.
11. Wiesmann M, Missler U, Gottmann D, Gehring S. Plasma S-100b Protein Concentration in Healthy Adults is Age-and Sex-Independent. *Clin Chem*. 1998; 44(5): 1056-1058.
12. Anderson RE, Hansson LO, Nilsson O, Dijlai-Merzoug R, Settergren G. High Serum S100B Levels for Trauma Patients without Head Injuries. *Neurosurgery*. 2001; 48(6): 1255-1260.
13. Mondello S, Muller U, Jeromin A, Streeter J, Hayes RL, Wang KK. Blood-based Diagnostics of Traumatic Brain Injuries. *Expert Rev Mol Diagn*. 2011; 11(1): 65-68.
14. Kiechle K, Bazarian JJ, Merchant-Borna K, Stoecklein V, Rozen E, Blyth B, Biberthaler P. Subject-Specific Increases in Serum S-100B Distinguish Sports-Related Concussion from Sports-Related Exertion. *PloS One*. 2014; 9(1): 849-877.
15. Ma R, Miller CD, Hogan MV, Diduch BK, Carson EW, Miller MD. Sports Related Concussion: Assessment and Management. *J Bone Joint Surg*. 2012; 94(17): 1618-1627.
16. Guskiewicz KM, Bruce SL, Cantu RC, Ferrara MS, Kelly JP, McCrea M, Putukian M, Valovich, McLeod TC. National Athletic Trainers' Association Position Statement: Management of Sport Related Concussion. *J Athl Train.* 2004; 39(3): 280-297.
17. Harmon KG, Drezner JA, Gammons M, Guskiewicz KM, Halstead M, Herring SA, Roberts WO. American Medical Society for Sports Medicine Position Statement: Concussion in Sport. *Br J Sports Med*. 2013, 47(1): 15-26.

18. Broglio SP, Sosnoff JJ, Shin S, He X, Alcaraz C, Zimmerman J. Head Impacts During High School Football: A Biomechanical Assessment. *J Athl Train*. 2009; 44(4): 342-349.
19. Casson IR, Viano DC, Powell JW, Pellman EJ. Twelve Years of National Football League Concussion Data. *Sports Health: A Multidisciplinary Approach*. 2010; 2(6): 471-483.
20. Mueller FO. Catastrophic Head Injuries in High School and Collegiate Sports. *J Athl Train*. 2001; 36(3): 312-315.
21. Mansell JL, Tierney RT, Higgins M, McDevitt J, Toone N, Glutting J. Concussive Signs and Symptoms Following Head Impacts in Collegiate Athletes. *Brain Inj*. 2010; 24(9): 1070-1074.
22. Teasdale TW, Engberg A. Duration of Cognitive Dysfunction after Concussion, and Cognitive Dysfunction as a Risk Factor: A Population Study of Young Men. *Br Med J*. 1997; 315(7108): 569-572.
23. McKinley J. New Challenges in Assessing and Managing Concussion in Sports. *Am Fam Physician*. 2007; 76(7):948-949.
24. Howell D, Osternig L, van Donkelaar P, Mayr U, Chou LS. Effects of Concussion on Attention and Executive Function in Adolescents. *Med Sci Sports Exerc*. 2012; 45(6): 1030-1037.
25. Dick RW. Is there a Gender Difference in Concussion Incidence and Outcomes? *Br J Sports Med*. 2009; 43(1): 46-50.
26. Broglio SP, Puetz *TW. The Effect of Sp*ort Concussion on Neurocognitive Function, Self Report Symptoms and Postural Control: A Meta-Analysis. *J Sports Med*. 2008; 38(1): 53-57.
27. Randolph C, Millis S, Barr WB, McCrea M, Guskiewicz KM., Hammeke TA, Kelly JP. Concussion Symptom Inventory: An Empirically Derived Scale for Monitoring Resolution of Symptoms Following Sport-Related Concussion. *Arch Clin Neuropsychol*. 2009; 24(3): 219-229.
28. Mondello S, Papa L, Buki A, Bullock MR, Czeiter E, Tortella FC, Wang KK, Hayes RL. Neuronal and Glial Markers are Differently Associated with Computed Tomography Findings and Outcome in Patients with Severe Traumatic Brain Injury: A Case Control Study. *J Cri Care*. 2011; 15(3): 156-183.
29. Mondello S, Jeromin A, Buki A, Bullock R, Czeiter E, Kovacs N, Barzo P, Schmid K, Tortella FC, Wang KK, Hayes RL. Glial Neuronal Ratio (GNR): A Novel Index for Differentiating Injury Type in Patients with Severe Traumatic Brain Injury. *J Neurotrauma*. 2012; 29(6): 1096-1104.
30. Mondello S, Linnet A, Buki A, Robicsek S, Gabrielli A, Tepas J, Papa L, Brophy GM, Tortella F, Hayes RL, Wang KK. Clinical Utility of Serum Levels of Ubiquitin C-Terminal Hydrolase as a Biomarker for Severe Traumatic Brain Injury. *J Neurosurgery*. 2012; 70(3): 666-675.
31. Mondello S, Robicsek SA, Gabrielli A, Brophy GM, Papa L, Tepas J, Robertson C, Buki A, Scharf D, Jixiang M, Akinyi L, Muller U, Wang KK, Hayes RL. Alphaii-Spectrin Breakdown Products (Sbdps): Diagnosis and Outcome in Severe Traumatic Brain Injury Patients. *J Neurotrauma*. 2010; 27(7): 1203-1213.
32. Iverson GL. Outcome from Mild Traumatic Brain Injury. *Curr Opin Psychiatry*. 2005; 18(3): 301-317.
33. Khurana VG, Kaye AH. An Overview of Concussion in Sport. *J Clin Neuroscience*. 2012; 19(1): 1-11.
34. Grubenhoff JA, Kirkwood M, Gao D, Deakyne S, Wathen J. Evaluation of the Standardized Assessment of Concussion in a Pediatric Emergency Department. *Pediatrics*. 2010; 126(4): 688-695.

35. Signoretti S, Lazzarino G, Tavazzi B, Vagnozzi R. The Pathophysiology of Concussion. *Am J Phys Med and Rehabil*. 2011; 3(10): 359-368.
36. Jantzen KJ, Anderson B, Steinberg FL, Kelso JS. A Prospective Functional MR Imaging Study of Mild Traumatic Brain Injury in College Football Players. *Am J Neuroradiology*. 2004; 25(5): 738-745.
37. Putukian M. The Acute Symptoms of Sport-Related Concussion: Diagnosis and On-Field Management. *Clin Sports Med*. 2011; 30(1): 49-61.
38. Xiong H, Liang WL, Wu XR. Pathophysiological Alterations in Cultured Astrocytes Exposed to Hypoxia/ Reoxygenation. *Prog Psych*. 2000; 31(3): 217-221.
39. Hu YY, Dong XQ, Yu WH, Zhang ZY. Change in Plasma S100B Level after Acute Spontaneous Basal Ganglia Hemorrhage. *Shock*. 2010; 33(2): 134-140.
40. Kleindienst A, Schmidt C, Parsch H, Emtmann I, Xu Y, Buchfelder M. The Passage of S100B from Brain to Blood is Not Specifically Related to the Blood-Brain Barrier Integrity. *Cardiovasc Psychiatry Neurol*. 2010; 2010(1): 1-8.
41. Undén J, Romner B. Can Low Serum Levels of S100B Predict Normal CT Findings after Minor Head Injury in Adults? An Evidence-Based Review and Meta-Analysis. *J Head Trauma Rehabil*. 2010; 25(4): 228-240.
42. Schatz P, Pardini JE, Lovell MR, Collins MW, Podell K. Sensitivity and Specificity of the ImPACT Test Battery for Concussion in Athletes. *Arch Clin Neuropsychol*. 2006; 21(1): 91-99.
43. Collins MW, Grindel SH, Lovell MR. Relationship between Concussion and Neuropsychological Performance in College Football Players. *JAMA.* 1999; 282(10): 964-970.
44. Clark S, Iltis PW. Effects of Dynamic Head Tilts on Sensory Organization Test Performance: A Comparison between College-Age Athletes and Nonathletes. *J Ortho Sports Phys Ther*. 2008; 38(5): 262-268.
45. Gysland SM, Mihalik JP, Register-Mihalik JK, Trulock SC, Shields EW, Guskiewicz KM. The Relationship between Subconcussive Impacts and Concussion History on Clinical Measures of Neurologic Function in Collegiate Football Players. *Ann Biomed Eng* 2012; 40(1): 14-22.
46. Guskiewicz KM, Mihalik JP, Shankar V, Marshall SW, Crowell DH, Oliaro SM, Hooker DN. Measurement of Head Impacts in Collegiate Football Players: Relationship Between Head Impact Biomechanics and Acute Clinical Outcome after Concussion. *Neurosurgery*. 2007; 61(6): 1244-1253.
47. McCrory P, Meeuwisse WH, Kutcher JS, Jordan BD, Gardner A. What is the Evidence for Chronic Concussion-Related Changes in Retired Athletes: Behavioral, Pathological and Clinical Outcomes? *Br J Sports Med*. 2013; 47(5): 327-330.
48. Abdesselam OB, Vally J, Adem C, Foglietti MJ, Beaudeux JL. Reference Values for Serum S100B Protein Depend on the Race of Individuals. *Clin Chem*. 2003; 49(5): 836-837.
49. Zongo D, Ribéreau-Gayon R, Masson F, Laborey M, Contrand B, Salmi LR, Lagarde E. S100-B Protein as a Screening Tool for the Early Assessment of Minor Head Injury. *Ann Emerg Med*. 2012; 59(3): 209-218.
50. Marion DW, Curley KC, Schwab K, Hicks RR. mTBI Diagnostics Workgroup. Proceedings of the Military mTBI Diagnostics Workshop, St. Pete Beach. *J Neurotrauma*. 2010; 28(4): 517-526.
51. O'Connor FG. SORT: Key Recommendations for Practice. *Am Fam Physician*. 2012; 85(2): 123-132.
52. Nuwer MR, Hovda DA, Schrader LM, Vespa PM. Routine and Quantitative EEG in Mild Traumatic Brain Injury. *Neurophysiol Clin*. 2005; 116(9): 2001-2025.

53. Marchi N, Bazarian JJ, Puvenna V, Janigro M, Ghosh C, Zhong J, Janigro D. Consequences of Repeated Blood-Brain Barrier Disruption in Football Players. *PloS One.* 2013; 8(3): 1 11.
54. Miller JR, Adamson GJ, Pink MM, Sweet JC. Comparison of Preseason, Midseason, and Postseason Neurocognitive Scores in Uninjured Collegiate Football Players. *Am J Sports Med*. 2007; 35(8): 1284-1288.
55. Biovendor Research and Diagnostic Products. *Human S100B ELISA: Product Data Sheet.* Ashville, NC. 2013.

VITA

Lucas Cameron Bianco MS, ATC, CSCS

EDUCATION____

University of Nevada- Las Vegas, Las Vegas, Nevada

Graduation: May 2014

Master of Science Degree in Kinesiology with an Emphasis in Athletic Training

Lynchburg College, Lynchburg, Virginia

Graduation: May 2012

Bachelor of Science Degree in Athletic Training

Minor in English- Writing

PROFESSIONAL CLINICAL EXPERIENCE_________________________

- Athletic Training, University of Nevada- Las Vegas
  --Provide coverage, treatment, rehabilitation and emergency care to student-athletes.

  -*Women's Softball (1/14-5/14)*

  -*Men's Football (8/12-1/14)*

  -*Men's and Women's Tennis (1/13-1/14)*

  -*Men's Club Hockey (11/13-2/14)*

SERVICE ACTIVITIES____________________________________

- 2013 National Athletic Trainers' Association National Meeting Volunteer
  - Assisting with the Indianapolis Convention Committee
- 2013 National Athletic Trainers' Association National Meeting Volunteer
  - Assisting with the Las Vegas Convention Committee.
- 2012 National Athletic Trainers' Association National Meeting Volunteer
  - Assisting with the St. Louis Convention Committee.
- 2011 NATA National Meeting Volunteer
  - Assisting with the New Orleans Convention Committee.